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ULTRASTRUCTURE OF EGGS OF ASCARIS LUMBRICOIDES LINNAEUS, 1758 I. EGG-SHELLS

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Abstract. Under the light microscope the chitin-protein layer of egg-shells in ascarids appears to be a regular, hyalinne and nonstructural layer of 1.5 to 2.00 μm in thickeness. The outer uterine layer is usually removed during the preparation. The lipid (ascaroside) layer covers the inner surface of the chitinous layer and seems to be irregularly undulated and regularly thick over the whole surface, with the thickeness up to 1 μm. In electron micrographs the fibrous structure of the lipid layer is not evident as a rule. This is probably due to washing the lipids away from this layer during the dehydration of deeper layers of egg-shells that are imperfectly fixed with glutaraldehyde. A very low permeability of the egg-shells is typical of geohelminth eggs. The layer lipid shows a distinct lamellate structure only after a prolonged fixation with osmium at higher temperature. This is supported by the studies using the method of freeze-fracturing.

The egg-shell of soil-transmitted worms (sometimes called geohelminths) is a structure providing by its complexity and function relatively stable physiological conditions required for the development of the enclosed embryo and then its survival for several years in the environment to which it is exposed. Therefore the egg-shells possess a high structural strenght and also resistance to the changing environmental factors. The resistance of the egg-shells to the action of chemical noxae had been thoroughly investigated in particular (Vasilkova 1949, Vasilkova and Nabokov 1955, Veber 1954, Beaver 1961). Therefore the study on the ultrastructure and chemical properties of the egg-shells and their layers has attracted much interest.

The structure of ascarid species has been studied with the transmission electron microscope by Tay et al. (1975), Foor (1967), Wharton (1980), with the scanning electron microscope by Ubelaker et al. (1975). According to Foor (1967) the egg-shells consist of the following layers:

1. muccopolysaccharide uterine layer forming on the egg surface typical irregularly thick structures of about 1.5 μm,
2. thin vitelline layer originating from the oolemma of the fertilized oocyte,
3. highly structural strong chitin-protein layer thick 2 μm, and finally,
4. inner lipid (ascaroside) layer reaching the thickeness of 0.8 to 1 μm being the extreme resistant and impermeable osmotic barrier protecting the developing embryo against chemical action.

During the study of the penetration process of ovicidal fungi through the egg-shells, the electron micrographs (in comparison with the results of other authors) showed rather different ultrastructure of the lipid layer of the egg-shells. In the present paper the results obtained by us are compared with those of other authors.

MATERIAL AND METHODS

The eggs of *Ascaris lumbricoides* were removed from live adult female worms obtained from slaughterhouse pigs 'guts' .The eggs were treated in the following three ways: 1. fixed with 3 % glutaraldehyde in 0.1 N phosphate buffer, the pH 7.2,f or 4 hours, then with 2 % osmium tetroxide in phosphate buffer, the pH 7.2, dehydrated with acetone and embedded in Durcupan ACM

(Fluka). 2. Fixed with 2 % osmium tetroxide in phosphate buffer for 24 hours at 40 °C. Dehydrated and embedded in Durcupan. From the specimens obtained by the above methods ultrathin sections were cut and observed with the electron microscope Tesla BS 613. 3. For a freeze-fracturing method the eggs were fixed with 3 % glutaraldehyde in 0.1 N phosphate buffer, the pH 7.2, for 4 hours. In Balzers' apparatus BA 360 M the carbon-platinous replicas were prepared by means of a freeze-fracturing technique and observed with the electron microscope Tesla BS 500 and Tesla BS 613.

RESULTS AND DISCUSSION

In the light microscope (Plate I, 1) the chitinous layer appears to be quite transparent and homogeneous. The thickeness of this layer remains unvariable over the whole surface of the egg. The lipid layer is an irregularly fibrous structure covering uniformly the whole inner surface of the egg-shells. It is closely associated with the chitinous layer adhering to it. In a completely mature egg capable for development, the monocellular embryo, which is round-shaped, touches the inner surface of the lipid layer in some places only. In other places the embryo and the lipid layer are separated by a diverse, visually empty space filled with a serous fluid which surrounds the developing embryo.

In the electron microscope studies of Foor (1967), Wharton (1980), and Tay et al. (1975) the chitin-protein layer is reported as completely homogeneous, non-structural. The thickeness of this layer supports the results obtained from the observations with the light microscope. The interpretation of the ultrastructure of the lipid (ascaroside) layer varies. On the electron micrographs from the studies quoted above this layer is missing so that the cytoplasmic membrane of the embryo adheres directly to the chitinous layer. A structure corresponding to the ascaroside layer has not been found (Foor 1967). Tay et al. (1975) refer to the ascaroside layer indicating it as the fourth layer (quarta capa), a thin, slightly electron-dense, hyalinne layer close under the chitinous layer. Foor (1967) and Wharton (1980) suggest the ascaroside layer to be a hyalinne, non-structural and electron-lucent layer between the chitinous layer and the cytoplasmic membrane of the embryo which considerably varies in the relative thickeness within the egg. However, this finding is contrary to the observations with the light microscope where the ascaroside layer shows a regular thickeness of about 0.001 mm. Close under the chitinous layer, however, irregular electrondense structures may be visible termed by Foor (1967) as dense material. However, where is the regular structure of the ascaroside layer which is responsible for the extreme osmoprotective function of the egg-shells?

Our material fixed with 3 % glutaraldehyde also gave similar electron micrographs (Plate I, 2). Conformably to Foor (1967), close under the chitinous layer we observed dense formations which, however, never formed a continuous layer. Therefore we could not consider them to be lipid layer of the egg-shells. The plasma mebrane of the embryo adhered to the whole surface against the chitinous layer of the egg-shells.

During the study of the process of penetration of ovicidal fungi through the egg-shells of worms, the attacked eggs penetrated by the fungus in one area at least inside the egg posseed a continuous layer of lamellar formations corresponding by their location, thickeness and structure to the lipid layer of the egg-shells. These lamellar formation were virtually identical with similar structures in the eggs of *Porrocaecum ensicaudatum* (Wharton 1980). The electron-density of the structures observed by us corresponded to the lipid structures intensified by the post-fixation with osmium (Plate I, 3). The ovicidal action of the fungus manifested in damaged egg-shells and especially in the impairment of osmotic barrier which enabled the penetration of glutaraldehyde and osmium through the egg-shells. Almost deeper layers of the egg-shells and the embryo were then fixed uniformly. The fixation of intact eggs with glutaraldehyde was not complete so that the incompletely fixed lipid structures were washed away during the dehydration of specimens with acetone. They left only remnants in the form of a dense material of irregular shape close under the chitinous layer of the egg-shells.

The lipid layer of the egg-shells was demonstrated also during a prolonged fixation with osmium at the temperature of 40 °C. Under those thermal conditions, the fixation with osmium penetrated completely all layers of the egg-shells, fixed perfectly even lipids of the ascaroside layer and caused a marked electron-density of lipids (Plate I, 4).

The study of the egg-shells by means of the method of freeze-fracturing demonstrated a lamellate structure in the lipid layer of the egg-shell. Its character and thickeness support the results obtained from the study under the light microscope. The lamellae of the lipid layer form a continuous, uniformly thick part of the egg-shell adhering to the inner surface of the chitin-protein layer (Plate II, 1, 2). It can be seen in the section that between the lipid layer and plasma membrane there is a visually empty space filled with a serous fluid in the live egg. This space is variously capacious in various areas and in some areas the plasma membrane of the embryo may adhere directly to the lipid layer. Lamellate structures of the lipid layer are remarkable when sectioned tangentially (Plate II, 2). Its stratification indicates that this layer of the egg-shell does not originate all at once, but is caused by aposition of several layers.

The results obtained by us suggest that the formations observed in ultrathin sections of electron microscopy and identified by some authors as a dense material are in fact the remnants of lipids that are not washed away from the lipid layer of the egg-shell. The hyalinne space under the chitinous layer in which the first pole corpuscle is found after egg-fertilization (Foor 1967) is not a component part of the egg-shells. This is a space between the egg-shells and the embryo proper, its cytoplasmic membrane.

УЛЬТРАСТРУКТУРА ЯИЦ *ASCARIS LUMBRICOIDES* LINNAEUS, 1758
I. ОБОЛОЧКА ЯИЦ

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Резюме. В световом микроскопе хитинопротеиновый слой в оболочке яиц аскарид регулярный, гиалинный и бесструктурный. Его толщина — 1,5—2 мкм. Внешний слой матки обыкновенно удаляется в течение приготовления. Липидный (аскарозидный) слой покрывает внутреннюю поверхность хитинового слоя и, вероятно, он нерегулярно волнистый. Его толщина (до 1 мкм) одинакова. В электронном микроскопе волокнистая структура липидного слоя обыкновенно не видима. Это вероятно причинено тем, что липиды вымываются из этого слоя в течение дегидратации более глубоких слоев оболочки яиц, несовершенно фиксированных глутаральдегидом. Очень низкая проницаемость оболочки яиц типична для яиц геогельминтов. Липидный слой имеет пластинчатую структуру только после продолжительной фиксации осмием при повышенной температуре. Это подтверждается изучением с помощью метода замораживания-переламания.

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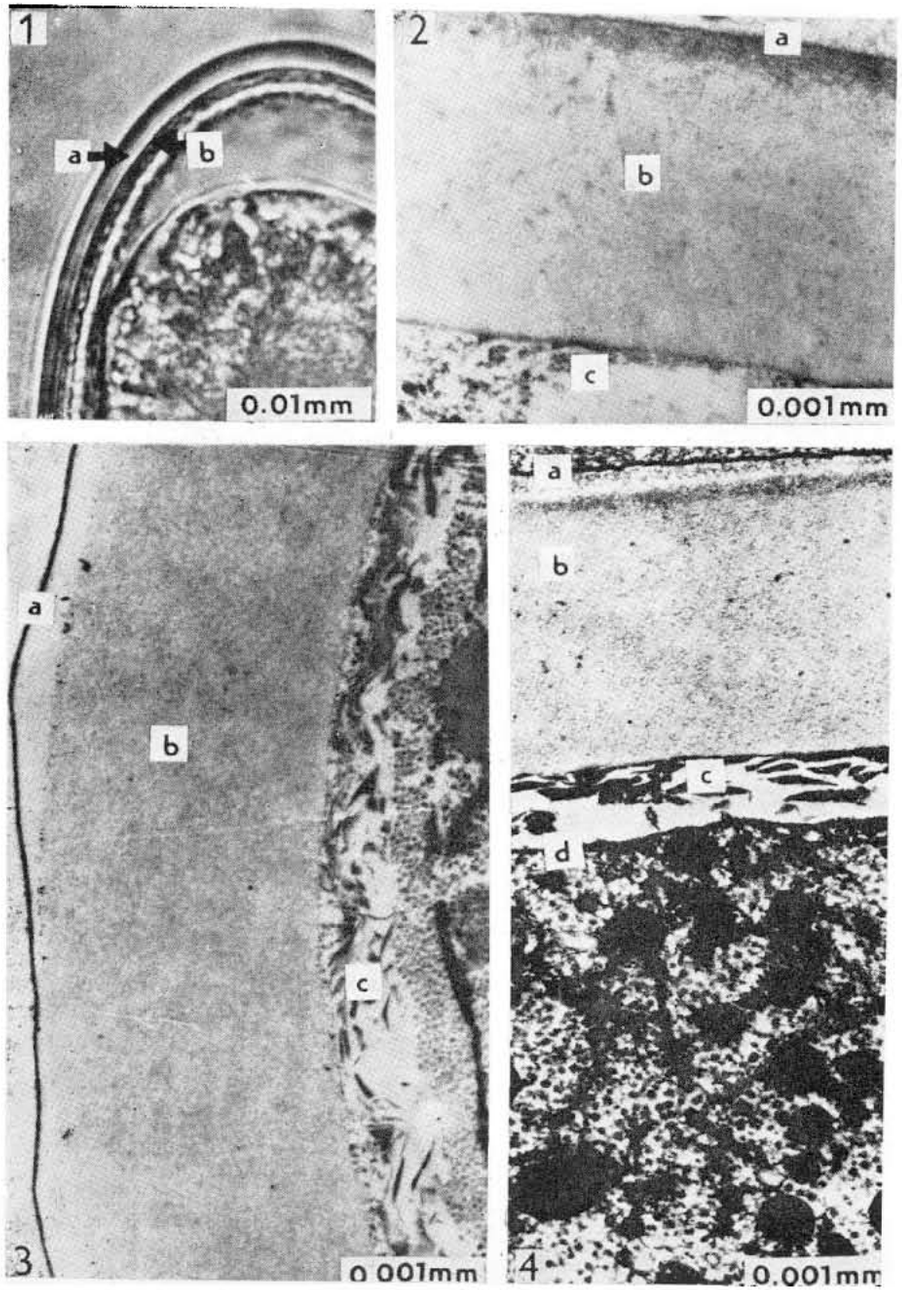
Fig. 1. The egg-shells of *Ascaris lumbricoides* under the light microscope. a) Hyalinne chitin-protein layer, b) lipid layer showing slightly undulated structure, not varying in thickeness over the whole surface of the egg-shells. The external uterine layer is missing. **Fig. 2.** The egg-shells after the fixation with glutaraldehyde. a) Thin vitelline layer, b) chitinous layer, c) dense material. **Fig. 3.** The egg-shells of the egg attacked by the ovicidal fungus. Glutaraldehyde fixation penetrated easily through an impaired osmotic barrier to deeper layers of the egg. a) Vitelline layer, b) chitinous layer, c) lamellae of the lipoprotein layer. **Fig. 4.** The egg-shells after a prolonged fixation with osmium at 40 °C. a) Vitelline layer, b) chitinous layer, c) lamellae of the lipid layer, d) cytoplasmic membrane of the embryo.

Fig. 1. Cross section by a freeze-fracturing method. a) External uterine layer, b) vitelline layer, c) irregularly fibrous structure of the chitinous layer, d) lamellate structure of the lipid layer, e) space between the internal surface of the egg-shell and the embryo, f) cytoplasmic membrane of a nonsegmentated embryo. **Fig. 2.** Tangential section by a freeze-fracturing method. a) Part of the chitinous layer, b) lipid layer, serval lamellae and their membrane nature are evident c) space between the internal surface of the egg-shell and the embryo, d) cytoplasmic membrane of the embryo.



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