Acta Medica Okayama

Volume 11, *Issue* 1 1957 *Article* 3

APRIL 1957

A Study on the Structure of Egg Shell of Enterobius Vermicularis (Linnaeus, 1758) Leach, 1853, with the Electron Microscope

Seiiti Inatomi*

*Okayama University,

A Study on the Structure of Egg Shell of Enterobius Vermicularis (Linnaeus, 1758) Leach, 1853, with the Electron Microscope*

Seiiti Inatomi

Abstract

1. The shell of the ova of Enterobius vermicularis is composed of two chitinous layers, a compact outer layer and a looser inner layer. Both surfaces of the two layers have a dense border. 2. The outer layer has innumerable tubules about 0.2 micron in diameter, and only those tubules found in the thinner part of the layer open to the outside and inside. 3. The outer layer of mature eggs is about 0.45 micron and the inner layer about 2 to 6 microns, in thickness. 4. The chitinous microfibrils in both layers form a rcticular structure which contains very fine granules in the mesh.

Acta Med. Okayama 11, 1, 18—22, (1957)

A STUDY ON THE STRUCTURE OF EGG SHELL OF *ENTEROBIUS VERMICULARIS* (LINNAEUS, 1758) LEACH, 1853, WITH THE ELECTRON MICROSCOPE

Seiiti INATOMI

Department of Parasitology, Okayama University Medical School. Okayama, JAPAN. (Director: Prof. S. Yamaguchi)

Received for Publication Feb. 21, 1957

ROGERS (1956) first studied the structure of the egg shell of *Ascaris lumbricoides var. suum* with the electron microscope and concluded that it consists of three layers. However, no investigation on the egg shell of *Enterobius vermicularis* has yet been worked out by any workers with the electron microscope.

LEUCKART (1876) assumed the existence of an operculum in the egg shell of the pinworm. ZAWADOWSKY and SCHALIMOV (1930) described the development and the structure of the egg of this worm. AKAGI (1924) reported the existence of a chitinous plug. NISHIO (1924) stated that the shell of the pinworm egg is constructed of two layers, a thin outer layer and a thick inner one, contrary to LEUCKART's claim of three layers.

The present paper deals with the structure of the egg shell of *Enterobius vermicularis* as seen through the electron microscope.

Materials and Methods

Living adult females of Enterobius were collected from the stool of a patient after treatment with tetrachlorethylene and small pieces of their body containing the eggs were fixed in a 1% buffered osmium tetroxide solution at pH. 7.2 according to the method of PALADE (1952) for 5 hours, washed for 2 hours, dehydrated for 3 hours, through a graded series of ethyl alcohol, and then infiltrated and embedded. A methacrylate monomer mixture consisting of 70% n-butyl and 30% methylmethacrylate with 3% benzoyl peroxide added was used for infiltration. The first infiltration which took 24 hours was carried out with an equivalent mixture of monomer and ethyl alcohol, for the second and third infiltrations monomer alone was used overnight. The infiltrated ova were embedded overnight in an incubater at 48°C, then sectioned to a thickness of about 0.02 micron using glass knives on a Nippon Microtome Spencer type rotary microtome. The thin sections were placed on celloidin coated copper-

mesh grids and observed with a TRS-50B AKASHI Works Electron Microscope.

Observations

The eggs of *Enterobius vermicularis* are clear and unstained, each measuring about 55 to 58 by 25 to 29 microns. They are asymmetrical with one side flattened, having somewhat the shape of a persimmon seed. The shell of the embryonated egg consists of two layers, a thin outer layer and a thick inner layer. The space between the larva and the egg shell is filled with fluid. (Fig. 1).

As observed by electron micrographs, the shell appears to be divided into two chitinous layers. (Figs. 2, 3, 4, 5 and 7).

The outer layer contains countless fine tubules, granules and fibrils, and is much more compact than the inner and about 0.45 micron in thickness. The outer surface of this layer is bordered by a smooth dense stratum about 0.041 micron in thickness and composed of fine granules and fibrils. The inner surface is bordered by a smooth membrane, about 0.08 micron in thickness, also containing fine granules and fibrils. The density to the electron beam of this outer layer is very strong. The tubules in the outer layer are radiating from inside to outside, with their diameter about 0.05 to 0.1 micron. The external openings of these tubules are slightly smaller than the rest of the tubules on the outer surface of the shell. The chitinous microfibrils less than a diameter of 0.006 micron form an irregular reticulum, with a large number of granules filling the interstices of the reticulum. The two poles as shown in Figure 7 of longitudinal section and the symmetrical lateral margins as shown in Figures 2 and 5 of cross section of the outer layer have a thickness of approximately 0.4 to 1 micron. The openings of the tubules in these parts are closed. The outer layer is thinnest on the convex side very close to the narrower pole. The outer layer in the immature egg is very thin, being about 0.16 micron. (Fig. 6).

The thickness of the inner layer is about 2 to 6 microns. (Figs. 3, 4 and 7). Both inner and outer surfaces of this layer are evidently bordered by a dense stratum about 0.08 micron in thickness and containing granules and fibrils. The density to the electron beam of this layer is poorer than that of the outer layer. Chitinous microfibrils contained in the inner layer are less than 0.004 micron in diameter and appear to form an irregular, fine network. The interspace among the fibrils of the reticulum is filled with a large number of fine granules, but the inner layer is, as a whole, of looser texture than the outer layer. The inner layer adheres

firmly to the outer layer but may be exfoliated by a little external force.

Discussion

LEUCKART (1876) reported that the egg shell of *Enterobius vermicularis* has an operculum, and AKAGI (1924) stated that this shell has a chitinous plug, but my observation with the electron microscope contradicts their views, though confirms the statement by NISHIO (1924) that the egg shell of *Enterobius vermicularis* is consisted of two layers. Further, the discovery of the countless tubules in the outer layer of the egg shell is to be pointed out. These tubules are probably concerned with the respiration of the embryo and with the control of humidity within the egg shell. The thinnest part of the outer layer corresponds exactly to the same spot that ruptures in hatching.

The outer layer of the shell is very thin in its early stage, but becomes gradually thicker with the development of the egg.

Summary

1. The shell of the ova of *Enterobius vermicularis* is composed of two chitinous layers, a compact outer layer and a looser inner layer. Both surfaces of the two layers have a dense border.

2. The outer layer has innumerable tubules about 0.2 micron in diameter, and only those tubules found in the thinner part of the layer open to the outside and inside.

3. The outer layer of mature eggs is about 0.45 micron and the inner layer about 2 to 6 microns, in thickness.

4. The chitinous microfibrils in both layers form a reticular structure which contains very fine granules in the mesh.

The writer wishes to express his deep appreciation to Prof. S. Yamaguti for his advice during the course of the study.

References

AKAGI, K.: (1924) Biological study of *Enterobius vermicularis*. Japan. J. of Med. Progr. 14; 905—950, 1049—1117. — LEUCKART, R.: (1876) Die menschlichen Parasiten und die von ihnen herruhrenden Krankheiten. Bd. 2. S. 285—351. — NISHIO, N.: (1924, 1925) A study on the Oxyuroidea. J. of Chiba Med. Soc. 2; 109—163. 3; 199—218. — PALADE, G. E.: (1952) A study of fixation for electron microscopy. J. of Experim. Med. 95; 285—297. — ROGERS, R. A.: (1956) A study of eggs of *Ascaris lumbricoides var. suum* with the electron microscope. J. of Parasit. 42; 97—103. — ZAWADOWSKY, M. M. and SCHALIMOV, L. G.: (1903) Die Eier von *Oxyuris vermicularis* und ihre Entwicklungsbedingungen, sowie über die Bedingungen, unter denen eine Autoinfektion bei Oxyuriasis unmöglich ist. Zeitschr. für Parasitenk. 2; 12—43.

Explanation of Figures

Fig. 1. Living embryonated egg. ×500.
Fig. 2. Tubules in outer layer of egg shell cut transversely. ×7000.
Fig. 3. Egg shell in embryonic stage. ×24000.
Fig. 4. Egg shell in embryonic stage. ×20000.
Fig. 5. Cross section of egg shell in embryonic stage. ×3000.
Fig. 6. Longitudinal section of egg shell in morula stage. ×3500.
Fig. 7. Longitudinal section of egg shell in embryonic stage. ×3700.

Fig. 1

Fig. 2

Fig. 3

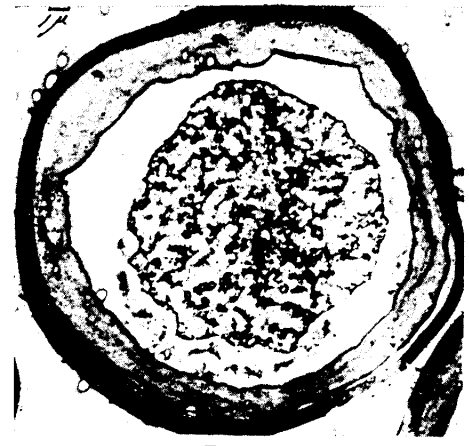

Fig. 5

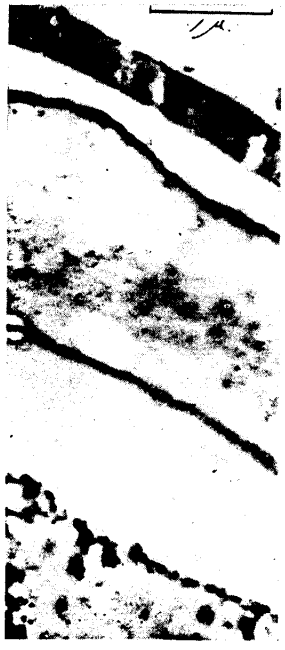

Fig. 4

Fig. 6

Fig. 7