[Jpn. J. Parasitol., Vol. 38, No. 2, 77–85, April 1989]

Morphological Study of the Stichocyte Granules of *Trichinella spiralis* Muscle Larvae

YUZO TAKAHASHI, TAKAKO UNO, NAOTO MIZUNO, HIDEKAZU SUZUKI, JUN YAGI, AKIYOSHI AISAKA AND TSUNEJI ARAKI

(Accepted for publication; February 14, 1989)

Abstract

An ultrastructure of the stichosome of *Trichinella spiralis* muscle larvae was described and polymorphism of stichocyte granules was revealed; at least 5 types of stichocyte granules were identified based on differences in shape, size, inclusions and distribution within the organ. All of these granules were surrounded by a plasma membrane. Granules termed as α0-granule were in the lower most stichocyte and devoid of inclusions; granules termed as α1-granule were equipped with a sharp inclusion; granules termed as α2-granule were equipped with a rather round inclusion and situated in the upper stichosome; β-granules were devoid of inclusions, and smaller than the α0-granules; granules termed as γ-granule were devoid of inclusions, much smaller than β-granules and situated in the upper stichosome.

Key words: *Trichinella spiralis,* stichosome, granule, electron microscopy, histology

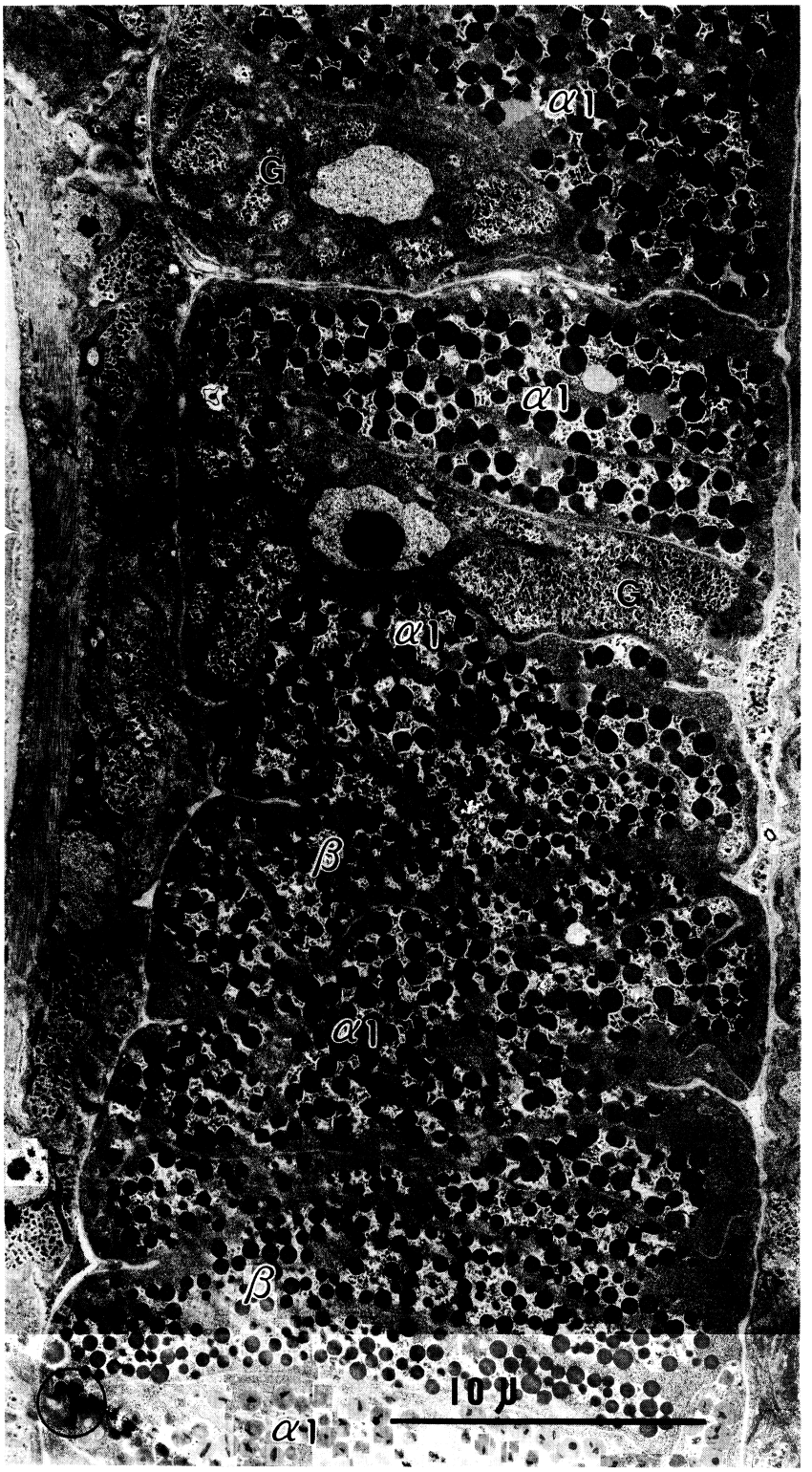
Introduction

The stichosome, which occupies the upper half of the cavity of *Trichinella spiralis* muscle larva, consists of a row of 50—55 discoid cells called stichocytes (Chitwood, 1930; Richels, 1955; Wu, 1955; Villela, 1970). Previous morphological studies have focused on the development of this organ (Villela, 1970; Despommier, 1974), but a description of its ultrastructure and function in the life cycle of the parasite is still incomplete. Two kinds of stichocyte granules have been characterized, those containing α-granules and those containing β-granules (Despommier, 1974). In the lower stichosome, the two types tend to appear usually alternatively but sometimes randomly. It is based on the unique antigenicity of the stichocyte granules that the stichosome has attracted a great deal of attention (Jackson, 1959; Brzosko et al., 1965; Despommier and Muller, 1970). The antigen associated with these granules is capable of inducing protective immunity against an oncoming infection (Despommier, 1977; Despommier and Laccetti, 1981; Gamble, 1985; Murrell and Despommier, 1984; Silberstein and Despommier, 1984, 1985a, 1985b), useful in immunodiagnosis (Gamble and Graham, 1984) and also endowed with stage specificity (Despommier and Muller, 1976). Recent progress in immunostaining technology allows the localization of antigens at the subcellular level disclosing the antigenic properties of each stichocyte granule (Takahashi et al., in press); however, the traditional classification of the stichocyte granules does not seem to be adequate for the precise description of results. This article centers on the definition of the morphological characteristics of each stichocyte granule.

Department of Parasitology, Nara Medical University, Kashihara Nara 634, Japan
Correspondence to: Yuzo Takahashi, M.D., 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　八木純　相坂章爾　荒木恒治（奈良県立医科大学寄生虫学教室）

Materials and Methods

Muscle larvae of *T. spiralis* (Polish strain) were isolated by pepsin-HCl digestion from ICR mouse and prepared for transmission electron microscopy according to the method described


α1
G
α1
α1
G
α1
β
α1
β
10μ
α1

previously (Takahashi et al. 1988a, 1988b). In brief, the excysted worms, approximately 100, were dissected into small pieces in the half strength Karnovsky fixative (Karnovsky, 1965) for better penetration by chemicals, post-fixed with OsO_4, dehydrated with alcohol and embedded in Epok 812 (Oken Syoji Co. Ltd., Tokyo). Ultrathin sections were stained with uranyl acetate and Reynold's solution, and observed under a JEOL 1200EX electron microscope.

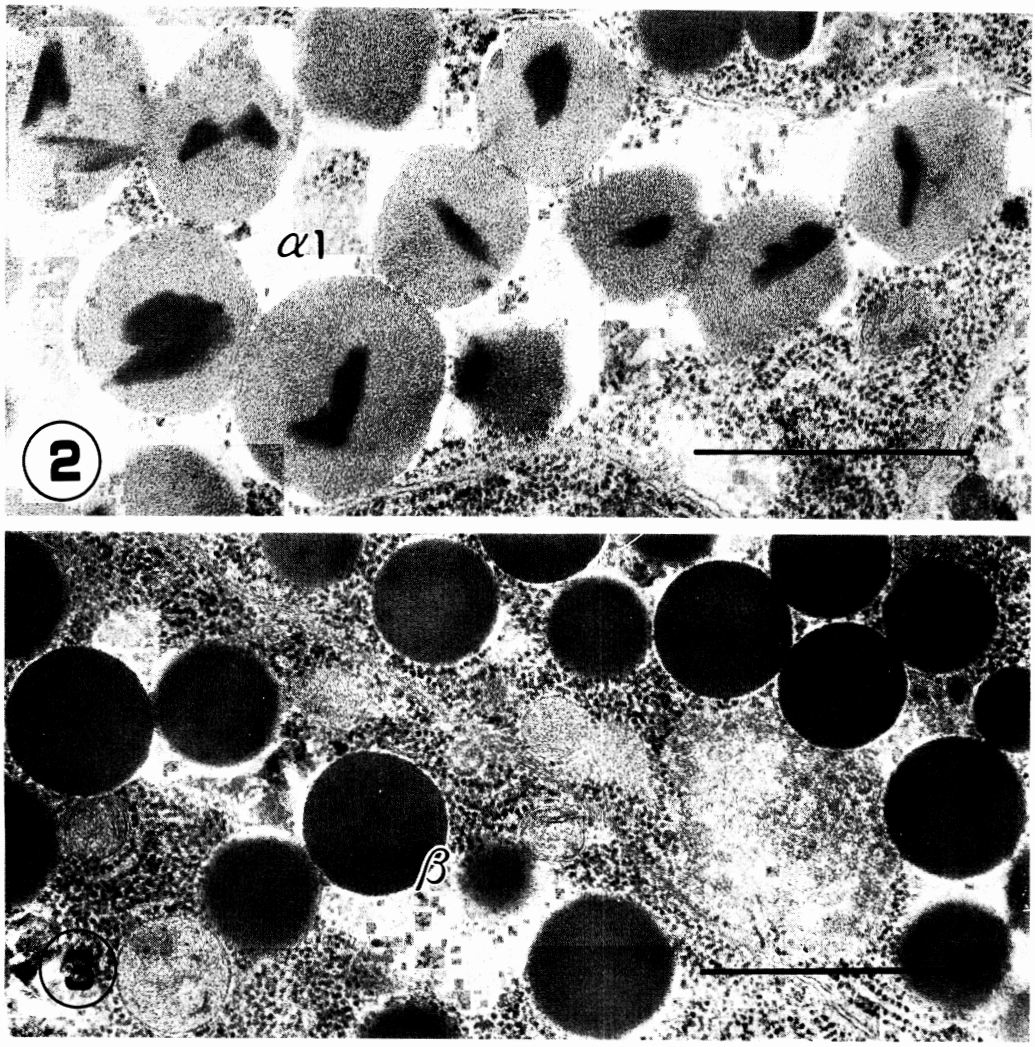


Fig. 2. A high power view of α1-granules (α1). Inclusions are of sharp appearance but the shape varies depending on an angle of sectioning.
Fig. 3. A high power view of β-granules (β).

A bar in photographs represent one μm, if not otherwise noted.

Fig. 1. A longitudinal section through the stichosome characterized by an alternative occurrence of α-stichocytes and β-stichocytes. Sometimes huge accumulation of glycogen aggregates (G) are seen in the cytoplasm. α1: α1-granules, β: β-granules.

Results

Cytoplasmic components

Cytoplasmic components of the stichocyte included granules, rough endoplasmic reticulum (rER), glycogen aggregates, mitochondria, lipid droplets and canalicular trees. Glycogen aggregates were scattered between granules (Figs. 1, 5), sometimes at a large extent (Figs. 1, 5, 6). The junction of the adjacent stichocytes was almost planar appearing segmented by a septum-like structure. This was not a cell membrane nor a basal membrane but rER-rich cytoplasm that was pushed toward the cell periphery by a great accumulation of granules and glycogen (Fig. 1).

α-granules

A crystalloid inclusion was the distinctive feature of α-granules as the electron density of the inclusion was always higher than the matrix.

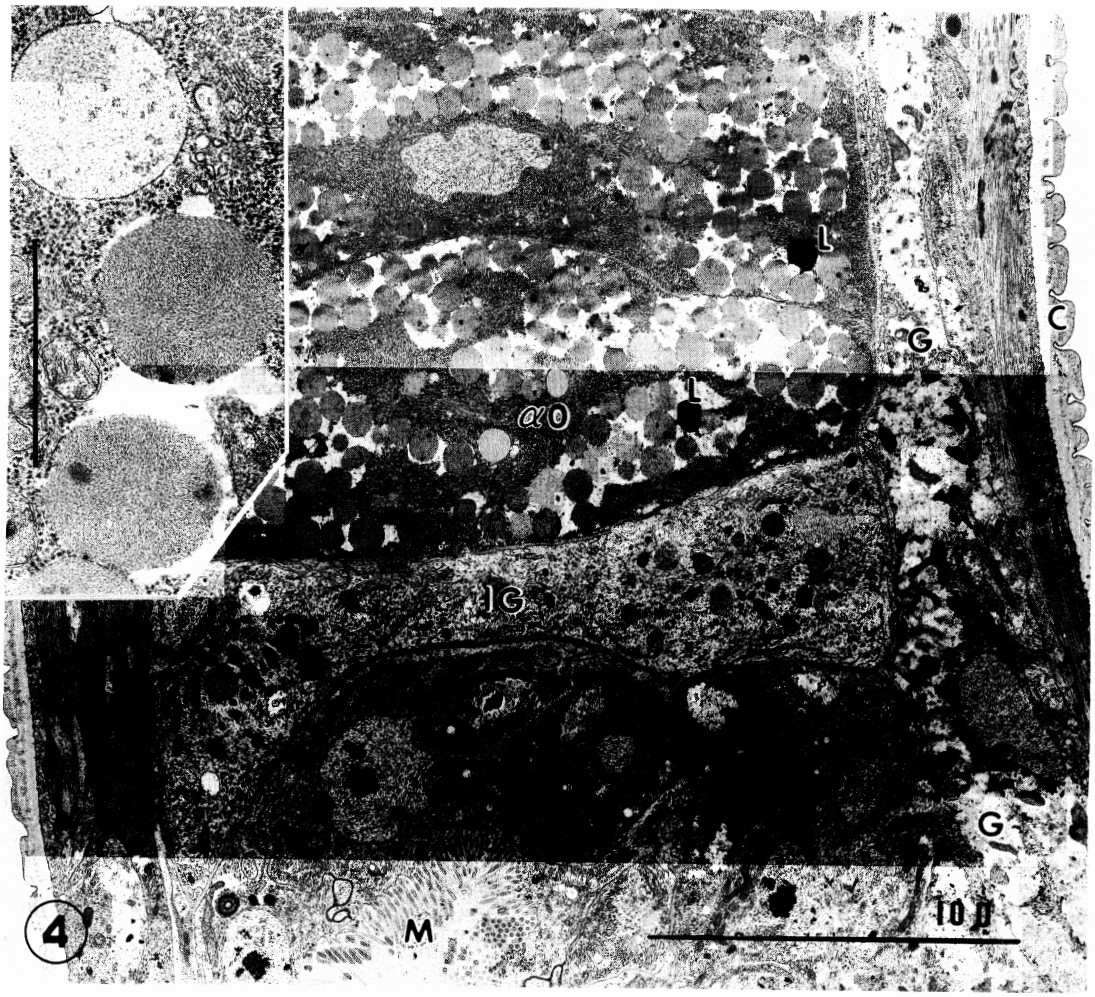


Fig. 4. A longitudinal section through the α-stichocytes, the intestinal gland cell (IG) and the midgut (M). On both sides of these organs, the body wall is seen consisting of the cuticle (C), hypodermis and muscle cells. A cluster of granules locating apparently in the lower most stichocyte are sometimes devoid of inclusions. None of these is equipped with sharp inclusions, therefore it is unlikely the absence of inclusions is an artifact by sectioning. G: glycogen aggregates, L: lipid droplet, α0: α0-granules. An insert is a high power of the granules.

Two kinds of inclusions were distinguished: a sharp crystal and a round crystal. The sharp crystal inclusion was embedded in a homogeneous matrix (sometimes granular at a high magnification) surrounded by a plasma membrane and measuring approximately 800 nm in diameter (Fig. 2). The α-granules of this type were exclusively localized in stichocytes of the posterior region of the stichosome and numbered between 10 and 13. The round crystal inclusions occurred in ellipsoidal shaped granules, measuring 700nm in long diameter and 400nm in short diameter. An α-granule with this type of inclusion was also surrounded by a plasma membrane, equipped with a completely homogeneous matrix (Fig. 6), and usually found in the upper stichosome that exhibits a banded appearance (Takahashi et al., 1987). Sometimes rectangular instead of round inclusions were found.

The lower most stichocyte, which is supposedly an α-stichocyte, often contained granules without inclusions or, if any, just a minute spot (Fig. 4). Those granules were also surrounded by a plasma membrane, and the matrix exhibited a fine granular appearance when observed at a higher magnification (insert in Fig. 4).

β-granules

A β-granule, lacking any inclusion, was the predominant granule in the stichosome (Fig. 1). The granule was 500nm in diameter and equipped

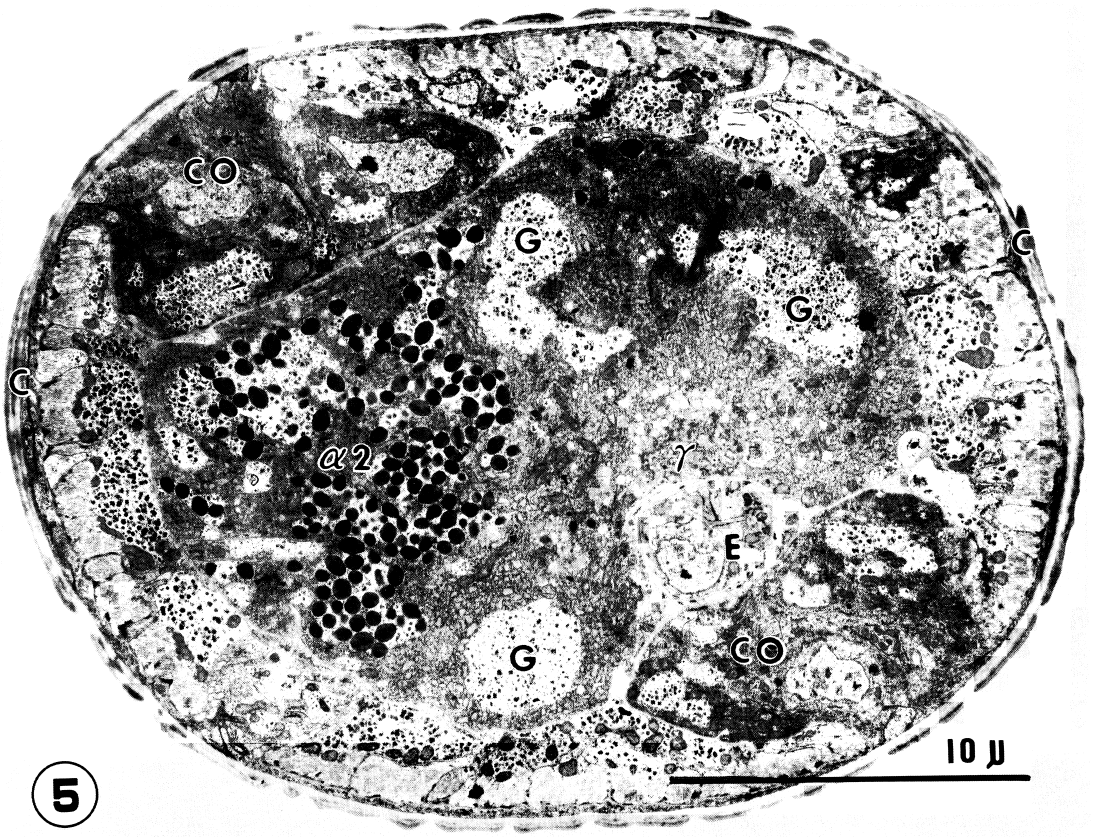


Fig. 5. A transverse section through the banded structure (Takahashi et al., 1987). A body wall is consisted of the cuticle (C), hypodermis and muscle cells. A cluster of α2-granules and γ-granules are seen. A huge accumulation of glycogen aggregates (G) often occurs in the cytoplasm. CO: cord, E: esophagus, α2: α2-granules, γ: γ-granules.

with a single membrane and an electron dense matrix (Figs. 1, 3). No morphological diversity was noted among β-granules.

Other granules

In the banded structure (Takahashi et al., 1987) there were cells with huge accumulations of glycogen aggregates and an extensive rER system (Figs. 5, 6). Synthesized product accumulated in the lumen of the reticulum where it appeared as a grey precipitate of protein (Fig. 7). The cisternae gradually distended and formed

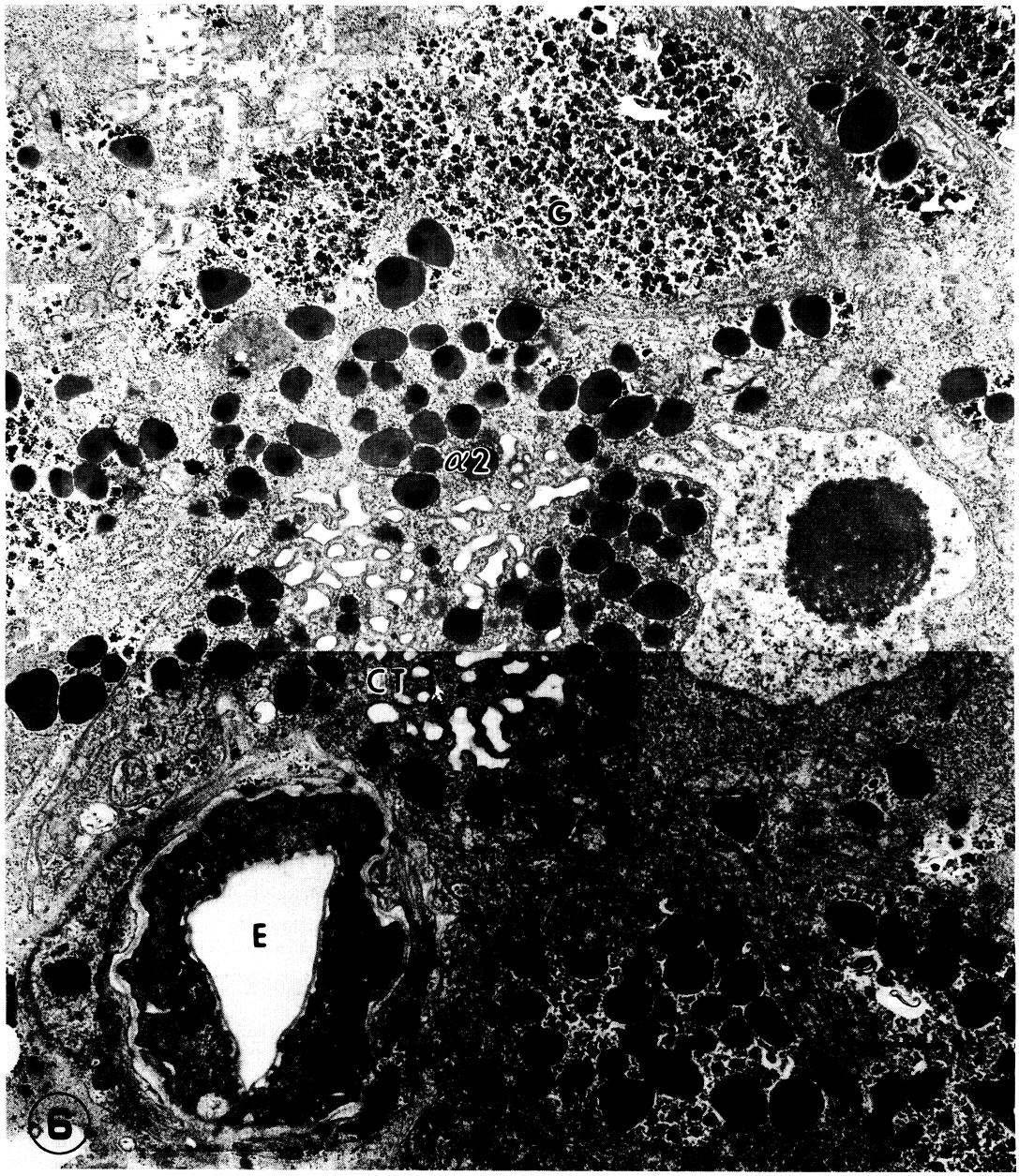


Fig. 6. A closer view of α2-granules (α2) associated with canalicular trees (CT) and the esophagus (E). Glycogen occurs (G) inbetween granules or in a huge accumulation.

secretory granules near the canalicular tree. These granules were devoid of inclusions, and smaller than α- and β-granules (300nm in diameter).

Usually stichocyte granules occurred in clusters and were closely associated with the canalicular tree (Figs. 6, 7).

Discussion

In order to obtain full knowledge of the stichocyte granules in *T. spiralis* muscle larvae, the stichosome was intensively observed at both low and high magnifications. The in situ location of each granule was identified based on visualization at low magnification.

Two kinds of α-granules were subsequently identified: one type containing sharp crystals and another type containing round crystals. Although these two types were thus morphologically distinct they have been indiscriminately referred to as α-granule (Despommier 1974). The most striking difference resided in their distribution; for granules with round inclusions were exclusively located in the upper stichosome, whereas granules with sharp inclusions were only found in the posterior region of the stichosome.

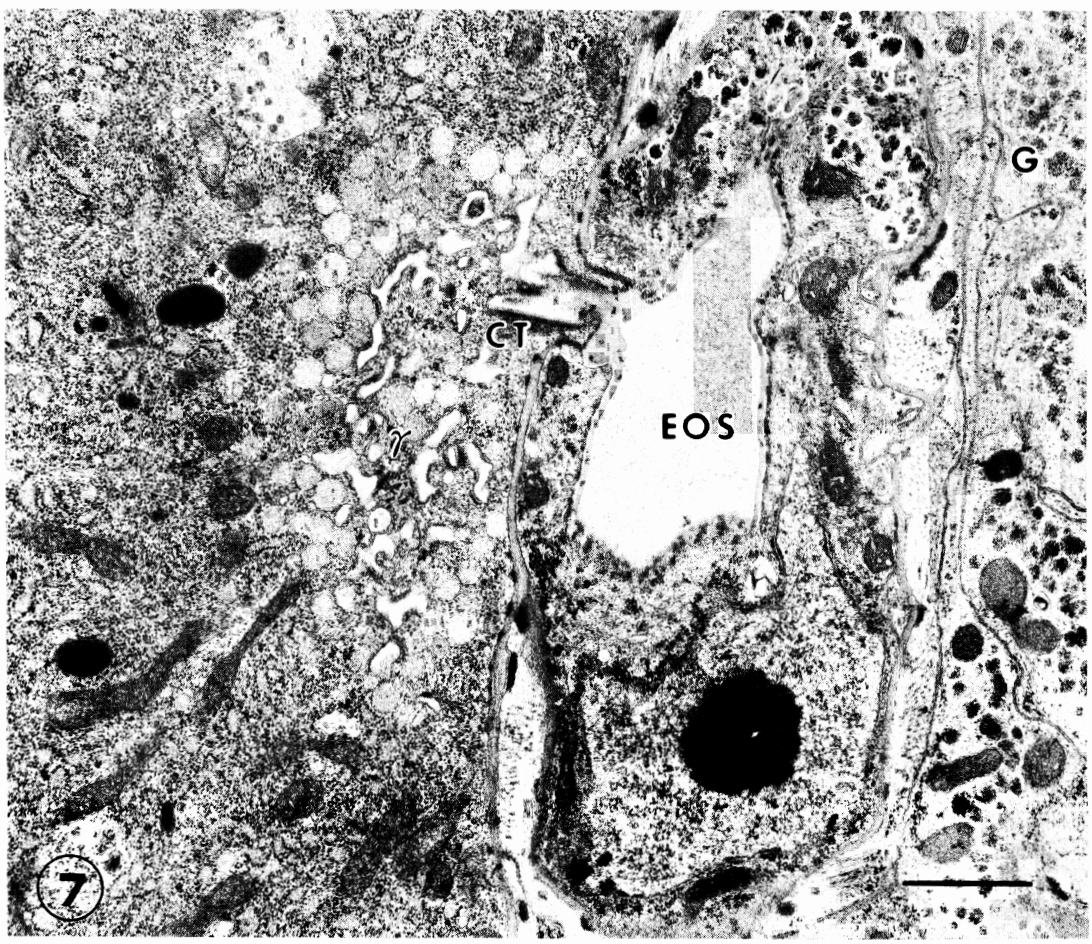


Fig. 7. A closer view of γ-granules (γ) associated with canalicular trees (CT) and the esophagus. The rest of the cytoplasm is occupied with extensive rER system. G: glycogen aggregate, EOS: esophagus occupying substance.

Furthermore, a difference was confirmed in antigenicity between the two types; granules with sharp inclusions were much more antigenic against Wistar rats during the initial infection than granules with round inclusions (Takahashi et al., in press).

Based on differences in antigenicity, the shape of inclusion and distribution within the stichosome, the subclassification of α-granules seems to be reasonable. We hereby termed granules with sharp inclusions "$\alpha 1$", and granules with round inclusions "$\alpha 2$".

In the lower most stichocyte, which is thought to be an α-stichocyte, some granules without inclusions (or just a minute spot if any) were sometimes observed. This type of granules tended to occur in clusters like other granules do, but none had sharp inclusions; while $\alpha 1$ granules in the adjacent area contained sharp crystal inclusions. Therefore, it seems to be reasonable to conclude that these granules, which have not been previously reported in literatures, are morphologically different from β-granules as to location, electron density and size. Therefore, granules located in α-stichocytes and devoid of inclusions are hereby termed as "$\alpha 0$" granule.

No morphological diversities were recognized among β-granules. Lectin staining, including Con A (*Canavalia ensiformis*), WGA (*Triticum vulgaris*), GS-II (*Griffonia simplicifolia*), DBA (*Dolichos biflorus*), UEA-I (*Ulex europaeus*) and lentil (*Lens esculenta*), also failed to show any heterogeneity among β-granules (our unpublished data). Consequently, subclassification of β-granules based on their morphology seems to be unnecessary.

The banded structure is a unique structure characterized by an alternative occurrence of bright and eosinophilic cells by hematoxylin-eosin staining (Takahashi et al., 1987). The eosinophilic cytoplasm consists of an extensive rER containing flocculent dense materials and granules. Rough endoplasmic reticulum may be transformed into granules by a progressive condensation of their contents. These granules are distinct from α- and

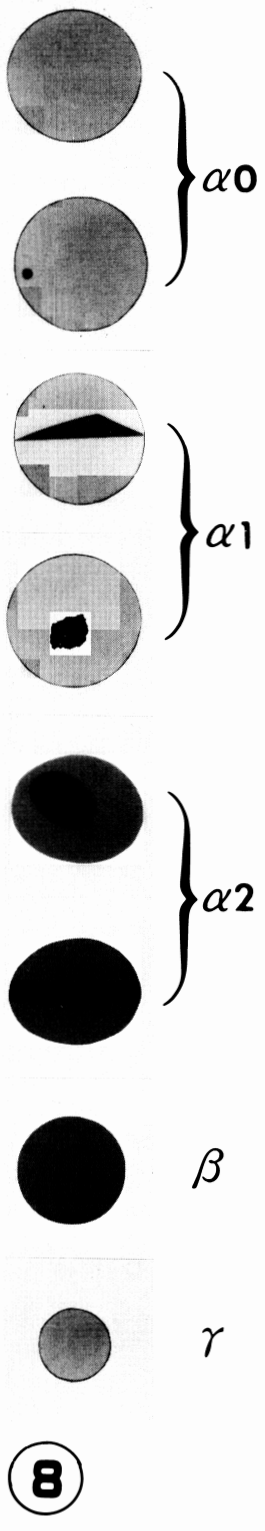


Fig. 8. A schematic illustration of stichocyte granules and tentative nomenclature.

β-granules as to their morphology, size, location and hereby are termed as γ" granules. These granules may also be secreted into the esophagus through the canalicular tree (Fig. 7).

In this contribution we describe the morphology of the stichocyte granules through extensive electron microscopic observations and propose a new classification as schematically illustrated in Fig. 8. Although this is exclusively based on morphological criteria, this new nomenclature will be contributory to a precise description of the physiological and immunological role of the stichocyte granules.

References

1) Brzosko, W., Gandarz, Z. and Nowoslawski, A. (1965): Immunofluorescence in the serological diagnosis of *Trichinella spiralis* infection. Exp. Med. Microbiol., 17, 355—365.
2) Chitwood, B. G. (1930): The structure of the esophagus in the Trichuroidea. J. Parasitol., 17, 35—42.
3) Despommier, D. D. (1974): The stichocyte of *Trichinella spiralis* during morphogenesis in the small intestine of the rat. In Trichinellosis Kim C., ed., Intext Educational Publishers, New York, 239—254.
4) Despommier, D. D. (1977): Immunity to *Trichinella spiralis*. Am. J. Trop. Med. Hyg., 26, 68—75.
5) Despommier, D. D. and Lacetti, A. (1981): *Trichinella spiralis*: Proteins and antigens isolated from a large-particle fraction derived from the muscle larva. Exp. Parasitol., 51, 279—295.
6) Despommier, D. D. and Müller, M. M. (1970): The stichosome of *Trichinella spiralis*: Its structure and function. J. Parasitol., 56 (Sect. II, Part I), Second International Congress of Parasitology, 76—77.
7) Despommier, D. D. and Müller, M. M. (1976): The stichosome and its secretion granules in the mature muscle larva of *Trichinella spiralis*. J. Parasitol. 62, 775—785.
8) Gamble, H. R. (1985): *Trichinella spiralis*: Immunization of mice using monoclonal antibody affinity-isolated antigen. Exp. Parasitol., 59, 358—404.
9) Gamble, H. R. and Graham, C. E. (1984): Monoclonal antibody-purified antigen for the immunodiagnosis of trichinosis. Am. J. Vet. Res., 45, 67—74.
10) Jackson, G. J. (1959): Fluorescent antibody studies of *Trichinella spiralis* infections. J. Infect. Dis., 105, 97—117.
11) Karnovsky, M. J. (1965): A Formaldehyde-Glutaraldehyde Fixative of High Osmolality for Use in Electron Microscopy. J. Cell. Biol., 27, 137A—138A.
12) Murrell, K. D. and Despommier, D. D. (1984): Immunization of swine against *Trichinella spiralis*. Vet. Parasitol., 15, 263—270.
13) Richels, I. (1985): Histologische Studien zu den Problemen der Zellkonstanz: Untersuchungen zur mikroskopischen Anatomie im Lebenszyklus von *Trichinella spiralis*. Zentralblatt fur Bakteriologie, Miklobiologie, und Hygiene. Series A 163, 46—84.
14) Silberstein, D. S. and Despommier D. D. (1984): Antigens from *Trichinella spiralis* that induce a protective response in the mouse. J. Immunol. 132, 898—904.
15) Silberstein, D. S. and Despommier, D. D. (1985a): Effects on *Trichinella spiralis* of host response to purified antigens. Science, 227, 948—950.
16) Silberstein, D. S. and Despommier, D. D. (1985b): Immunization with purified antigens protects mice from lethal infection with *Trichinella spiralis*. J. Parasitol., 71, 516—517.
17) 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 a longitudinal sectioning. Jpn. J. Parasitol., 36, 361—366.
18) Takahashi, Y., Uno, T., Furuki, J., Yamada, S. and Araki, T. (1988a): The morphology of *Trichinella spiralis*: ultrastructural study of the mid- and hindgut of the muscle larvae. Parasitol Res., 75, 19—27.
19) Takahashi, Y., Uno, T., Yagi, J., Yamada, S., Araki, T. (1988b): Morphology of the alimentary tract of *Trichinella spiralis* muscle larva with emphasis on the esophagus. Parasitol Res., 75, 42—49.
20) Takahashi, Y., Uno T., Mizuno, N., Yamada, S. and Araki, T.: Ultrastructural localization of antigenic substances of *Trichinella spiralis*. Parasitol. Res., 75, 316—324.
21) Villella, J. B. (1970): Life cycle and Morphology. In Trichinosis in man and animals Gould S. E., ed., C. C. Thomas, Springfield, Illinoi. 19—60.
22) Wu, L. Y. (1955): The development of the stichosome and associated structures in *Trichinella spiralis*. Can. J. Zool., 33, 440—446.