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## Submicroscopic structure of the egg shell of helminth II. A study on *Trichuris vulpis*

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# Submicroscopic structure of the egg shell of helminth II. A study on *Trichuris vulpis*\*

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## **Abstract**

Electron microscopic structures of the egg shell and the plug of the ova of *Trichuris vulpis* have been demonstrated. The shell is of one thick membrane of about 4 microns in thickness and consisted of several opaque and less opaque layers arranged in parallel and alternatively. The plug is of transparent substance having opaque limiting membrane on the surface and being consisted of meshwork of microfibrils.

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## SUBMICROSCOPIC STRUCTURE OF THE EGG SHELL OF HELMINTH

### II. A STUDY ON *TRICHURIS VULPIS*

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In the previous paper (4) the author reported of the structure of the egg shell of *Enterobius vermicularis* as revealed by electron microscope observation, demonstrating that the egg shell consists of two transparent chitinous layers penetrated by numerous tubules in the outer layer.

The fine structure of the egg shell of *Trichuris vulpis* is presented in this paper as revealed by observing thin sections with an electron microscope.

#### MATERIAL AND METHOD

Several adult female *Trichuris vulpis* Froehlich, 1789 obtained from dogs, which were provided by the Health Center of Okayama City, served as materials. Small pieces of these worms with eggs were fixed with 1% buffered osmium tetroxide solution at pH 7.2 for 2 hours, washed with distilled water for 2 hours, dehydrated for 12 hours through graded series of ethanol. These were infiltrated and embedded with methacrylate monomer mixture, consisting of 80% n-butyl and 20% methylmethacrylate added with 3% benzoyl peroxide. The other procedures in detail are the same with those appearing in the previous paper (4). Besides these, same eggs were observed under light microscope on wet samples prepared by the routine method.

#### OBSERVATIONS AND DISCUSSION

Under light microscope the eggs of *Trichuris vulpis* appear transparent brownish barrel-shaped shell of 60 to 70 by 37 to 40 microns in size. In the fertilized egg the shell consisted of two layers, thicker outer layer and thin inner one, can be distinguished. On both longitudinal ends the shells are discontinuous, where the translucent polar plugs are situated (Fig. 1).

Under electron microscope the shell appears as one dense layer of about 4 microns in thickness, which is probably of chitin, lacking the layer corresponding to the inner layer as observed by light microscope (Fig. 2). Within this

dark shell nineteen to twenty layers can be distinguished, arranged in parallel and alternatively with the layers of opaque and less opaque, resembling the annual rings of wood (Figs. 5 and 6). No distinct border lines can be recognized between each layer. Each layer increases in its thickness toward outside, especially marked in less opaque layer, about 0.3 micron in dark six layers of about 0.4 micron in 7th and 8th dark zones, 0.6 micron in the 9th, 0.8 micron in 10th zone and 0.3 micron in outer one. No limiting membrane can be seen both at the outer and inner surfaces of the shell though the dark layers on surface seem to increase in their opacity. The plug, approximately 7 to 10 micron thick and 7 to 9 microns in diameter, appears transparent but at the outer and inner surfaces it has opaque thin limiting membrane of about 0.004 micron in thickness and the transparent mass consisted of the moderately opaque fine microfibrils less than 0.001 micron in diameter forming irregular fine network, probably of mucoid nature (Figs. 3 and 4). There is not any clear border line at the part of conjugation of the plug and the shell. From these observations it can safely be said that the egg shell of *Trichuris vulpis* is actually of one membrane. It appears to have two layers under light microscope but the inner membrane is probably of light refraction but not the actual membrane as it can never be seen under electron microscope. Electron microscope observation reveals the egg shell consisted of several layers but it is another different structure from the light microscopic inner membrane. The shells have no tubular structure and are completely different in their structure from that of *Enterovius* eggs.

## SUMMARY

Electron microscopic structures of the egg shell and the plug of the ova of *Trichuris vulpis* have been demonstrated. The shell is of one thick membrane of about 4 microns in thickness and consisted of several opaque and less opaque layers arranged in parallel and alternatively. The plug is of transparent substance having opaque limiting membrane on the surface and being consisted of meshwork of microfibrils.

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EXPLANATION OF PLATES

A micrograph (Fig. 1.) shown is under light microscope and other five micrographs are taken under electron microscope.

- Fig. 1. Living fertilized egg. The egg shell appears transparent brownish barrel-shaped and as two layers. The translucent polar plugs are situated on both poles of the egg shell.  $\times 1200$ .
- Fig. 2. Section cut longitudinally through both poles of egg shell. The shell appears as one dense layer. The perivitelline space of the egg has not any kind of structures.  $\times 1400$ .
- Fig. 3. Section cut longitudinally through the plug of egg shell. The plugs appear transparent and have opaque thin limiting membrane on the outer and inner surfaces. There is not any clear border line at the part of conjugation of the plug and the shell.  $\times 12000$ .
- Fig. 4. Section cut longitudinally through the plug of an egg shell. The plug has an irregular fine network of opaque microfibrils.  $\times 33000$ .
- Fig. 5. Section cut longitudinally through a fertilized egg shell. The shell can be distinguished into nineteen to twenty layers and are arranged in parallel and alternatively with the layers of opaque and less opaque, resembling the annual rings of wood. There are no distinct border lines between each layer and no limiting membrane on both the outer and inner surfaces of the shell.  $\times 12000$ .
- Fig. 6. Cross section through a fertilized egg shell. This picture is the same as that in Fig. 5.  $\times 21000$ .

