Recent Advances in Insect Embryology in Japan and Poland
Edited by H. Ando and Cz. Jura
Arthropod. EmbryoL. Soc. Jpn.
(ISEBU Co. Ltd., Tsukuba) 1987

Oogenesis of *Tetrodontophora bielanensis* (Waga) (Collembola). Preliminary Ultrastructural Studies of the First Egg Envelope Formation*

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and

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Synopsis

Formation of the first egg envelope at final stage of oogenesis, by means of TEM was studied. The material for envelope accumulates in perioocytic space, between microvilli of oolemma. At the same time, in peripheral layer of ooplasm of the oocyte numerous electron-dense vesicles are observable. The formed first egg envelope is 4 μm thick and outer one 0.2 μm thick. The outer layer is covered by concentrations, oval in shape, built of substance of very high electron-density. Between these concentrations and follicular cell a structure is situated similar to the basal lamina. The ultrastructure of the first egg envelope, described in this paper, closely resembles the ultrastructure of chorion described by Wójtowicz (1978).

Introduction

In the recent years, the formation and structure of the egg envelopes of Pterygota have been subject of many studies (Furneaux and Machay, 1976; Hinton, 1981; Margaritis, 1985; Regier and Kafatos, 1985), but the studies concerning Apterygota are rather scanty.

According to Matsuzaki (1973) and Palévody (1976), the vitelline membrane in

* Supported by Grant CPBP 04-01-5-03.

Collembola is produced by oocyte, whether the chorion is produced by the cells of oviduct (Matsuzaki, 1973).

In this paper we describe the first egg envelope, produced by the oocyte at final stage of oogenesis. Knowingly, we call it "first egg envelope", because at present it is difficult to decide whether it represents vitelline membrane or chorion (see also Discussion).

Material and Methods

The specimens were collected in the vicinity of Kraków. In the species studied, the process of oogenesis is synchronized in all egg chamber of the ovaries (Krzysztofowicz, 1971, 1975; Biliński, 1976). The oogenesis begins in August and ends in October or early November. The egg envelopes are formed by the end of oogenesis. In nature, the temperature influences the oocyte maturation. The maturation takes place after temperature dropping below −10℃, following 2−4 days with temperature above 0℃. Such condition happens, however, at different time by the end of October or beginning of November, with fluctuation up to 14 days. This causes difficulty in collection of the material in proper phase of egg envelope formation and in understanding of their nature.

Adult females were decapitated and fixed in 2% osmium tertoxide for 1.5 hr in phosphate buffer at pH 7.4 with saccharose (256 mg/10 ml of fixative). Following dehydration, in series of alcohol and aceton, the specimens were embedded in Epon 812. Ultrathin sections were stained with uranyl acetate followed by lead citrate, and examined in Tesla BS-500.

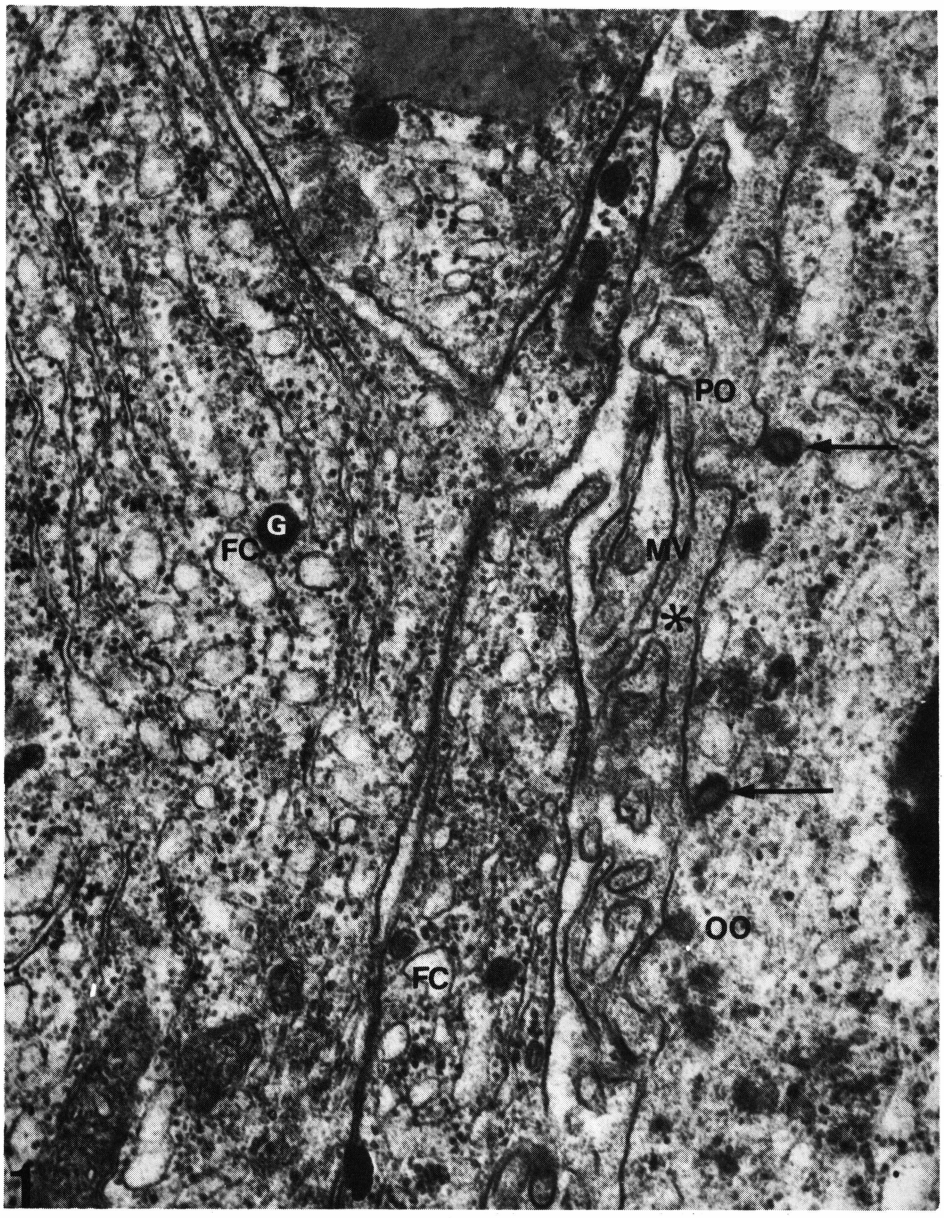
Results

By the end of oogenesis, the oocyte produces numerous microvilli on its surface. Between microvilli a fibrous material appears of medium electron-density.

As the maturation of the oocyte progresses, the number of microvilli and amout of fibrous material increase. At the same time, within the oocyte peripheral ooplasm numerous vesicles form, filled with electron-dense substance. A part of these vesicles is connected with oolemma of the oocyte, another part is lying deeper in periplasm. At the level of connections with oolemma, the vesicles extrude the electron-dense substance outside of the oocyte into the perioocytic space (Figs. 1−3). The extruded substance condenses and progressively fills the perioocytic space (Figs. 1−6).

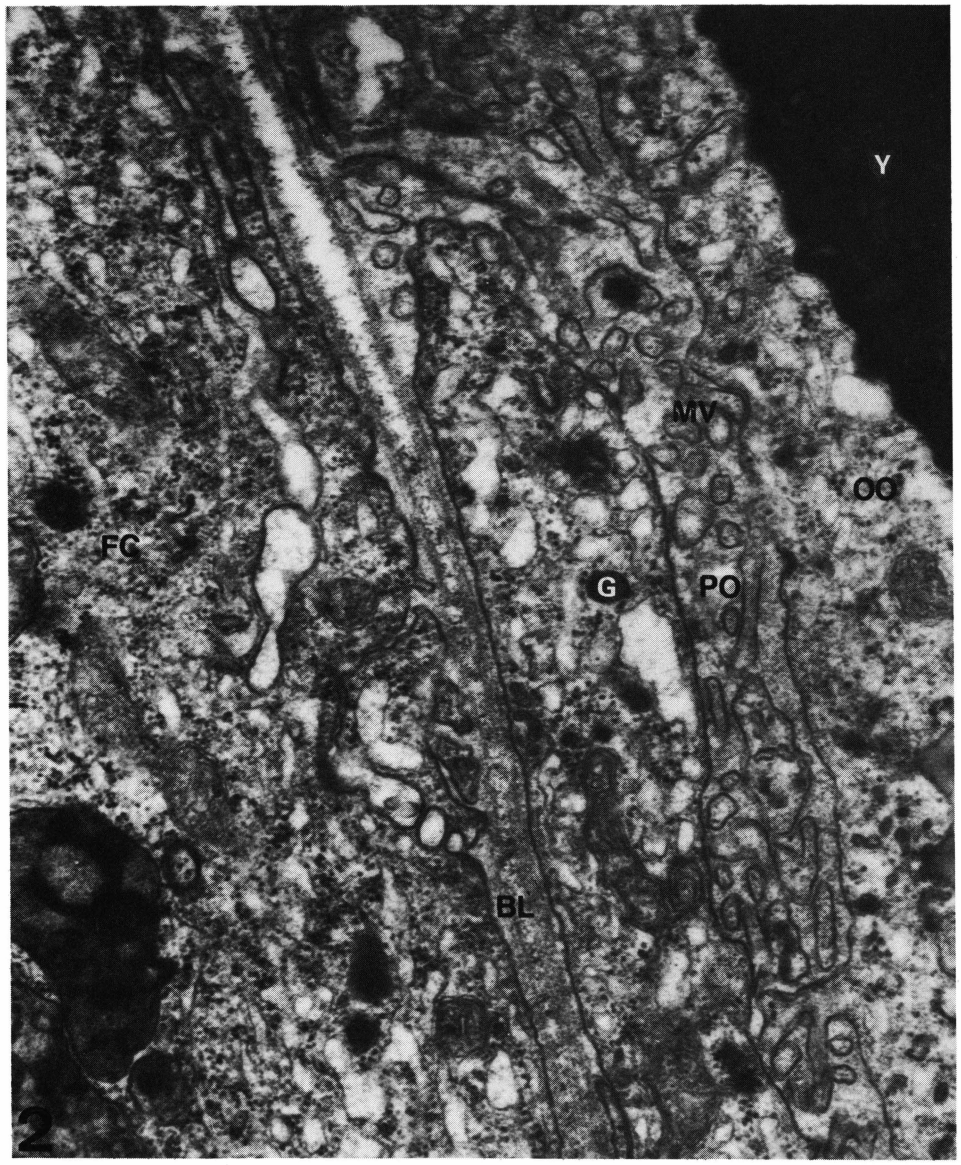
The above described processes represent preparatory phase for the formation of the first embryonic envelope.

During the preparatory phase, the follicular cells, surrounding the oocyte, themselves do not form microvilli, but their cytoplasm is rich in RER and electron-dense granules. Similar granules are also observable in the perioocytic space. At the late phase of the first egg envelope formation (see below), the cytoplasm of the follicular cells is poor in both RER and electron-dense granules (Figs. 5, 6), and finally these structures are not longer observable.



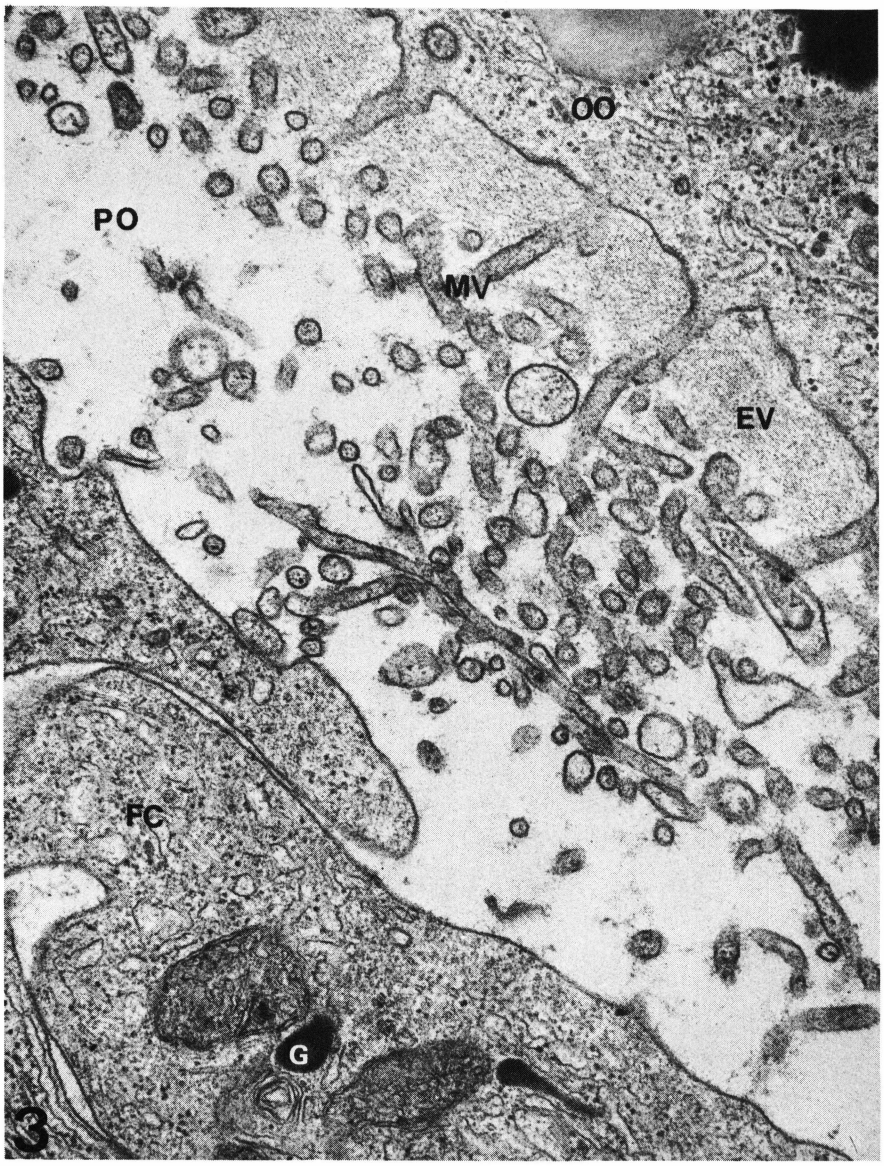
Figs. 1. Successive stages of first egg envelope formation. (1st of 6)

FC, follicular cell; G, dense granule; MV, microvilli; OO, oocyte; PO, perioocytic space. Note material for first egg envelope (asterisk) and vesicles with electron-dense substance in peripheral ooplasm (arrows). × 49,000.



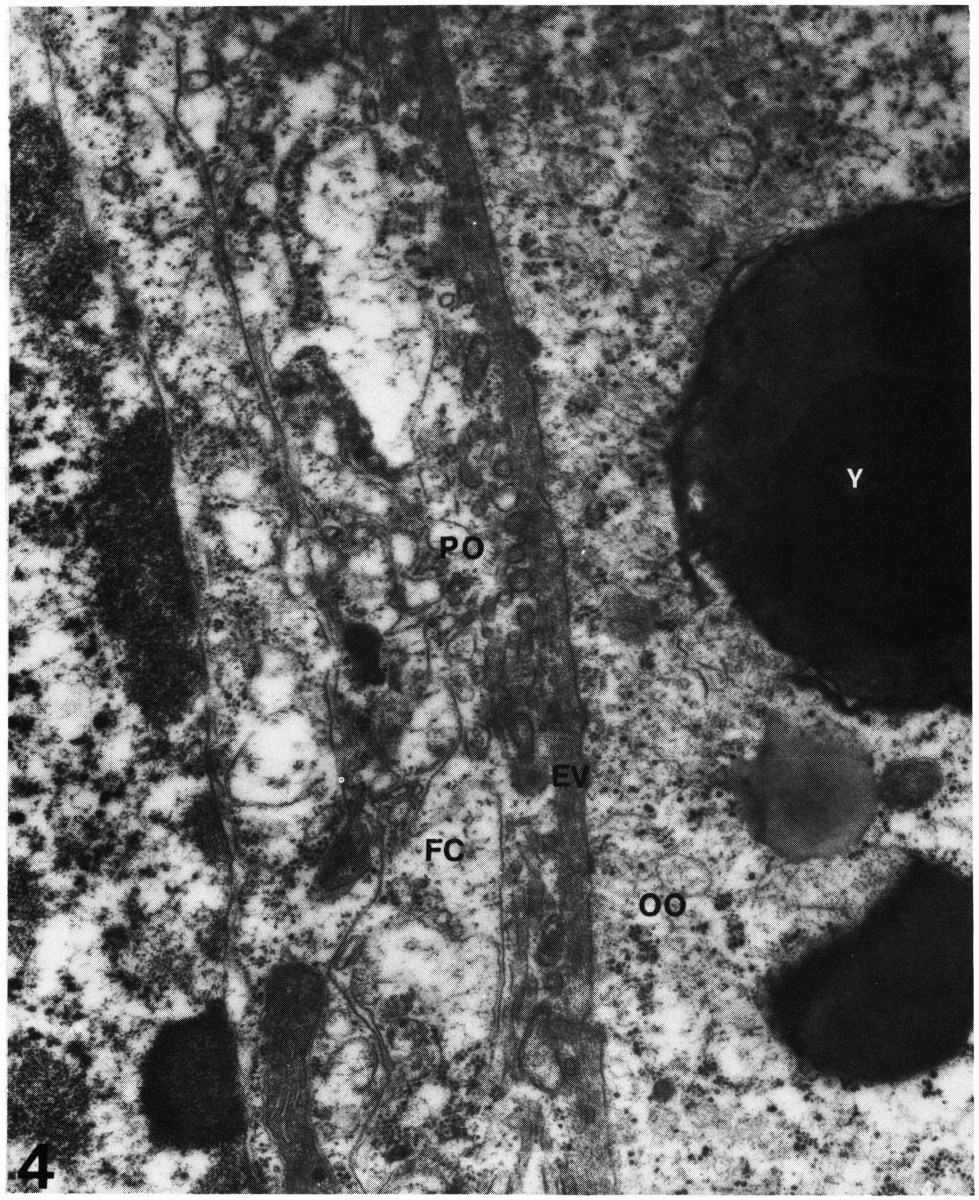
Figs. 2. Successive stages of first egg envelope formation. (2nd of 6)

BL, basal lamina; FC, follicular cell; G, dense granule; MV, microvilli; OO, oocyte; PO, perioocytic space; Y, yolk. × 39,000.



Figs. 3. Successive stages of first egg envelope formation. (3rd of 6)

EV, first egg envelope in formation phase; FC, follicular cell; G, dense granule; OO, oocyte; PO, perioocytic space. × 32,000.

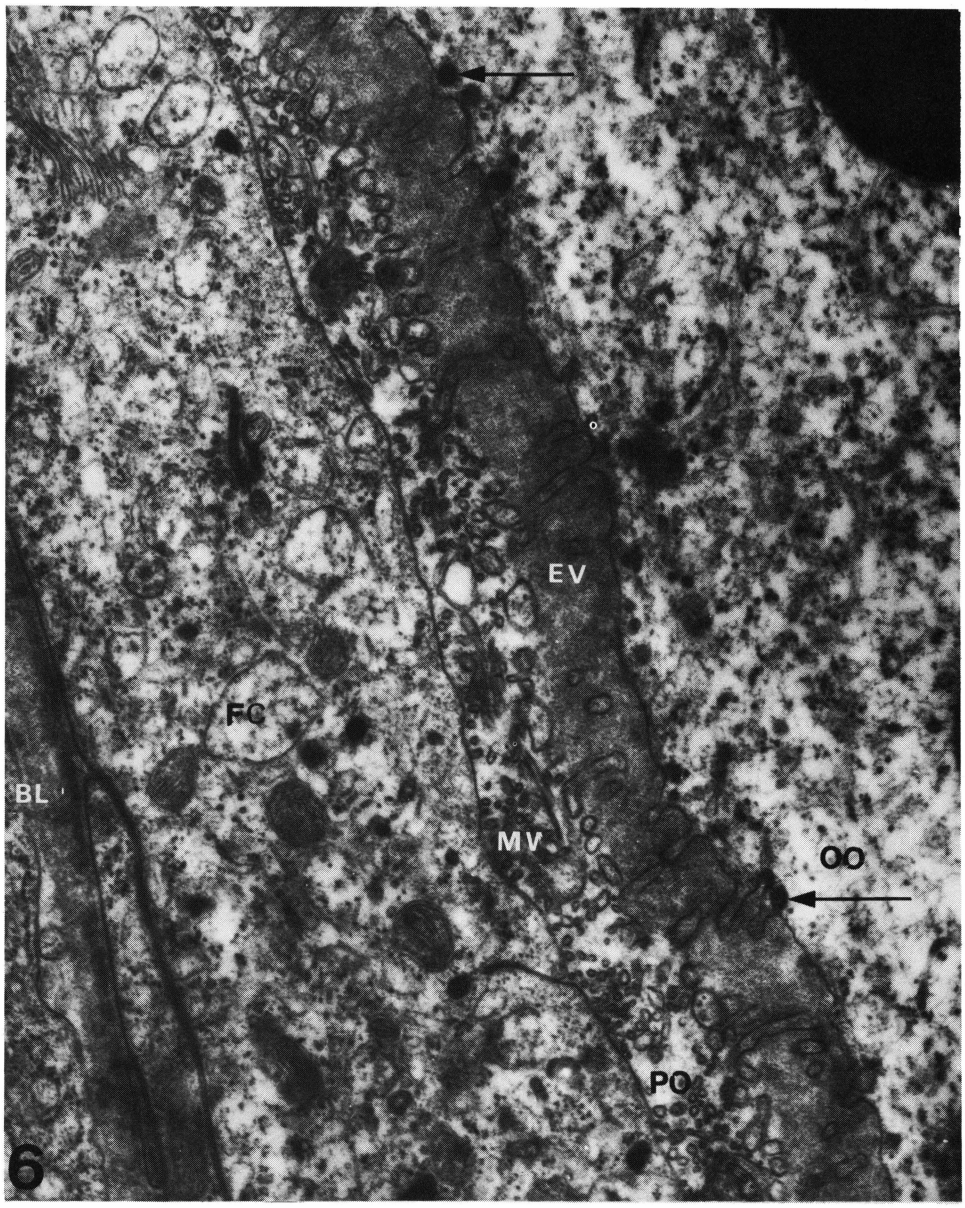


Figs. 4. Successive stages of first egg envelope formation. (4th of 6)

EV, first egg envelope in formation phase; FC, follicular cell; OO, oocyte; PO, perioocytic space; Y, yolk. × 30,000.

Figs. 5. Successive stages of first egg envelope formation. (5th of 6)

BL, basal lamina; EV, first egg envelope; FB, fat body; FC, follicular cell; MV, microvilli; OO, oocyte. × 30,000.



Figs. 6. Successive stages of first egg envelope formation. (6th of 6)
BL, basal lamina; EV, first egg envelope; FC, follicular cell; MV, microvilli; OO, oocyte. Note secretion from ooplasm into perioocytic space (arrows). × 30,000.

During the preparatory phase, the layer of material destined for the first egg envelope reaches the thickness of about 0.5 μm. Above this layer upper portions of microvilli of the oocyte are observable, arranged in a chaotic pattern. Tips of some microvilli join together and form compact aggregations (Fig. 6).

The middle phase of the first egg envelope formation was not observed in this study. The final phase is illustrated in Figs. 7 and 8.

The first egg envelope, at its final phase of formation, is about 4 μm thick and is composed of two layers. The inner layer, neighbouring the oolemma of the oocyte, is about 3.8 μm thick and shows a high electron-density. The second layer, bordering the perioocytic space, is only 0.2 μm thick and shows much less electron-density. At this final stage of first egg envelope formation, the oolemma develops numerous evaginations filled with peripheral ooplasm. The width of the perioocytic space measures now about 0.6 μm, and is enclosed by the element similar in structure to the basal lamina, 0.4 μm in thickness. Within the perioocytic space aggregations of heterogenic material occurs, showing high electron-density (Fig. 7). Between these aggregations the flocculent material is loosely dispersed (Figs. 7, 8). Above the material, similar in structure to the basal lamina, the oocyte is surrounded by the follicular cells showing now signs of degeneration. The basal lamina of the follicular cells, at this time, is very thin.

Discussion

Matsuzaki (1973) and Palévody (1976) studied the first egg envelope formation in several collembolan species and named it the "vitelline membrane" . According to them, the collembolan eggs are covered also by the second envelope the chorion. However, they did not studied the formation of the second envelope, only Palévody speculated that chorion is produced in oviduct.

The egg of *Tetrodontophora bielanensis* is covered by two envelopes (Wójtowicz, 1978): the vitelline membrane and chorion. The structure of the first egg envelope, described in this paper, is very similar to the chorion described by Wójtowicz. The only difference concerns the absence of the pits on the surface of the first egg envelope described by Wójtowicz, but the oval structures shown in this paper in Fig. 7 may represent the pits in stage of formation.

The first egg envelope of *T. bielanensis* completely differs from the vitelline membrane described in the same species by Krzysztofowicz (1986a, b). It is therefore reasonable to postulate that in the species studied, as the first egg envelope the chorion is formed and then the vitelline membrane.

The process of formation of egg envelopes in other apterygotan orders than the Collembola is better known. Bitsch (1980) studied vitelline membrane and chorion formation in Thysanura. According to him, both envelopes arise in the same manner as in Pterygota. Protura were analysed by Biliński and Klag (1977) and Diplura by Biliński (1983), but only process of chorion formation was studies, which in both orders is produced by follicular cells.

The process of the first egg envelope formation in *T. bielanensis* is similar to that occuring in Myriapoda and Crustacea. Egg membrane formation of Myriapoda were

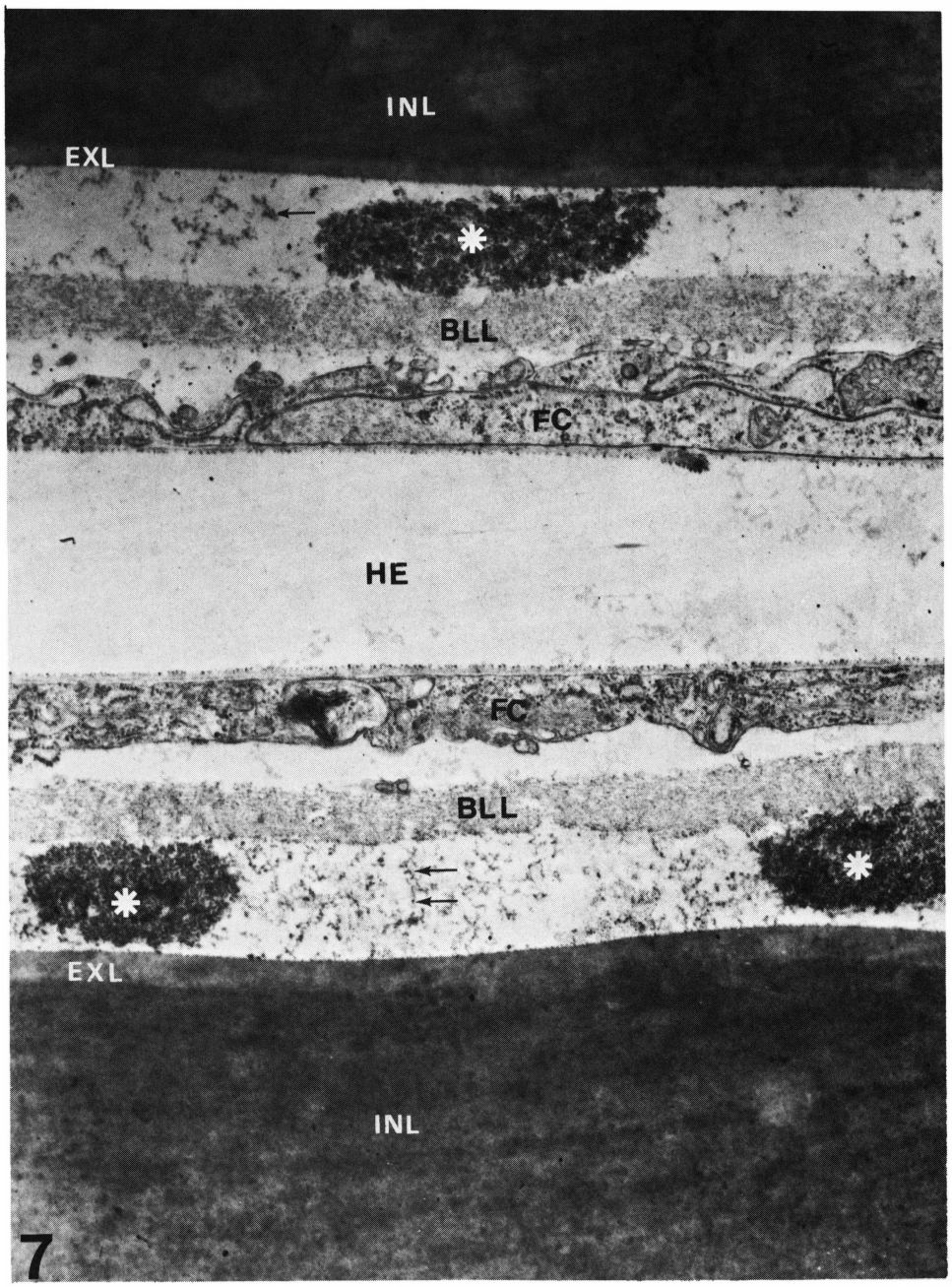


Fig. 7. Fragments of two egg chambers with first egg envelope. BLL, basal-like lamina; EXL, external layer of first egg envelope; FC, follicular cell; INL, Internal layer of first egg envelope; Note oval heterogenic structures (asterisk) and between them flocculent material (arrows). × 18,000.

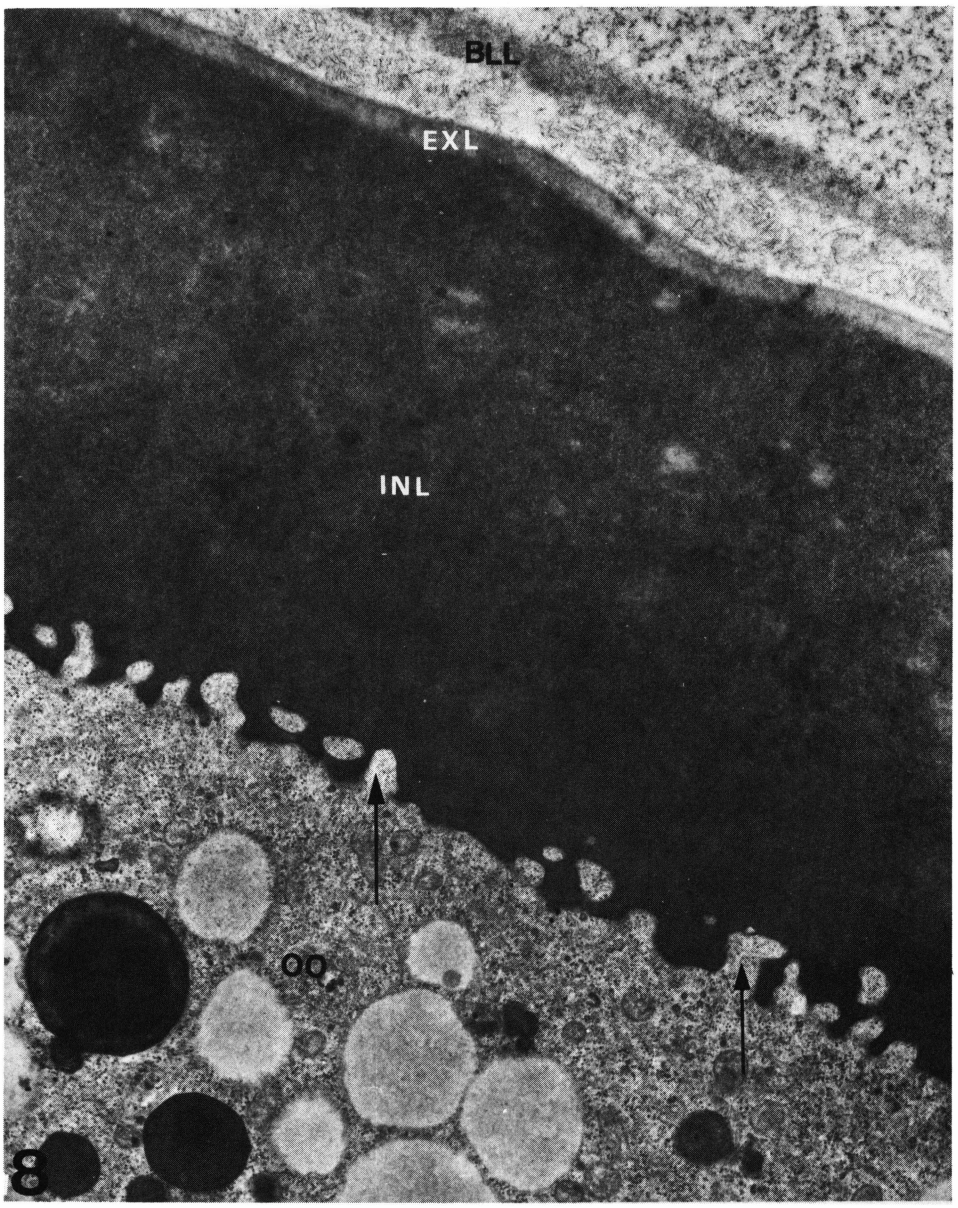


Fig. 8. Fragment of first oocyte envelope. BLL, basal like lamina; EXL, external layer of first egg envelope; INL, internal layer of first egg envelope; OO, oocyte. Note invaginations of oolemma (arrows). × 18,000.

studied by Herbau (1974), however, the author named the first egg envelope as vitelline membrane, which according to him degenerates before chorion formation. The chorion is formed by the epithelial cells of the ovary. According to Biliński (1981) in Symphyla, precursors of chorion are most likely synthesized by follicle cells.

Crustacea were studied by Souty (1980), Zerbib (1980) and Talbot (1981). However, both mentioned authors used very confusing nomenclature for naming egg envelopes and because of this, at present any discussion concerning the analogy of these structures in Arthropoda is premature.

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