Boll. Zool. 56: 291-298 (1989)

HUBBARD

Chorionic adhesive material of the egg of the mayfly *Habrophlebia eldae* (Ephemeroptera, Leptophlebiidae): morphology and synthesis

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ABSTRACT

In Ephemeroptera, in spite of the absence of accessory glands in the female genitalia, the eggs frequently show adhesive coats. Ultrastructural observations of the female reproductive apparatus of *Habrophlebia eldae* demonstrated that the elaboration of chorionic adhesive layers takes place in ovarioles and oviducts. In ovarioles, follicle cells synthesize viscous material that is released on the chorionic surface, and that coalesces to form: a) electron-dense masses closely packed on both sides of the ridges that characterize the fully formed chorion in this species; and b) a thin granular layer that covers the egg surface before ovulation. During passage through the oviducts, the chorion is enriched by products secreted by the cells delimiting the oviductal lumen.

KEY WORDS: Ephemeroptera; Adhesive material; Chorionic envelopes.

ACKNOWLEDGEMENTS

Research financially supported by the Ministry for Public Education (MPI): in part under «Popolamento animale del Mediterraneo Occidentale» and in part under other M.P.I. funds.

(Accepted 12 May 1989)

INTRODUCTION

It is common to find adhesive layers on the egg chorion of Ephemeroptera (Degrange, 1960; Koss, 1968; 1970; Koss & Edmunds, 1974; Soldàn, 1979; Kopelke & Müller-Liebenau, 1981a, b, 1982; Gaino & Mazzini, 1984; Mazzini & Gaino, 1985). They may constitute an adhesive device in eggs that lack the well differentiated chorionic attachment structures that usually fulfill this purpose (Koss & Edmunds, 1974; Gaino & Mazzini, 1987). A recent ultrastructural study of the egg chorion of *Electrogena* and *Rhithrogena* species showed the differentiation of species-specific knob-terminated coiled threads that ensure adhesion to the substrate (Sowa & Soldàn, 1986; Alba-Tercedor & Sowa, 1987; Gaino & Mazzini, 1987; Gaino *et al.*, 1987; Gaino *et al.*, 1989).

In *Habrophlebia*, the chorion lacks attachment projections and the shell surface presents ridges arranged in longitudinal series (Gaino & Mazzini, 1984). Each ridge is composed of columns, supporting the outermost chorionic layer; the columns are separated by chambers filled with viscous material that also covers the inter-ridge egg surface (Mazzini & Gaino, 1985).

Owing to the absence of accessory reproductive glands in the female genitalia (Brinck, 1957), the adhesive layers were believed to be deposited by the follicle cells after the synthesis of the egg envelopes (Koss, 1970). The presence of adhesive material on eggs dissected from mature nymphs suggests that this coat is deposited before fertilization. However, the origin and deposition of the adhesive material have never been demonstrated.

In this study, we examine both the ovarian follicles and the oviducts of *Habrophlebia eldae* Jacob & Sartori, 1984 to investigate the deposition of adhesive layers on the eggs.

MATERIAL AND METHODS

The reproductive apparatus was dissected from young nymphs of *Habrophlebia eldae* at the developmental stage characterized by wing pads enveloping wings with readily visible veins (Gaino, 1987).

For transmission electron miscroscope (TEM) analysis, selected material was fixed for 1 hr in 5% glutaraldehyde-4% formaldehyde in 0.1 M cacodylate buffer at pH 7.2 (Karnovsky, 1965). After rinsing in buffer, the material was post-fixed for 1 hr in 1% osmium tetroxide in 0.1 M cacodylate buffer at pH 7.2, dehydrated in a graded series of ethanols and embedded in an Epon-Araldite mixture. Sections were cut on a Reichert ultramicrotome, mounted on Formvar-coated copper grids and examined in Philips 300 and 400 microscopes after staining in aqueous uranyl acetate and lead citrate.

For histochemical investigations, specimens were fixed in Karnovsky's medium, rinsed in cacodylate buffer, dehydrated in a graded ethanol series and embedded in JB4 resin. Sections were cut with a Reichert OM U2 ultramicrotome and stained with Alcian blue (pH 2.5).

RESULTS

Previous transmission electron microscopy findings in the panoistic ovarioles of *Habrophlebia eldae* demon-

strated that oogenesis ends after chorionic envelope deposition by the follicle cells (Mazzini & Gaino, 1988). The mature eggs then pass from the ovarioles to the lateral oviducts which join in a common tract, known as the «ovipositor» or «egg guide», which is in communication with the external environment (Gaino & Mazzini, unpubl. data). Oocytes released from the oviducts or stored in the ovipositor are covered with layers of viscous material.

Our observations of the female reproductive apparatus of this species show that both ovarioles and lateral oviducts synthesize the adhesive material.

Synthesis in the ovarioles

According to Mazzini & Gaino (1988) the chorion of *H. eldae* presents three distinct layers: a) the inner layer adjacent to the vitelline envelope, consisting of membrane-like units regularly spaced; b) the middle layer consisting of loosely structured fibrillar material; and c) the outermost double layer. The middle chorionic layer is the core of the columnar projections arising from the egg surface, which form the system of ridges running along the major axis of the egg (Fig. 1).

The follicle cells overlying the oocytes are competent in egg envelope deposition and display the characteristics of metabolically active glandular tissue. In fact, they are characterized by extensively developed parallel rows of rough endoplasmic reticulum (Fig. 2) and Golgi elements.

Egg envelope deposition starts with the synthesis of precursor material which is then released at the follicle cell-oocyte interface. Choriogenesis proceeds with the secretion of the prominent ridges that characterize the shell surface in this species (Fig. 2). In this secretory phase microvilli protrude from follicle cells into the space adjacent to the chorionic surface (Fig. 3). The fine granular material filling their cytoplasm is released into the space surrounding the egg chorion, where it coalesces to form a closely apposed electron-dense amorphous region on both sides of the ridges (Fig. 3). This so-called «mucus-like material» or «mucous mass» (Mazzini & Gaino, 1985) gave a positive reaction to Alcian-blue.

Chorionic membrane secretion terminates with the deposition of the continuous outer envelope that borders the egg shell on the outside (Fig. 4). At this stage the follicle cells lose all microvillar projections, and show a thin coat of amorphous material in the continuous space between the plasma membrane and the chorionic surface (Fig. 4). The viscous material close to the ridges undergoes organizational changes: condensation and crystallization. Indeed, more electron transparent bodies of a paracrystalline appearance become visible within the granular homogeneous matrix peripherally lined by a meshwork of filamentous bundles (Fig. 5). The space between the follicular epithelium and the egg surface shows a fibrillar component associated with a granular matrix (Fig. 5). Finally, this fibrous, Alcian-blue positive sheet completely covers the fully formed chorion and overlaps the electron-dense material tightly packed on both sides of the ridges (Fig. 5).

Synthesis in the oviducts

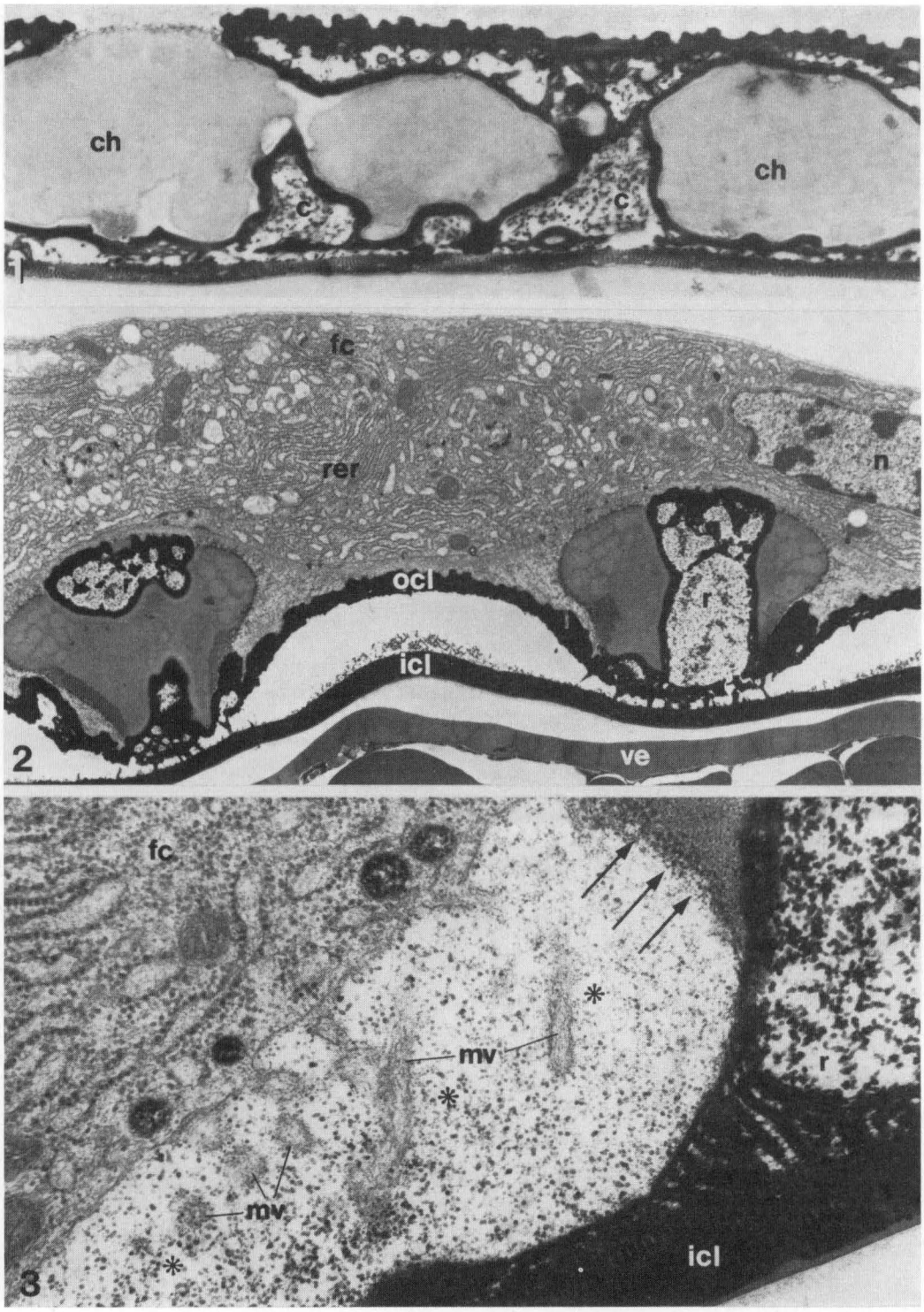
After ovulation, the eggs move through the two lateral oviducts that flow into a single tract. As the eggs travel down the oviduct (Fig. 6), the adhesive coat, secreted by the follicle cells, is enriched with a fine layer of fibrous material (Figs. 7, 8). This is connected with the glycocalyx of the epithelial cells lining the oviduct walls. These cells show an irregularly folded plasma membrane that sometimes has a microvillar border (Fig. 9) and a large central nucleus with dispersed chromatin (Figs. 8, 9). Budding vesicles are visible at the apices of the microvilli (Fig. 9). A layer of circular muscle surrounds the epithelial cells (Fig. 8) involved in the passage of the eggs. Thick strands of fibrous material, probably dependent on the secretory activity of the oviduct cells of the upper level, are found in the lower part of the lateral oviducts where the epithelium delimiting the lumen is covered by a cuticular intima (Fig. 10). The epithelial cells, very closely attached to each other, show large masses of heterochromatin (Fig. 10).

DISCUSSION

As in many other groups of Insecta, the follicle cells of Ephemeroptera are closely associated with the developing oocyte and are involved in vitelline and chorionic envelope synthesis (Mazzini & Gaino, 1988). In the mature eggs of mayflies, an adhesive layer is associated with the surface of the chorion: a feature also observed in Coleoptera (Biemont *et al.*, 1981), Lepidoptera (Barbier & Chauvin, 1974a, b; Chauvin *et al.*, 1974) and Diptera (Mazzini & Santini, 1983).

The present study shows that, in *H. eldae*, the elaboration of the Alcian-blue positive material, which constitutes the adhesive layer in this species, occurs in two parts of the reproductive system: the ovarioles and oviducts.

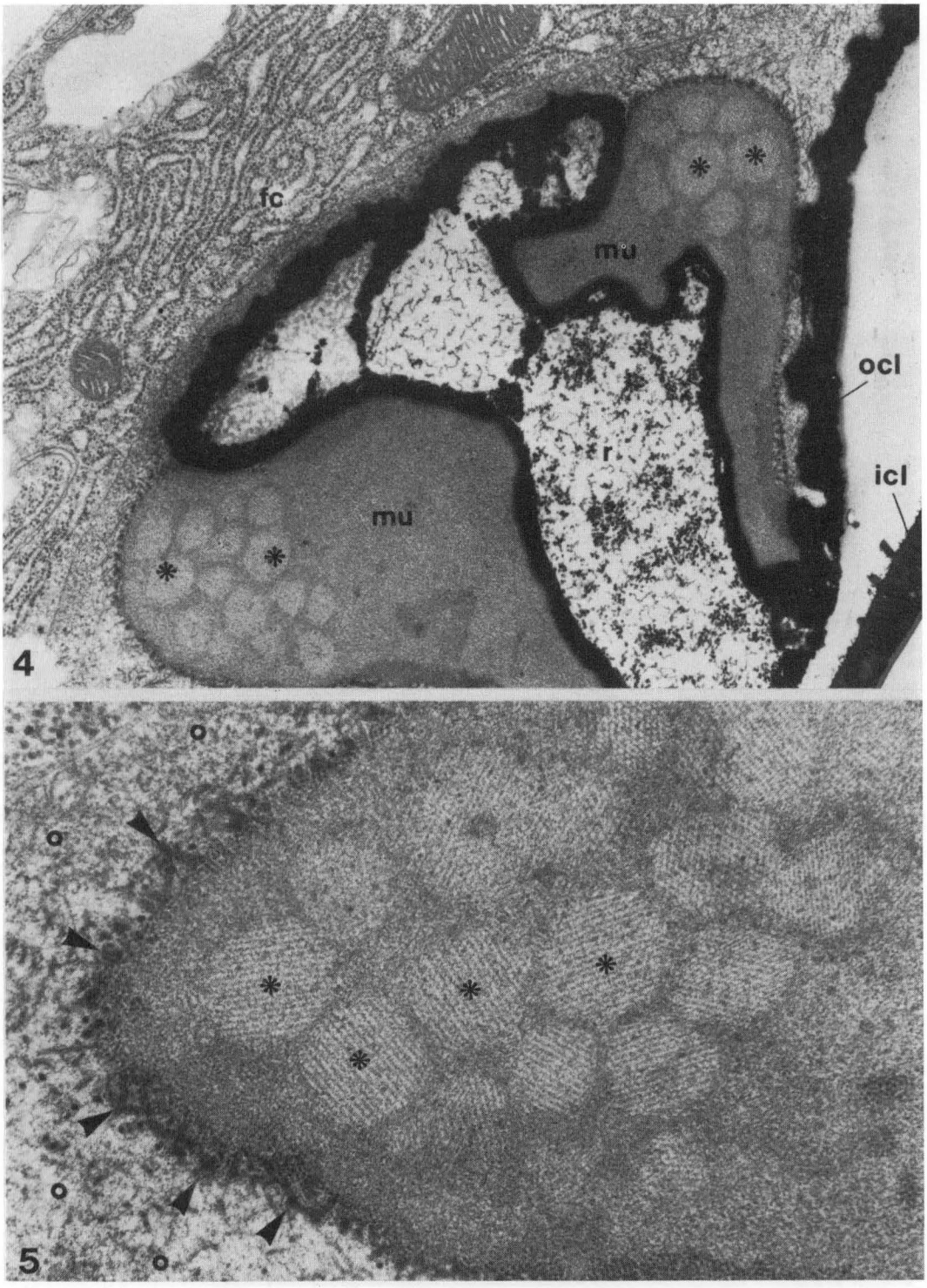
Transmission electron microscopy of the ovarioles demonstrates that the deposition of such a material involves the follicle cells and that the process constitutes a well-defined phase of their secretory activity. The role of the follicle cells in synthesis is consistent with the ultrastructural features of extensively developed parallel rows of rough endoplasmic reticulum, microvillar projections, secretory vesicles and granular cytoplasm as is usual in insects (Mathew & Rai, 1975; Norton & Vinson, 1982). The origin within the follicle cells of an amorphous component used in the formation of the adhesive layers, suggests that this precursor material is a true chorionic constituent, previously de-



Figs. 1-3 - 1. Mature egg of *H. eldae*; the section goes through one of the chorionic ridges composed of columns (c) separated from each other by chambers (ch) filled with mucous material. × 14 000. 2. A follicle cell (fc) adhering to the chorion of an almost mature egg. Two chorionic ridges (r) project from the chorionic surface. icl, inner chorionic layer; n, follicle cell nucleus; ocl, outer chorionic layer; rer, rough endoplasmic reticulum; ve, vitelline envelope. × 8 300. 3. Granular material released from a follicle cell (fc), accumulates in the space close to the chorionic surface (asterisks) and condenses (arrows) on both sides of a ridge (r). icl, inner chorionic layer; mv, microvilli. × 52 000.

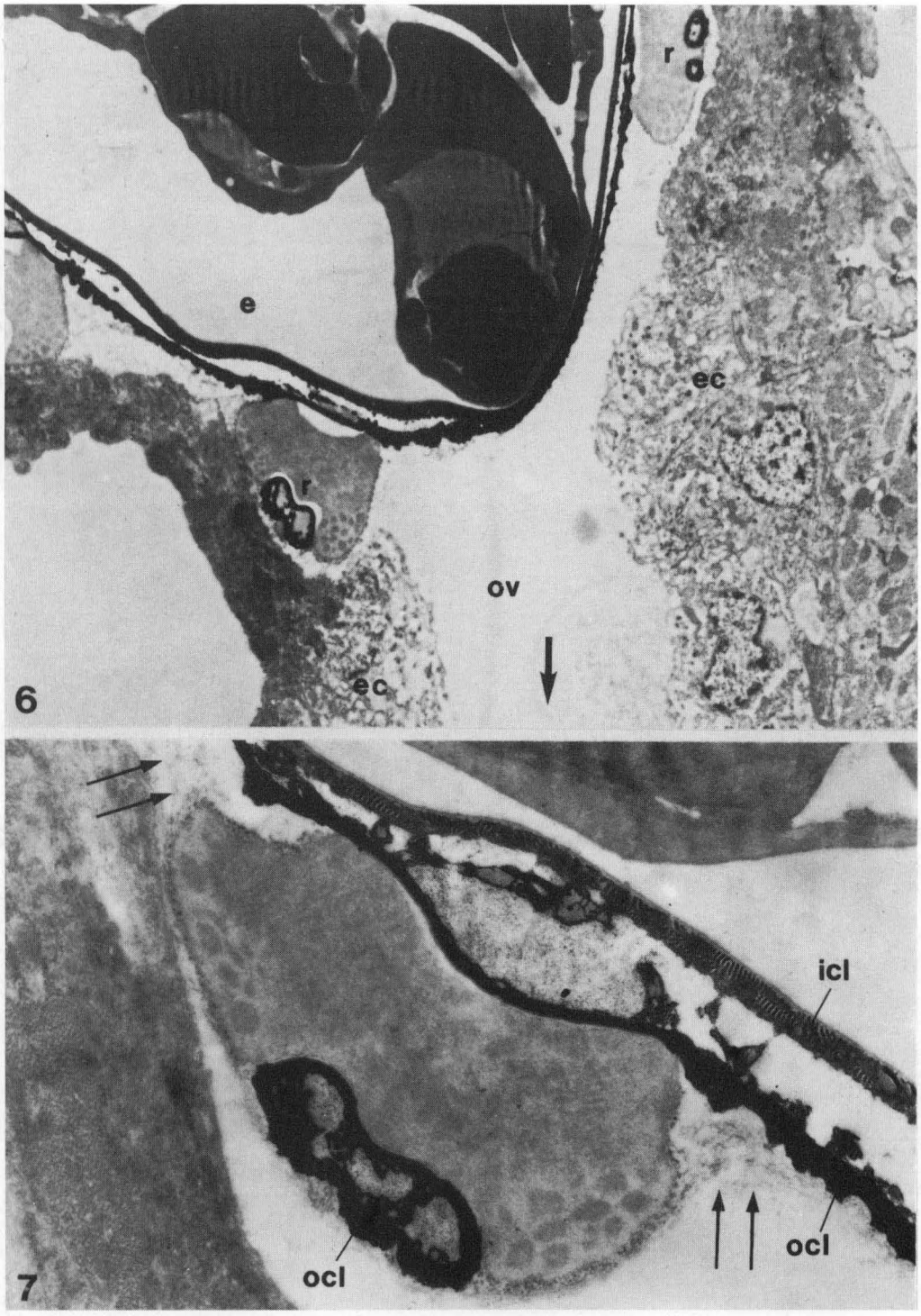
posited as a granular matrix on both sides of the columns of the longitudinal ridges. During the subsequent phase of outer chorion deposition, viscous material fills the chambers of the ridges. Morphological changes occur within this adhesive material: paracrystalline bodies and a filamentous meshwork border become visible.

When the chorion is fully secreted, the adhesive



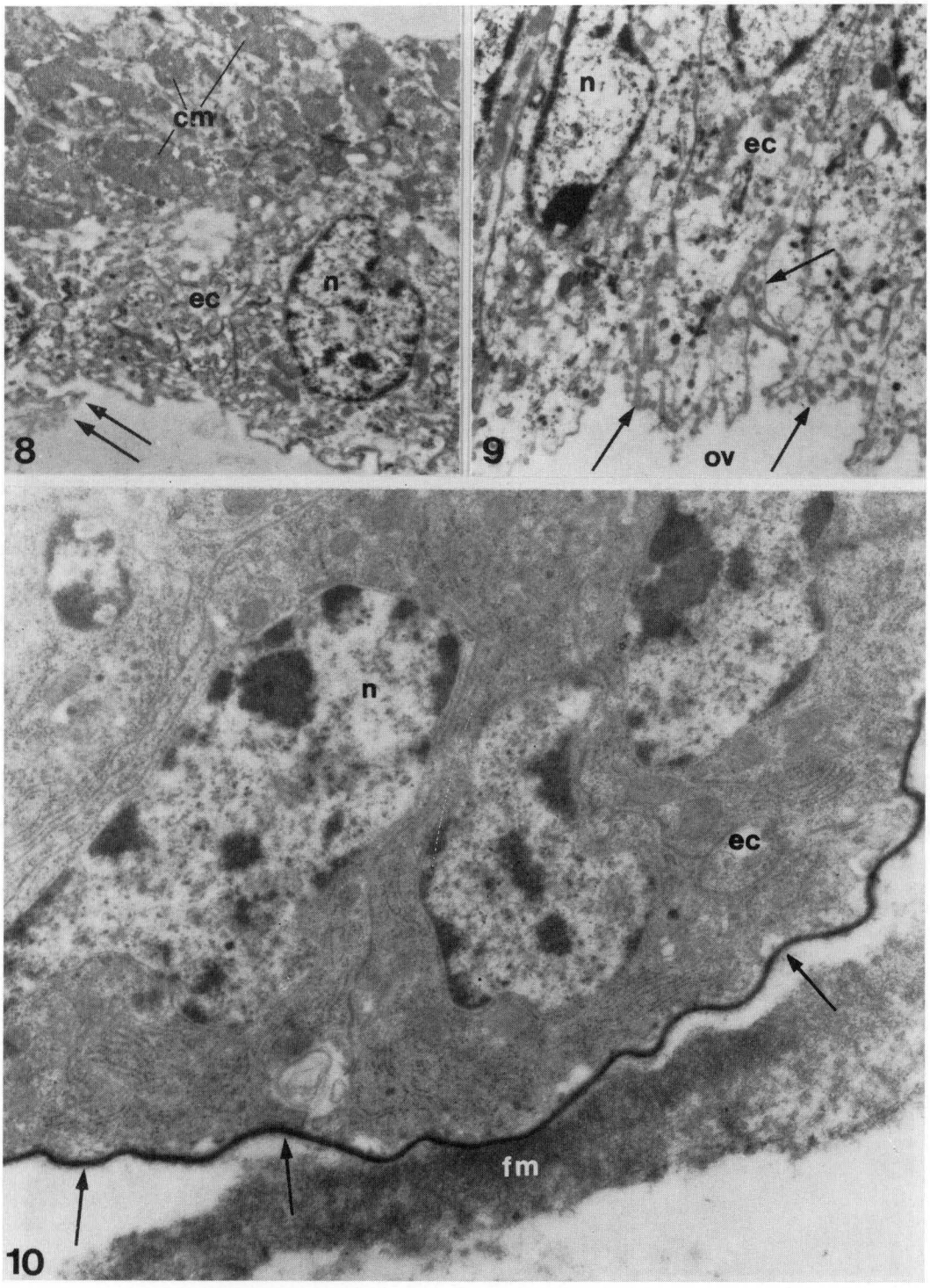
Figs. 4-5 - 4. Transverse section of a mature egg enveloped by a follicle cell (fc) that lacks microvilli. A ridge (r) of the fully formed chorion with mucous material (mu) densely packed on both sides. Note the paracrystalline bodies within the mucous masses (asterisks). icl, inner chorionic layer; ocl, outer chorionic layer. × 27 000. 5. Higher magnification showing the organization of the viscous material close to the ridges with the paracrystalline bodies (asterisks) in a granular matrix enclosed by a fibrillar meshwork (arrowheads). A thin layer (circles), composed of granules associated with fibrils, envelops the entire egg surface. × 66 000.

layers are enriched with other material that accumulates in the space between the egg shell and the follicle cells. As a consequence, the eggs that begin moving down the oviducts are completely enveloped by a more complex adhesive layer. As in other Ephemeroptera, the genital apparatus of *H. eldae* has no accessory reproductive glands. The cells lining the lumen of the two lateral oviducts therefore perform a secretory func-



Figs. 6-7 - 6. Downward passage of a mature egg (e) through the lateral oviduct whose lumen is lined with epithelial cells (ec). Note several ridges (r). Arrow indicates outward direction. ov, oviductal lumen. × 6 400. 7. Higher magnification of Figure 6 showing the outermost chorionic layer (ocl) and the fibrous material (arrows) added by the epithelial cells of the oviduct during the passage of the egg. icl, inner chorionic layer. × 19 000.

tion, as in coleopteran species (Hamon *et al.*, 1982). Fibrous material, such as electron-dense masses, is found in connection with the glycocalyx of the epithelial oviductal tract. The presence of the budding vesicles at the apices of the microvillar border of these cells indicates secretory activity. Although oviductal products adhere to the chorion during the downward passage of the eggs, their main function seems to be to help the passage and to preserve the mature eggs during storage in the lateral oviducts. Fibrous strands



Figs. 8-10 - 8. Oviduct wall showing a luminal side of an epithelial cell (ec) whose surface is connected with fibrous material (arrows). cm, circular muscle; n, nucleus. × 8 000. 9. Detail of the oviduct border delimited by epithelial cells (ec) showing budding vesicles and traces of the fibrous material at the apices of their microvilli (arrows). n, nucleus; ov, oviduct. × 13 000. 10. Section of a lateral oviduct showing a strand of fibrous material (fm) located over the cuticular intima (arrows). ec, epithelial cells; n, nucleus. × 21 000.

found in the lumen of the lower portion of the oviducts could serve for this protective function. Deposition of the adhesive layers occurs before fertilization and thus might constitute a mechanism by which to trap spermatozoa, as observed in some Amphibia (McLaughlin & Humphries, 1978; Jego *et al.*, 1983; Yoshizaki, 1985). There is no doubt that adhesive layers improve the mutual adhesion of eggs to be laid in water. According to Grandi (1943, 1955), before deposition, the eggs of *H. eldae* (cited as *H. fusca*, see

review in Belfiore & Gaino, 1985) form a clump of 1-2 mm that adheres to the last abdominal segment. In agreement with Grandi's observations (1943), although the chorion lacks any differentiated adhesive apparatus, eggs of *H. eldae* adhere to the substratum after deposition in water. The viscous material apposed to the sides of the ridges and filling the egg chambers accomplishes this adhesive function after swelling in water. Egg adhesive material of Ephemeroptera may increase the rate of reproductive success of many species by fixing eggs to the substrates and by preventing them from floating away. A similar role is played by a very unusual viscous material found on the chorionic surface of a lepidopteran species (Chauvin & Chauvin, 1980). This material forms club-like structures which are again important for the survival of the species after egg deposition.

In conclusion, the presence of the adhesive layers both on the chorion and within specialized ridges of the chorion of *H. eldae*, suggests that in Ephemeroptera the environment has a great effect on the survival of the eggs once they have been laid in water.

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