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THE FORMATION OF ASCAROSID LAYER OF THE EGG SHELL IN ASCARIS LUMBRICOIDES

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Abstract. The ultrastructure and formation of the ascarosid layer of the egg shell in *Ascaris lumbricoides* were studied. The formation of the third egg shell starts immediately after fertilization and after the formation of the chitin-protein complex. Individual components of the layer, i.e. refringent granules surrounded by fat vacuoles, fuse with one another in form of wedges which probably makes the layered structure of the ascarosid layer.

The ultrastructure, chemical composition and structure of egg shells in *Ascaris* were studied by many authors, as Lams (1952) (ex Foor 1967), Monné and Hönig (1954) (ex Foor 1967), Rogers (1956), Foor (1967), Wharton (1980) and others. In our Department, the egg shells of *A. lumbricoides* have been studied by freeze-fracturing and transmission electron microscopy (Lýsek et al. 1985) and further studies are in progress.

The formation of all four egg shells is stimulated by the fertilization. Already Rogers (1956) demonstrated in the electron microscope the presence of the vitelline layer on the outer surface of chitinous envelope. This vitelline layer originates by the modification of oolemma as the first egg shell and is described as a three-layered, membrane-like structure. During the formation of further egg shells this layer becomes thicker, but keeps its membrane character.

In the structure-less zone filling the space between the oolemma and cytoplasm surface originates the chitin-protein layer consisting of N-acetylglucosamine units of chitin. The initial substrates for chitin synthesis are glucose, ammonia and acetate.

The third shell protecting the egg contents is the ascarosid layer, which is chemically a proteolipid containing 25 % of protein and 75% of lipid. The ascarosid layer consists of refringent granules adhering to fat droplets. The refringent granules occur already in young oocytes; at the later stage they possess a complete membrane and are connected with endoplasmic reticulum. Before the fertilization the refringent granules are freely dispersed in the cytoplasm, but after fertilization they migrate below the chitinous layer where they accumulate, gradually fuse and form the ascarosid layer (Foor 1967). The last shell — the uterine layer — is secreted by uterine cells. The formation of the four shells is terminated after about 24 h.

The study of the shell ultrastructure is difficult due to the uneasy preparation of the material, since the fixatives hardly penetrate through the very resistant egg shells. The first methods used were imperfect (Bird 1971), but satisfactory results were obtained by Foor (1967) and Bird (1968) who used the fixation with 3 % glutaraldehyde and formaldehyde for 1.5—24 h. This method, however, could not be used for the fixation of the sacarosid layer. Only the fixation with 2% OsO_4 in 0.1 M phosphate buffer at pH 7.2 for 28 h at the temperature of 40 °C (Lýsek et al. 1985) enabled a perfect penetration through deeper layers of the egg shells and fixation of even the ascarosid layer. The same effect was obtained after fixation at the temperature of 25 °C for 72 h.

The interest in the studies of the egg shell structure is caused by the fact that the egg shells play and important role at the developmental stage of the parasite outside the host, as they enable its surviving in the outer environment and infecting another host. The present study deals with the course of formation and ultrastructure of the ascarosid layer which ensures the impermeability of the egg shell.

MATERIAL AND METHODS

Adult *A. lumbricoides* specimens were recovered from pigs and the eggs used for the electron microscopy were taken from them at the site between oviduct and uterus. The eggs were immediately fixed in 2 % OsO_4 in 0.1 M phosphate buffer at pH 7.2 and left at the temperature of 25 °C for 72 h (the same effect was obtained at the temperature of 40 °C for 28 h). After fixation the eggs were washed in 0.1 M phosphate buffer (pH 7.2) for 10 min. After dehydration through a graduated acetone series the eggs were placed in 100 % propylenoxide for 30 min and then in a mixture of propylenoxide and Durcupan I for at least 2 h. Then the mixture was decanted and the eggs were saturated in the mixture of Durcupan I and II in a slow rotation mixer. The eggs were then kept overnight at 40 °C in a thermostat. On the next day, they were again saturated in Durcupan II. The samples were then embedded into gelatine capsules and polymerized at the temperature of 60 °C for three days.

Series of semithin and ultrathin sections were cut with Reichert ultramicrotome and examine with Tesla BS 613 electron microscope.

RESULTS AND DISCUSSION

The formation of the ascarosid layer in the egg shells of *A. lumbricoides* and its layered structure are the topic of this paper. The studies follow from the results obtained by Lýsek et al. (1985), who studied the ultrastructure of egg shells of *A. lumbricoides* using the method of freeze-fracturing in confrontation with transmission electron microscopy in ultrathin sections. Both methods showed the laminal character of the ascarosid layer (Plate I, Figs. 1, 2).

The process of formation of the chitinous and ascarosid layers, which is induced by the penetration of the sperm into the egg, seems to be very rapid. In the majority of eggs isolated from the beginning of uterus (from the site where fertilization takes place), the two basic layers of egg shells were already formed. Only in some of them we managed to observe the initial phase of ascarosid layer differentiation. At first the chitin-protein complex is formed and then the ascarosid layer arises. The beginning of this process is shown in Plate II, Figs. 1, 2. Refringent granules surrounded with fat vacuoles move immediately below the chitin layer where they accumulate and form an almost continuous, linear, electron-lucent layer. The surface membrane structures of fat vacuoles fit together in form of wedges and they are most probably the base of the lamellar structures of the ascarosid layer.

A more advanced phase of formation of this layer could not be observed, which indicates that this process is very rapid.

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ОБРАЗОВАНИЕ АСКАРОЗИДНОГО СЛОЯ ОБОЛОЧКИ ЯИЦ У *ASCARIS LUMBRICOIDES*

Д. Шромова

Резюме. Изучали ультраструктуру и образование аскарозидного слоя оболочки яиц у *Ascaris lumbricoides*. Образование этого третьего слоя оболочки яиц начинается непосредственно после оплодотворения и после образования комплекса хитин-белок. Отдельные компоненты слоя, т. е. преломные гранулы, окруженные вакуолями жира, соединяются друг с другом в форме клинов, что, по-видимому, образует слоистую структуру аскарозидного слоя.

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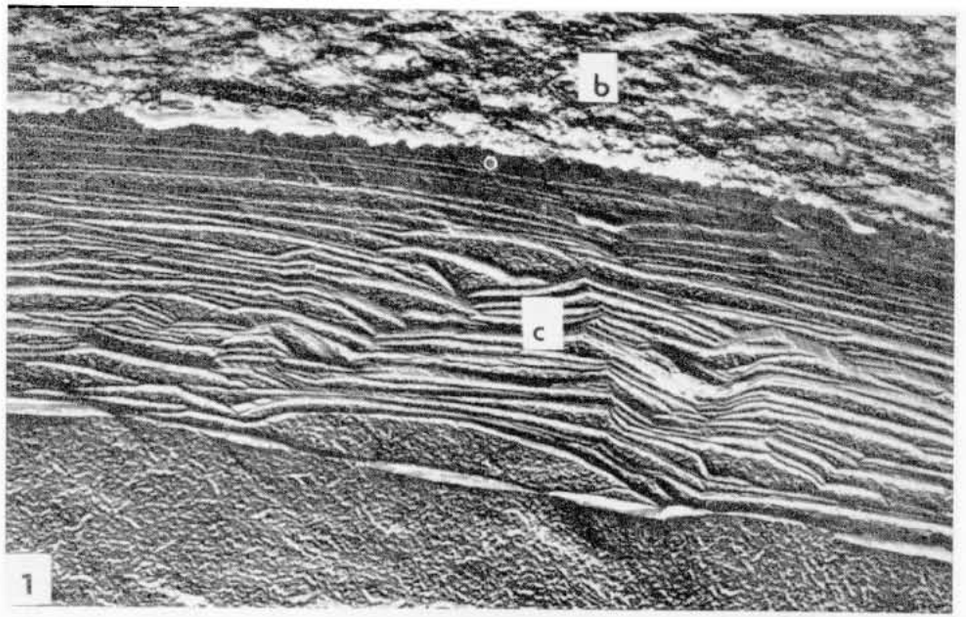
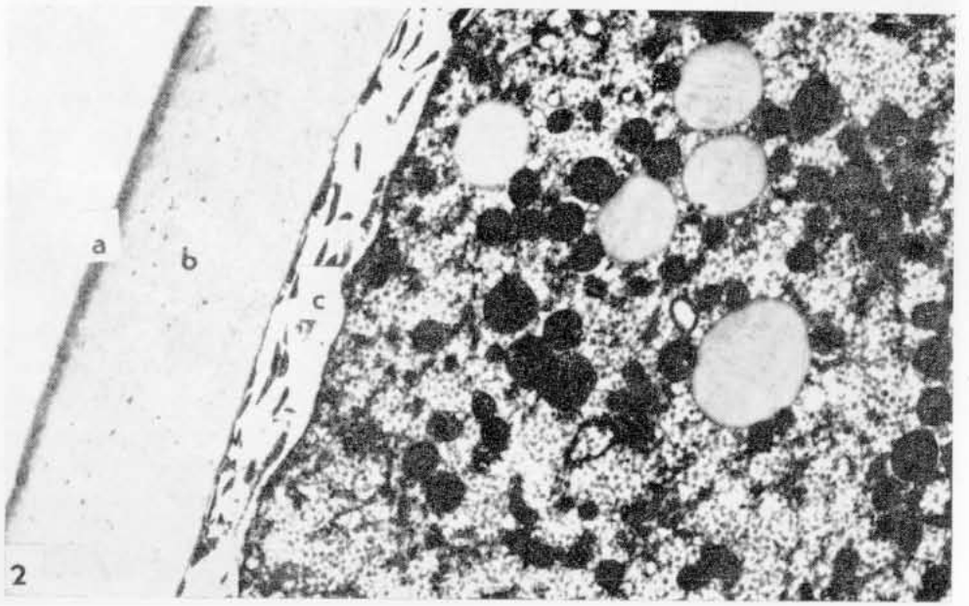
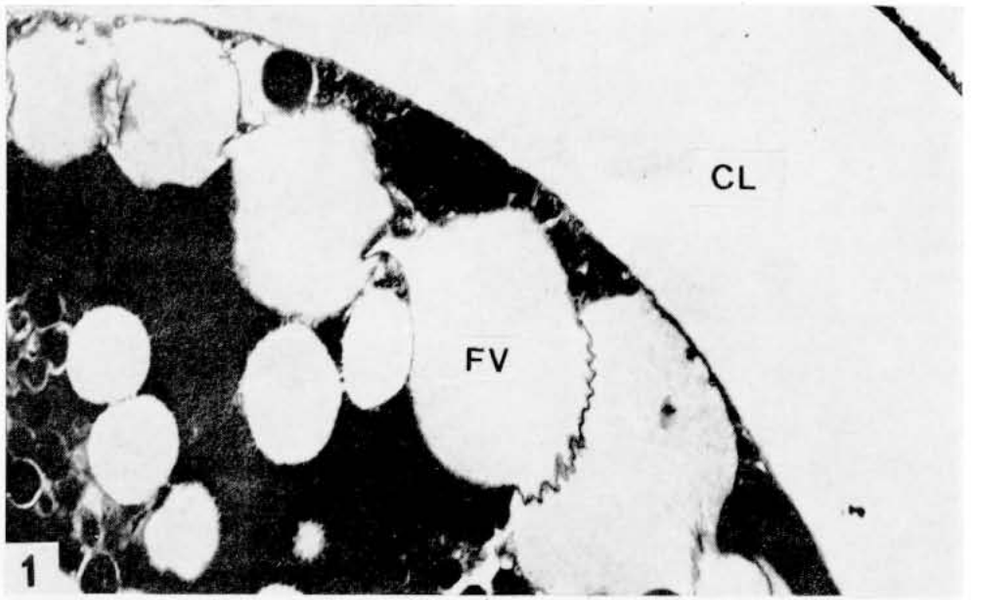
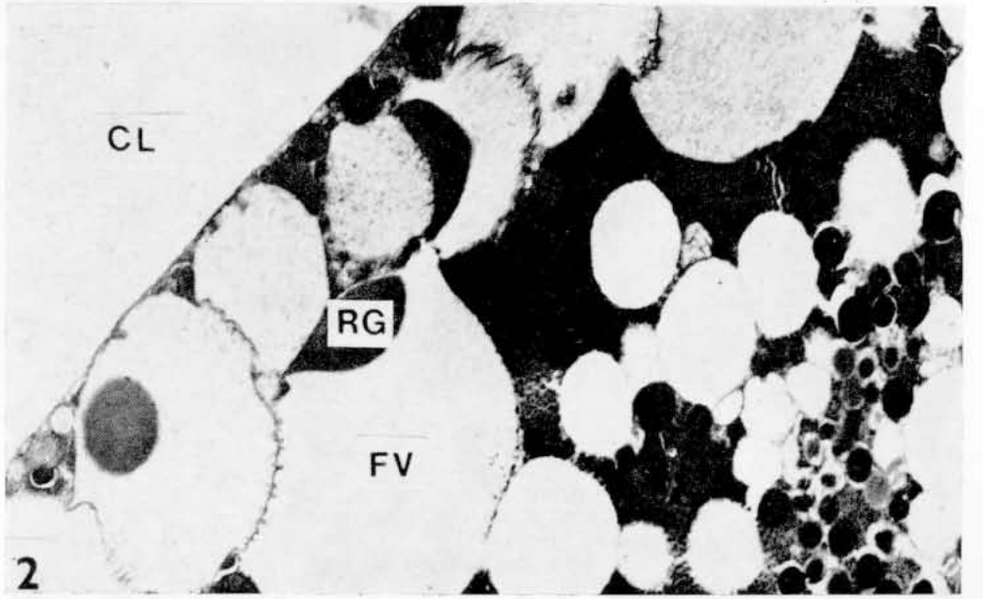




Figs. 1, 2. Egg of *Ascaris lumbricoides* with formed egg shells: a — vitelline layer, b — chitinous layer, c — ascarosid layer. Note the structure and even thickness of the layer. **Fig. 1.** (obtained by the courtesy of Prof. Dr. H. Lýsek) (×30 000). **Fig. 2.** (×9 400).





Figs. 1, 2. Refringent granules (RG) adhering to fat vacuoles (FV) and accumulated under the chitinous layer (CL). The refringent granules and fat vacuoles are fusing. (×9 400).