

6-19-1986

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Recommended Citation

Meng, Xianqin; Wang, Songshan; Zhou, Wenqin; Wang, Boxia; Han, Wensu; and Wang, Li (1986) "The Operculum-Plug Area and Membranous Structure of the Eggs of *Trichuris Trichiura*," *Scanning Electron Microscopy*: Vol. 1986 : No. 3 , Article 20.

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The Operculum-Plug Area and Membranous Structure of the Eggs of *Trichuris Trichiura*

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THE OPERCULUM-PLUG AREA AND MEMBRANOUS STRUCTURE OF THE EGGS OF TRICHURIS TRICHIURA

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(Received for publication March 28, 1986, and in revised form June 19, 1986)

Abstract

Eggs of *Trichuris trichiura* were prepared for scanning electron microscopy (SEM) by the dimethylsulfoxide freeze-cracking method. The egg-shell and oocyte were examined by SEM. The egg has a chitinous shell which consists of more than 10 layers of dense lamellae. The shell is bordered by a limiting membrane. An operculum and a collar made of chitinous shell together form the opercular area. The operculum is an empty cavity. The chitinous fibers of the egg-shell in this area are diffuse and loose, with numerous micropores or spaces. The egg-shell in this area therefore appears to form a fine tubular network. The oocyte is an undifferentiated cell with a biconcave drum-like shape. The perivitelline space is conspicuous at both ends of the cell.

Introduction

In a series of scanning electron microscope (SEM) studies on the fine membranous structure of the egg shell in some helminths (3-5, 8), the eggs of *Trichuris trichiura* were examined with the dimethylsulfoxide (DMSO) freeze-cracking method, to show the substructure of the operculum area and the membranous structure of the egg.

Materials and Methods

Adult worms of *T. trichiura* were collected at autopsy from the lumen of the large intestine of a seriously infected patient. The worms were washed thoroughly in normal saline and then stored in a 10% formalin solution (= 4% formaldehyde) for two years at 4°C. Subsequently, the worms were washed several times in normal saline for one hour and fixed in a 2.5% glutaraldehyde solution in 0.1 M phosphate buffer (pH 7.4) for 30 min. After washing in the same buffer solution they were fixed in 1% osmium tetroxide for one hour. After another rinse, the specimens were transferred to a 25% and 50% DMSO solution for 30 min each. The specimens were fractured in cold DMSO according to the DMSO freeze-cracking method of Tanaka (7) with the TF-1 cracker. Then the specimens were washed in buffer for one hour, fixed in 0.1% osmium tetroxide three times for 30 min, washed in distilled water three times for one hour, and finally postfixed in 1% osmium tetroxide for one hour. After a rinse in distilled water (30 min) they were dehydrated in an increasing concentration of ethanol and critical point dried. The specimens were sputter-coated with gold (about 10 nm in thickness) and examined in the SEM at 25 kV.

Results

Eggs of *T. trichiura* generally are barrel-shaped and have a smooth surface (Fig. 1). At higher magnification (x 10000), the surface appears rough, and extensive uterine secretion is scattered randomly over the outer surface of the egg. An opercular plug area is located on each end of the egg (Figs. 2 and 3). As already shown by transmission electron microscopy (TEM) by Inatomi (2), there is a chitinous shell around the oocyte, consisting of more than 10 layers of dense lamellae. SEM showed that

KEY WORDS: parasitology, *Trichuris trichiura*, dimethylsulfoxide freeze-cracking, operculum plug area, shell membrane, eggs.

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some lamellae were separated from each other (Figs. 4 and 5). No limiting membrane can be seen between the lamellae. The egg-shell narrows gradually at both ends into a collar-like structure and forms the operculum plug or opercular area (Fig. 3). The chitinous layer is limited by a dense membrane, the vitelline layer (9). This membrane ends abruptly at the two poles and does not cover the plug. The chitinous shell located in the opercular plug area suddenly widens towards the opercular cavity and forms a circle of swollen tissue. The opercular cavity therefore appears to be columnar in form at the location of the opercular plug. The chitinous fibers in this area are apparently different from those in the rest of the egg shell. They are loosely and randomly arranged and form a reticular structure with numerous micropores and spaces, resulting in a fine tubular system (Fig. 6).

The operculum is circular or oval in shape with larger, rough granules adhering to the lid (Fig. 3). There is a circular gap, about 0.1-0.2 μm wide, between the operculum and the collar margin. The top of the operculum extends beyond the collar. In the fractured surface, this lid appears as an empty cavity, and an outer limited membrane is closely connected with the chitinous shell (Figs. 6 and 7). Viewed from the inner surface of the egg cavity, the operculum is situated in the inner collar circle. It can apparently easily be separated from the egg-shell, since the connection between the collar and the egg-shell is very loose (Fig. 8). The oocyte is an undifferentiated cell located at the center of the perivitelline space, with a biconcave drum-like shape. The size of the yolk granules is variable. Some times, large granules fuse, forming a rough-textured pattern (Figs. 4 and 6).

A schematic diagram clarifying the structure of the operculum plug area is shown in Fig. 9. The sizes of the various structures are summarized in Table 1.

Discussion

Controversy still exists about the recognition of the membranous structure of whipworm eggs, as well as those of *Ascaris*. Inatomi (2) reported that the egg-shell of *T. vulpis*, as viewed in TEM, was composed of one thick membrane. Belding (1), on the other hand, stated that the *T. trichiura* egg had a two-layered shell. Wharton and Jenkins (9) observed in TEM that the egg-shell of *T. suis* was divided into three layers: the vitelline layer, chitinous shell, and lipid layer. Monne and Hoenig (6) found that the egg of *T. ovis* contains an outer lipid layer, an external quinone-tanned protein layer and an internal layer. The differences in these descriptions may be due to the different methods used. Our observations show that the *T. trichiura* egg has only one layer of chitinous shell. The shell is limited by a dense, narrow membrane, the vitelline membrane or fertilized membrane. The protein layer and lipid layer were not observed by us.

There is a difference of opinion about the nature of the egg plug. Inatomi (2) reported that the plug was probably mucoid in nature, but Wharton and Jenkins (9) stated that it was made of chitin.

Table 1

Some dimensions in *Trichuris trichiura* eggs

Egg size	43-56 x 20-25
Thickness of the chitinous shell	1-1.3
Limiting membrane of the shell	0.05-0.1
Diameter of the operculum	5-6.5
Operculum height	5.6-6.8
Outer diameter of the outer collar	5.8-7.4
Inner diameter of the outer collar	3.5-4.0
Thickness of the outer collar	1-1.2
Outer diameter of the inner collar	10
Inner diameter of the inner collar	5-6
Thickness of the inner collar	2-3
Oocyte size	25-28 x 14-16
Size of the yolk granule	0.1-0.2

All measurements in μm .

Figure Captions

Fig. 1: Cracked surface of eggs in the uterus of the worm.

Fig. 2: The outer surface of the egg-shell is smooth, with some randomly scattered uterine secretions.

Fig. 3: The operculum (O) is circular, and surrounded by a collar (C), which is separated by a circular gap (G) from the operculum.

Fig. 4: Cracked transverse surface through the middle part of the egg. The oocyte is surrounded by a limiting membrane, the oocyte membrane (arrow).

Fig. 5: The chitinous shell consists of several layers of dense lamellae: some lamellae are separated from each other (arrow).

Fig. 6: The cracked vertical surface of the opercular area shows the fine tubular system (TS), inner collar (IN) and chitinous fibers (arrow).

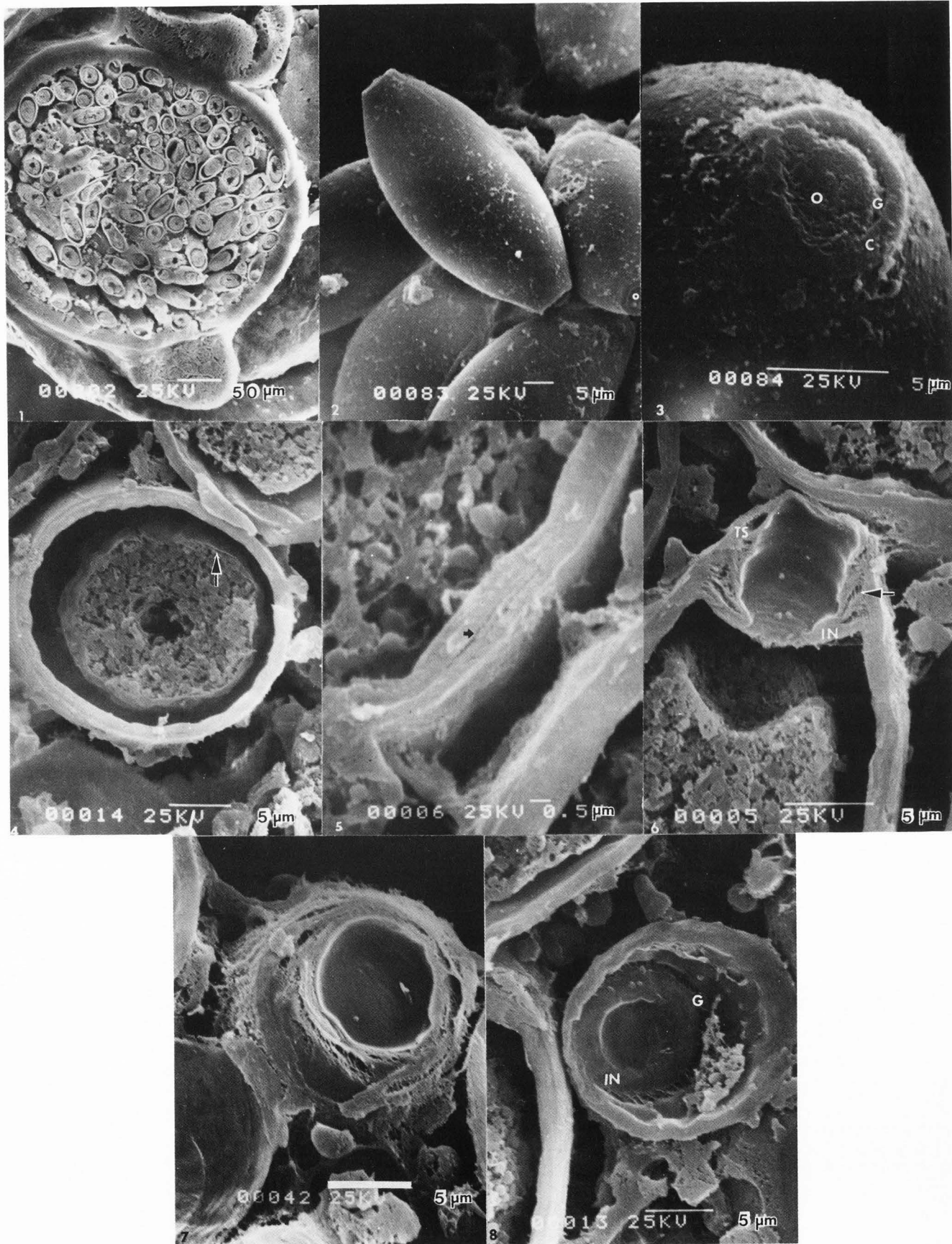
Fig. 7: The cracked transverse surface near the bottom of the operculum, bordered by a dense limiting membrane and loose network-like tissue.

Fig. 8: The opercular area viewed from the inner surface of the egg cavity, showing the inner collar (IN) and gap (G).

In our investigation it was found that the plug was an empty cavity, with a clearly recognizable limiting membrane. This cavity may originally have contained mucous materials which were dissolved during preparation for SEM.

The egg-shell is dense and thick. The basic units of the shell are the chitinous fibers. Wharton and Jenkins (9) reported that these fibers consisted of complex substances of chitinous protein. In fact, they are chitinous microfibrils surrounded by a protein coat. We have also demonstrated in this investigation that the chitinous fibers are not arranged tightly and regularly in the opercular area. There are micropores or greater spaces of variable size in this area. These are connected to form a network structure. This network which is only found in the operculum-plug area forms the fine tubular system of the egg. It is clear that this system is a weak area from which rhabditiform larvae can hatch after breaking the lid, or where enzymes produced by the larvae can dissolve or corrode the operculum, allowing hatching.

STRUCTURE OF THE EGGS OF *TRICHURIS TRICHIURA*



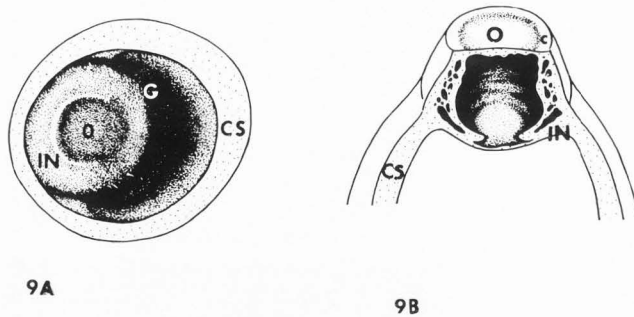


Fig. 9: Diagrams of the operculum-plug area. (A) A view from the inner surface of the egg cavity. (B) A longitudinally cracked surface of the operculum area. CS: cracked surface of the shell, O: operculum, C: collar, IN: inner collar, G: gap.

Acknowledgement

The authors would like to acknowledge the help of Prof. Takashi Makita in preparing this paper.

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Editor's Note: All questions raised by the reviewers of this paper were answered by text changes. There is therefore no 'Discussion with Reviewers'.