A STUDY OF EGGS OF ASCARIS LUMBRICOIDES VAR. SUUM WITH THE ELECTRON MICROSCOPE

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The egg of Ascaris has been widely used as a subject for cytological investigations. Nelson (1851) described the development of the egg of Ascaris mystax. Leuckart (1886) and Wharton (1915) reported the Ascaris lumbricoides egg to be surrounded by a shell and an outer, albuminous layer. The appearance of unfertilized eggs of this species was described by Otto (1932) and Keller (1933).

The number of layers surrounding the egg of ascarids has been reported as three (Nelson, 1851, Ascaris mystax; Leuckart, 1886, Ascaris lumbricoides; Ackert, 1931, Ascaridia lineata; Wottge, 1937, Parascaris megalcephala; Christenson, 1940, Ascaris lumbricoides), four (Kreuzer, 1953, Ascaris lumbricoides; Frenzen, 1954, Ascaridia galli, and five (Zavadovsky, 1928, Ascaris). A variety of techniques has been used by different authors in studying the egg layers, including microscopic observations of the penetration of substances into the egg, polarizing and fluorescent microscopy, centrifugation, and chemical tests on the membranes. The chemical composition of the layers has been investigated by several workers (Fauré-Fremiet 1913, 1913a; Chitwood 1938; Christenson, et al. 1942; Jacobs 1940; Timm 1950; Monné and Hönig 1954; Fairbairn 1955) and, in general, have led rather consistently to the conclusion that there are three membranes. Wottge (1937) described the endogenous formation of the egg layers of Ascaris megalocephala, whereas Christenson (1940) believes the outer layer of the Ascaris egg to be of exogenous formation.

Morita (1953) examined paraffin sections of the egg shell of Ascaris megalocephala with the electron microscope and concluded that the layer “consists of completely individualized microfibrils.” In the present investigation, thin sections were examined with the electron microscope to determine structural details of ova and the order of formation of the egg coverings of Ascaris lumbricoides var. suum.

MATERIALS AND METHODS

Living adult Ascaris were removed from the intestines of freshly killed pigs and thin slices from various parts of the egg-containing uterus were placed in fixative. The eggs were fixed in either 1% osmium tetroxide according to the method of Palade (1952) for 2 hours, or Carnoy’s fixative (acetic acid 20%, absolute

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alcohol 80%) for 18–24 hours. Following fixation, the ova were washed, dehydrated, and infiltrated with a mixture of 72% n-butyl- and 28% methyl-methacrylate. The eggs were sectioned at 0.025 microns with an International Minot rotary microtome, the thin sections then placed on celloidin-coated grids and examined with the aid of a model EMU–2B RCA electron microscope.

**OBSERVATIONS**

*Organization of the Ascaris egg.*

The egg cytoplasm of *Ascaris lumbricoides* var. *suum* is surrounded by three layers: an outer protein coat, a middle chitinous shell, and an inner lipoid lining. These layers, as observed in the living state by means of light and phase microscopes, are shown in Figure 1. The protein coat and the chitinous shell when observed in eggs removed from the uterus of the adult worm, are usually colorless and transparent. Striations are observed in the lipoid layer. Between the cytoplasm and the lipoid layer in the mature, fertilized egg is a fluid-filled cavity, the perivitelline space. Some of the egg layers and the perivitelline space are absent in unfertilized eggs (Fig. 2).

*Structure of the egg by electron microscopy.*

**Protein Coat**

The external margin of the protein layer is usually mamillated (Fig. 3). The mamillations vary greatly, appearing obtuse, undulate, or very irregular. The surface is granular, and when interrupted, the ends of fibers arising from the protein coat can be observed. In fully-formed eggs, a distinct separation between the protein and the shell layer is observed, and the protein coat terminates abruptly at the outer margin of the shell.

The protein coat appears as a very dense, reticulated material (Figs. 4 and 5) containing granules without apparent structure. The fibrils of the reticulum have a diameter of approximately 150 Angstrom units (Å) and branch irregularly from the granules. The coat appears uniform in density except where it is very broad, in which case the branches are more widely separated at the outer margin.

**Chitinous Shell**

The chitinous shell attains a thickness of approximately three to four microns in the mature egg. The inner surface of the shell is limited by a membrane (Fig. 4) containing small granules and fibrils. The outer surface of the shell is bordered by a dense zone or membrane (Figs. 4 and 5), approximately 0.4 micron in thickness, containing granules and fibrils. At the margin of this membrane, the junction between the protein layer and the shell is marked by a narrow line (Fig. 5) approximately 200 Å in thickness. The membrane is joined at intervals by the chitinous fibers of the shell. These chitinous microfibers (Fig. 5) vary in diameter from approximately 75 Å to 400 Å. The majority of fibers branch when reaching a length of 250 Å or less to form a loose, irregular reticulum. A lamellation of the chitinous fibers was not observed.

Electron micrographs which are believed to represent successive stages in the formation of the chitinous shell are shown in Figures 6 through 11. Figure 6 shows small granular projections attached to the plasma membrane along its entire
margin. These surface projections may extend outward from the plasma membrane, or they may be elongated, attached as an inverted-U (Fig. 7), or scattered irregularly along the membrane. The projections may have been formed from the dark granules within the cytoplasm of the egg. A material which forms around the surface projections (Fig. 8), is possibly a precursor of the chitin fibers and may have been carried to the surface by cytoplasmic vacuoles (Wottge, 1937), or secreted by the projections. The chitin structure as well as the disappearance of the surface projections, is observed in Figure 9. Later stages in the process of shell formation are shown in Figures 10 and 11, where the chitinous shell has become much thicker. The plasma membrane is visible on the inner surface and the protein material is beginning to be attached on the outer surface of the shell. The embedding medium has not been removed from the latter two figures; hence the fibrous arrangement of the chitinous shell is not as immediately apparent as in Figure 9. A section through sperm is shown external to the egg in the upper part of Figure 7. All photographs in Plate III are taken at the same magnification, and the appearance of size differences is due to the part of the egg through which the section was cut.

**Lipoid Layer**

The lipoid layer is soluble in all common fat solvents, and therefore dissolved in the process of preparing the eggs for sectioning. The layer is not fixed by one percent osmium tetroxide or Carnoy’s fixative, and the method of freeze-drying was found not to be satisfactory for its study. It was, therefore, not possible with any of these methods to obtain electron micrographs of the lipoid layer.

**Perivitelline Space**

The perivitelline space, which is filled with fluid as a result of “deutoplasmolysis” (Lams, 1952), appears optically empty; no structures of any kind were observed with the methods of fixation used.

**Cytoplasm**

Large numbers of granules and vacuoles are located within a very coarse cytoplasmic reticulum (Figs. 12 and 13). The vacuoles, varying in size from 0.3 to 1.7 microns in diameter, are optically empty; a fine, fringed border is sometimes seen surrounding the inside of the wall. The vacuoles may be concentrated in the central portion, or scattered at random in the cytoplasm.

The granules of the cytoplasm, called “brown granules” by Boveri (1910), vary in diameter from 0.1 to 0.8 microns. No structural details were observed and whether or not some of the granules are mitochondria is uncertain.

In the immature egg, large, homogeneous, ovoid structures called “hyaline spheres” by van Beneden (1883) were observed (Fig. 13). They vary in diameter from 1 to 4 microns and are enclosed by a granular membrane. At the outer margin of some immature eggs, material which appears similar to that contained within the spheres was observed. The hyaline spheres are dissolved in acetic solutions (Fauré-Fremiet et al., 1953, 1954) and were observed only with osmium tetroxide fixation.

The nucleus, or pronucleus (Fig. 14), approximately four microns in diameter, is surrounded by a granular membrane. It contains dark bodies, presumably chromatin material, as well as a loose protoplasmic reticulum.
DISCUSSION

The nature of the egg envelope and therefore a knowledge of its ultrastructure are of interest. The controversy which exists concerning the number of membranes in nematode eggs was summarized by Jacobs (1940) who indicated, "the difference in the number of membranes described by various authors can . . . be attributed to the variety of techniques used in studying the eggs".

The electron micrographs presented herein support the belief that there are three layers surrounding the egg cytoplasm of *Ascaris lumbricoides* var. *swum*, viz. the outer protein coat, the middle chitinous shell, and the inner lipoid layer. The report of Frenzen (1954) that the egg envelope of *Ascaris lumbricoides* includes four layers is not supported by the present work. The portion of the egg indicated as a separate layer by him is probably the zone of separation between the protein coat and the chitinous shell. This zone, although more dense, is not believed to represent a distinct egg layer, since close observation at high magnification reveals a typical chitin structure. Furthermore, the report of Zavadovsky (1928) that the egg cytoplasm of *Ascaris* is enclosed by five layers is not supported by the present investigation, since a lamellation of the chitinous shell was never observed.

Jaskoski (1952) suggested that the outer protein coat represented an important auxiliary barrier against the passage of materials in or out of the ascarid egg. Germans (1954) concluded that the "slime layer" confers protection against desiccation. Wottge (1937) reported that the protein coat contained a lipoid substance, and this material might be located in the interstitial vacuoles of the protein reticulum. The network arrangement of the protein coat with interposed lipoid material, as well as the limiting membranes separating the egg layers, may lend support to these conclusions.

The endogenous formation of the protein coat of the egg of *Ascaris megalocéphala*, advanced by Wottge (1937) was based on the presence of a protein covering around unfertilized eggs. Chitwood (1938), however found eggs without this layer and suggested that the coat was a uterine secretion added after the two inner layers had formed. In support of this latter point of view, Lowry et al., (1941), observed granules within the cytoplasm of the uterine cells of *Ascaris equorum*, and suggested that the granules represented an albuminous substance which is secreted into the uterus, and surrounds the eggs as they pass along it. The electron micrographs of the pig *Ascaris* egg fail to show the protein coat surrounding either the oocyte as described by Wottge (1937), or the outer margin of those eggs in which the chitinous shell was in the process of formation. In view of these observations, the order of formation of the egg envelope is believed to occur as follows: the chitinous shell is formed first, followed by the appearance of the lipoid lining, both of these layers of endogenous formation; the protein layer of the egg is added last as a product of uterine secretion.

Kreuzer (1954) suggested that the chitinous shell provides mechanical protection to the egg and preserves stability of form. Wottge (1937) referred to this layer as the “homogeneous membrane” because of the clearness and transparency of the layer, and Schmidt (1936) indicated that the shell substance was homogeneous and composed of non-fibrillar material. Observations of the chitinous shell with the electron microscope indicate that the shell is distinctly fibrillar. The suggestion of Monné and Höning (1954) that the shell of *Ascaris* consists of chitin...
fibers and protein lamellae which alternate with each other seems not to be warranted on the basis of these observations. The photograph published by Morita (1953) as a cross-section through the shell, actually appears to be a photograph of the outer protein layer. The size of the chitin fibers, as reported by this author, probably represents the measurements of the protein strands of the outer coat, and consequently the lower limits are much too large. In addition, it is believed that the cross-section of the shell, in any case, would not have the appearance pictured.

Monné and Höning (1954) indicated that the lipoid coat is the portion of the egg envelope which protects the egg against penetration by harmful chemicals, and Kreuzer (1954) suggested that this layer produces a completely effective diffusion barrier to ions. The ultrastructure of this region will be of interest when fixation techniques for the study of this material are devised.

Fauré-Fremiet, et al. (1953, 1954) reported that the hyaline spheres were distributed at random in the cytoplasm of the oocyte, and passed to the periphery after fertilization, where they coalesced and disappeared into the perivitelline space. The role and significance of the spheres remain undetermined.

**SUMMARY**

1. Studies of ova of *Ascaris lumbricoides* var. *suum* with the electron microscope support the belief that the egg envelope is composed of three layers which in order of formation include a middle chitinous shell, an inner lipoid layer, and an outer protein layer. The protein coat and the chitinous shell are bordered by limiting membranes.

2. The protein coat of the egg, whose margin may be smooth or mamillated, appears as a dense, reticulated material, with fibrils having a diameter of about 150 A.

3. The chitinous shell is composed of highly branched microfibers which vary in diameter from approximately 75 A to 400 A. A lamellation or orientation of the chitin fibers in any one plane of the egg was not observed. Electron micrographs, which may represent successive stages in the formation of the chitinous shell, are presented.

4. The lipoid layer, in the living state, has a striated appearance. A suitable method for the fixation of the layer, however, was not found.

5. Electron micrographs fail to reveal structures of any kind in the perivitelline space of the egg.

6. Large numbers of granules and vacuoles are located within a very coarse cytoplasmic reticulum inside the egg itself. The hyaline spheres, observed in immature eggs, are described.

7. The highly branched arrangement of the protein coat and the chitinous shell, as well as the limiting membranes separating the egg layers, may help explain the extreme resistance of *Ascaris* ova to many environmental changes.

**References Cited**


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EXPLANATION OF PLATES

PLATE I

Fig. 1. Living fertilized egg. P protein coat, S chitinous shell, L lipoid layer. ×1500
Fig. 2. Living unfertilized egg. P protein coat. ×700
Fig. 3. Fertilized egg. P protein coat, S chitinous shell, C cytoplasm. Carnoy’s fixative. Embedding medium not removed. ×12,000

PLATE II

Fig. 4. Mature egg. P protein coat, S chitinous shell. Carnoy’s fixative. ×25,600
Fig. 5. The fibrous structure of the protein coat P and the chitinous shell S are shown. Osmium tetroxide fixation. ×64,000

PLATE III

Figs. 6-11. Successive stages in the formation of the chitinous shell. Figures 6-7. Osmium tetroxide fixation. Figures 8-11 Carnoy’s fixative. Embedding medium not removed in figures 10-11. All figures ×12,000.

PLATE IV

Fig. 12. Mature egg. P protein coat, S shell, N nucleus. Carnoy’s fixative. ×7200

PLATE V

Fig. 13. Cytoplasm of an immature egg in which the egg envelope has not yet formed. H hyaline sphere, V vacuole, G brown granule. Osmium tetroxide fixation. ×12,000
Fig. 14. Nucleus of egg enclosed by granular membrane. N nucleus. Carnoy’s fixative. ×18,400