Follicle Cell Activity in the Ovarioles of *Habrophlebia eldae* (Ephemeroptera: Leptophlebiidae)[1]

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Abstract. Ultrastructural analysis carried out on the panoistic ovarioles of *Habrophlebia eldae* (Ephemeroptera: Leptophlebiidae) demonstrated that the organization and activity of the follicular epithelium change according to secretory function performed during oogenesis. In previtellogenic ovarian follicles, the cuboidal follicle cells are packed into a columnar epithelium and are connected by gap junctional plaques, forming an oocyte-follicle cell epithelium complex tightly interlocked by way of microvilli. The transition to vitellogenic growth is marked by the appearance of a complex endocytic apparatus in the cortical ooplasm. The plasma membrane folds to form coated pits and vesicles, both being adorned with a clathrin lattice-work. Upon initiation of vitelline envelope deposition, follicle cells flatten, retract their microvilli, and interconnect by means of septate junctions. Chorionic envelopes are laid down in sequence, creating a three-layered structure whose organization varies according to the chorionic pattern of longitudinal ridges. Each ridge is composed of several columnar projections. In the final secretory phase, follicle cell membranes become folded and release closely packed microgranules: (1) that adhere to both sides of the chorionic columnar projections; and (2) that form a sheet of amorphous material enveloping the egg surface prior to ovulation. The synthesis of these complex egg coverings and their role in this primitive insect group also are discussed.

Ephemeropteran ovaries consist of several ovarioles arranged side by side to form parallel rows. Each ovariole is made up of follicles and exhibits a panoistic organization (Brinck, 1957; Soldán, 1979). In a recent ultrastructural study, follicle cells were shown to be involved in vitelline and chorionic membrane deposition (Mazzini & Gaino, 1988).

The follicular epithelium is a cellular system highly specialized for a secretory function. As presumed for the panoistic ovarioles of the stick insect *Bacillus rossius*, egg pinocytosis appears to be stimulated by extracellular protein secretion by follicle cells following interaction with vitellogenin (Mazzini & Giorgi, 1984). The follicular epithelium thus represents the primary target for juvenile hormone, and may have metabolic control over early vitellogenesis (Giorgi & Mazzini, 1984; Mazzini & Giorgi, 1984, 1986). Nevertheless, follicle cells are important for synthesis of precursor material, which in turn, is released for egg envelope organization (Mathew & Rai, 1975; Norton & Vinson, 1982). Chorionic layers are complex; their formation in a well-defined sequence requires energy expenditure by follicle cells. Organization of the chorion is requisite among insects laying eggs in water (e.g., Ephemeroptera), in which case species survival is enhanced by specialized egg envelopes (Koss & Edmunds, 1974). Follicle cells, then, constitute a primary cell system for insect egg growth and protection.

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A detailed ultrastructural analysis carried out on the ovarioles of *Habrophlebia eldae* Jacob & Sartori, 1984 provided information on those events occurring in follicle cells during oogenesis.

Materials and Methods

For transmission electron microscopy (TEM), ovarian follicles dissected from larval stages of *Habrophlebia eldae* Jacob & Sartori, 1984 were fixed in Karnovsky's medium (1965), rinsed in 0.1 M cacodylate buffer (pH 7.2) at 4°C, and postfixed in 1% osmium tetroxide in cacodylate buffer. Selected material was rinsed, dehydrated in a graded ethanol series, and embedded in Epon 812 or in mixture of Epon-Araldite. Sections were cut with a Reichert ultramicrotome, mounted on Formvar-coated copper grids, and stained with conventional uranyl acetate and lead citrate.

For freeze-fracturing, ovarioles were fixed as described above and infiltrated gradually with glycerol to a final concentration of 30%. They were frozen in Freon 22 cooled to the temperature of liquid nitrogen. Fracture and carbon coating were carried out in a Balzer BAF 301 freeze-etching apparatus set at −115°C. Replicas were digested with Chlorox and mounted on Formvar-coated copper grids.

Thin sections and freeze-fracturing preparations were examined with Philips 300 and 400 transmission electron microscopes.

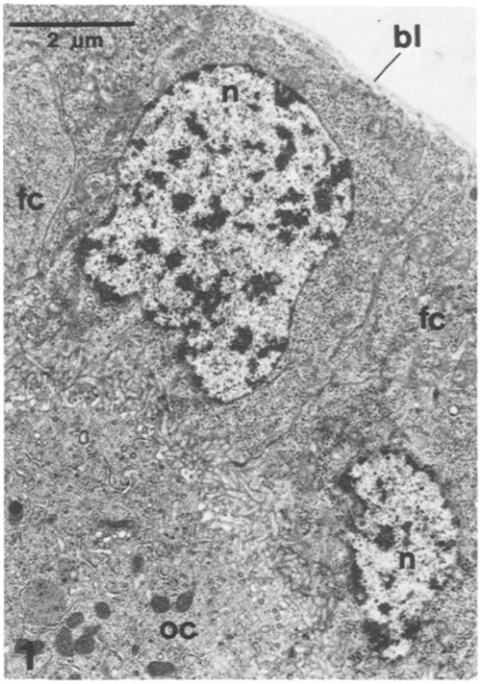
Results

The reproductive system of *Habrophlebia eldae* consists of two ovaries, each made up of numerous ovarioles. Each ovariole terminates in a thin filament, which anchors the ovariole to the internal body wall and joins it to the basal oviduct. Ovarioles are made up of two major regions, the distal germarium and the proximal vitellarium.

Oogonia fill the apex of the germarium; oocytes are found in its basal portion (Soldán, 1979). Follicle cells surround oocytes undergoing gradual development in the vitellarium. The oocyte-follicular epithelial complex forms a single follicle, and a series of follicles increasing in size constitutes an ovariole.

In previtellogenic and early vitellogenic growth, the follicular epithelium surrounds the oocyte and is characterized by cuboidal cells bounded by a basal lamina (Fig. 1). Follicle cells have a large nucleus with dispersed chromatin. No specialized structures such as Golgi complexes and endoplasmic reticula are recognizable in the cytoplasm. Mitochondria are sparse, and the remaining cytoplasm is filled with free ribosomes. Freeze-fractured tissue reveals gap junctional plaques connecting follicle cells (Fig. 2). At the follicle cell-oocyte interface, the plasma membrane forms microvilli that interdigitate with those extending from the oocyte (Fig. 3). Between adjacent microvilli, coated pits adorned with a clathrin lattice-work occur. They extend into the cortical egg cytoplasm where clathrin-coated vesicles also are visible (Fig. 4).

Upon initiation of vitelline membrane deposition, the follicle cells become flattened, lose their microvilli, and become interconnected by septate junctions (Fig. 5). An intercellular space separates the plasma membrane of the oocyte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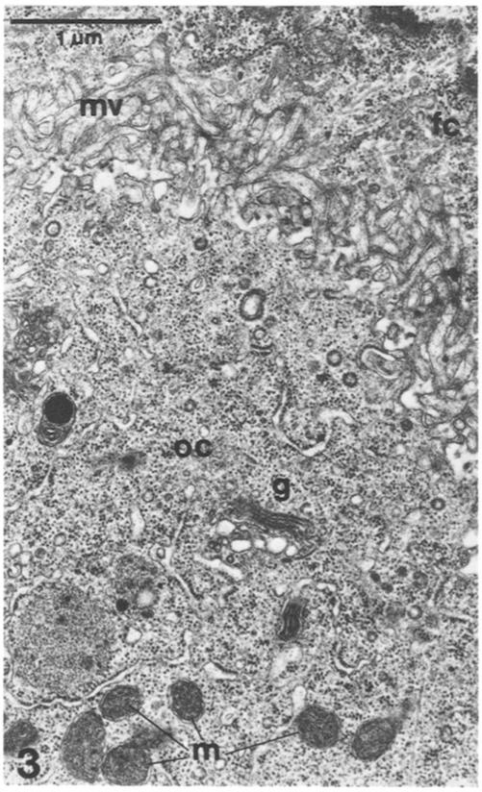
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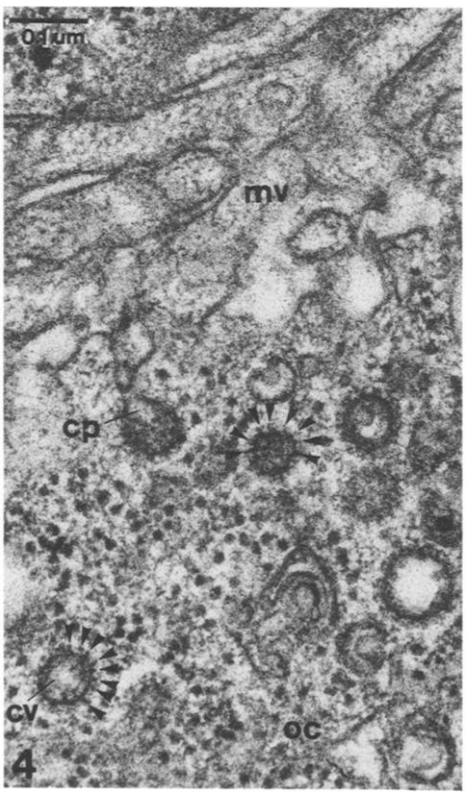
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from that of the follicular epithelium. In the follicle cells, the rough endoplasmic reticulum is abundant, and precursor material of the vitelline envelope is evident within their cytoplasm. Such material is released at the follicle cell-oocyte interface, where it coalesces into irregularly shaped plates that are first scattered and then organize to form a discontinuous sheet (Fig. 6). In this phase of their secretory activity, follicle cells present an elongated nucleus occupying a significant portion of the cytoplasm (Fig. 6). The vitelline membrane is formed by the coalescence of its electron-dense structural units. These fuse extensively to build up a continuous sheet in the intercellular space. During the sequence of events leading to vitelline membrane formation, follicle cells have at their apical ends narrow and discontinuous bands of electron-dense material (Fig. 7). This indicates the onset of chorionic membrane deposition. The portion adjacent to the vitelline plates is characterized by a periodic sequence of electron-dense units giving a maze-like configuration to the inner chorionic layer (Fig. 8).

In addition to this region, the fully formed chorion of *H. eldae* has two other superimposed layers, the intermediate and the outermost, which are organized differently. Indeed, in agreement with earlier observations, the chorionic surface has a pattern of ridges running along the major axis of the egg (Gaino & Mazzini, 1984). Each ridge is formed by columnar projections separated from each other by chambers (Mazzini & Gaino, 1985). The intermediate chorionic layer forms the central core of such columns (Fig. 9). As observed during vitelline membrane and inner chorionic layer deposition, precursor material of the intermediate chorionic layer is visible within the follicle cells as granules with a more electron-dense border and a microgranular core (Fig. 10). Following their release, granules fuse to form the particulate matter that fills the columns (Fig. 9). Microvilli extend from follicle cells toward the interposed space containing a granular ground matrix that is packed closely near the ridges (Figs. 9, 10). Microgranules forming this matrix are more dense around and near the apex of the microvilli, but the cortical cytoplasm also presents a granular appearance (Fig. 10). In the fully formed chorion, the intermediate layer supports the outermost electron-opaque sheet (Fig. 11) resulting from a continuous double-layered structure (Fig. 12). Even though the vitelline membrane has not yet formed a continuous envelope, most plates are fused (Fig. 11). Before ovulation, the mature egg is enveloped by a matrix of amorphous material filling the space between the follicular epithelium and egg surface (Fig. 11).

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FIG. 1. Follicle cells (fc) bounded by basement lamina (bl) and arranged around the oocyte (oc). FIG. 2. Follicle cell epithelium showing gap junctions (arrows) connecting follicle cells (fc); freeze-fracture. FIG. 3. Microvillar interdigitations (mv) at follicle cell (fc)/oocyte (oc) interface. FIG. 4. A coated pit (cp) and some coated vesicles (cv) with clathrate pattern (arrowheads) that penetrate within oocyte cytoplasm (oc). g, Golgi complex; m, mitochondria; mv, microvilli; n, follicle cell nucleus.

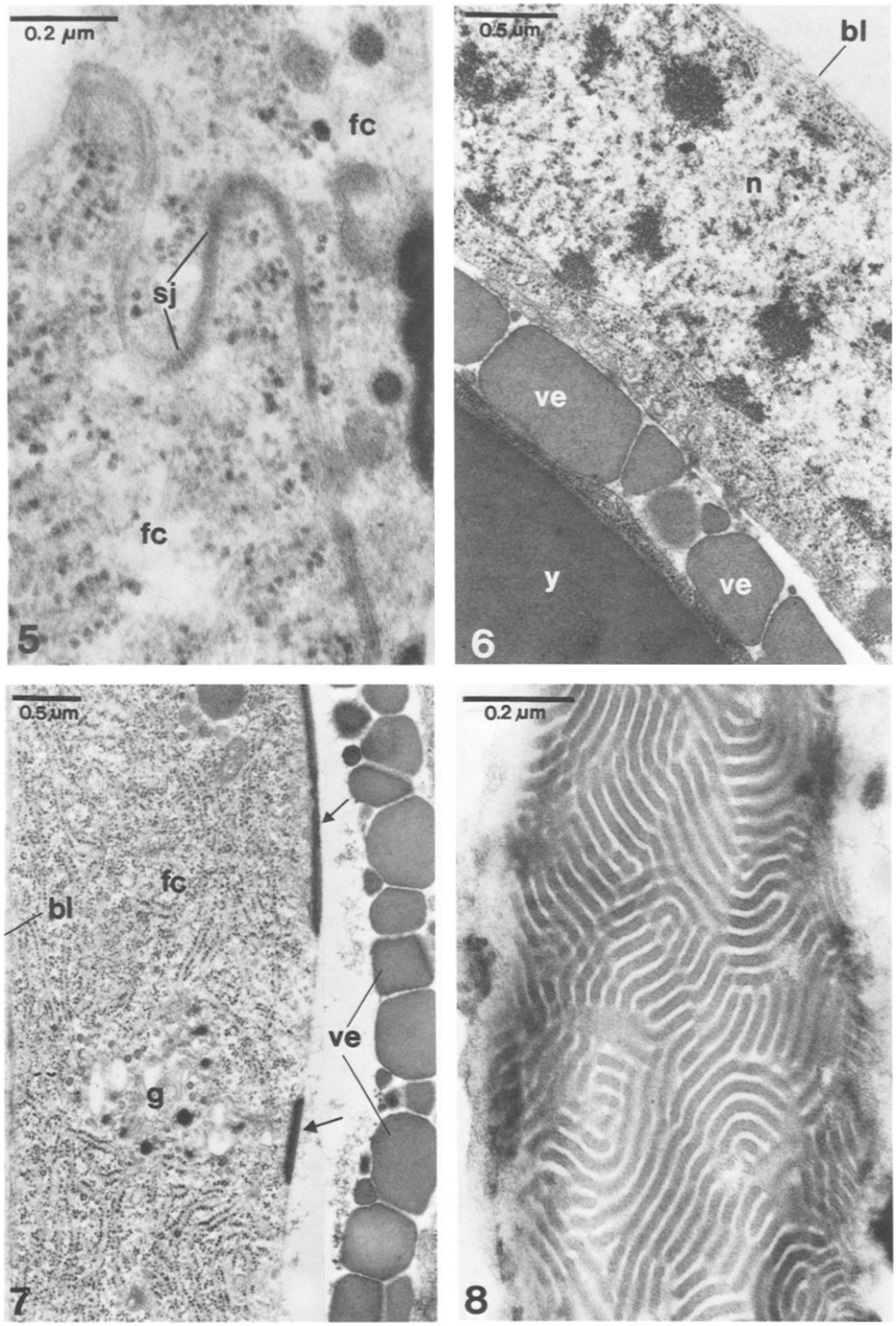

FIG. 5. A septate junction (sj) connecting follicle cells (fc). FIG. 6. Electron-dense plates which coalesce to form the vitelline envelope (ve). FIG. 7. Inner chorionic layer precursors (arrows)

DISCUSSION

In most insects investigated, the follicular epithelium is the source of vitelline and chorionic membrane deposition (Beams & Kessel, 1969; Favard-Séréno, 1971; King & Kich, 1963; Mathew & Rai, 1975; Norton & Vinson, 1982). During previtellogenic or early vitellogenic growth, follicle cells are cuboidal, tightly juxtaposed, and coupled. Heterologous coupling also has been recognized between follicle cells and oocytes (Huebner, 1981; Postlethwait & Giorgi, 1985) which are commonly interlocked by way of microvilli. Gap junctions should assure cell communication capable of coordinating both synthesis and release of precursor material that forms vitelline and chorionic membranes (Caveney & Berdan, 1982; Giorgi, 1977; Telfer, 1979). The involvement of gap junctions in regulation of endocytosis should not be disregarded. Indeed, heterologous coupling is assumed generally to have a nutritional role for the oocyte (Eppig, 1985) and to influence vitellogenesis (Mazzini & Giorgi, 1985). During early oocyte growth, the cortical ooplasm is enriched by coated pits and vesicles that assure the uptake of nutrients. The endocytic apparatus develops along tracts where follicle cells and oocytes are interlocked by way of microvilli. This interaction may be essential for the onset of vitellogenesis.

In Ephemeroptera, oogenesis takes place during the aquatic stages, which are the only trophic phases. In early vitellogenic growth, the eggs of *Habrophlebia eldae* are characterized by a complex endocytic apparatus. This is consistent with the assumption that also among ephemeropterans yolk deposition stimulates inception through the synthesis of some activating substances which are transferred to the egg by way of junctional complexes.

Cytoplasmic features observed in *H. eldae* indicate that mayfly follicle cells also are capable of egg envelope synthesis. The onset of vitelline and chorionic layer elaboration stimulates follicle flattening and their coupling by septate junctions. The extensive development of the endoplasmic reticulum, numerous Golgi elements, and secretory granules follow these transformations. A firmer connection between cells contributes to the organization of a thin epithelium around each growing egg.

Egg envelopes are derived from precursor material secreted within the cytoplasm and released at the follicle cell-oocyte interface. Within this space, the material accumulates and coalesces to form continuous and well-differentiated envelopes.

As observed in other insects (Anderson & Spielman, 1971; Beams & Kessel, 1969), precursor material of the vitelline membrane, in the form of spherical droplets, accumulates at the follicle cell-oocyte interface. Also, the inner chorion layer is organized early as a discontinuous sheet along the apical border of the follicle cells. Suggested as well for an ichneumonid parasitoid (Norton & Vinson, 1982; Rotheram, 1973), the maze-like configurations of the inner chorionic

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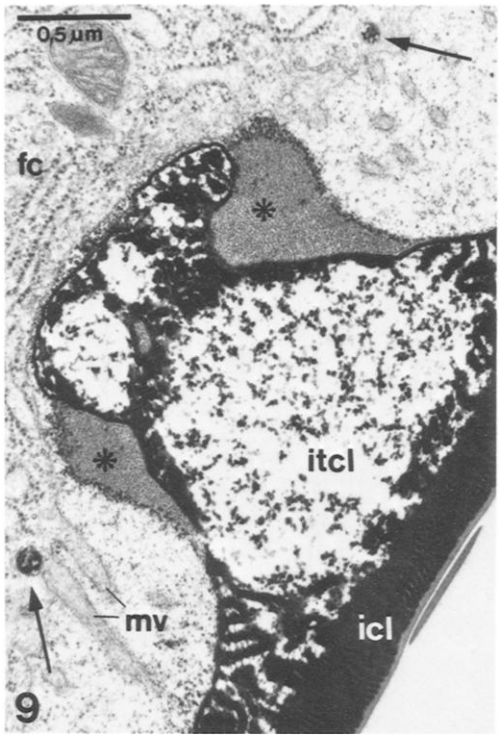
accumulate at the apical part of a follicle cell (fc). FIG. 8. Higher magnification of the inner chorionic layer showing its maze-like configuration. bl, basement lamina; g, Golgi complex; n, follicle cell nucleus; ve, vitelline envelope; y, yolk.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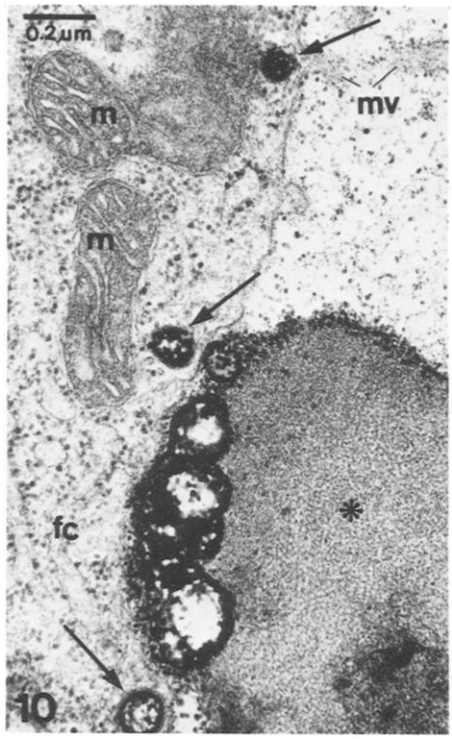
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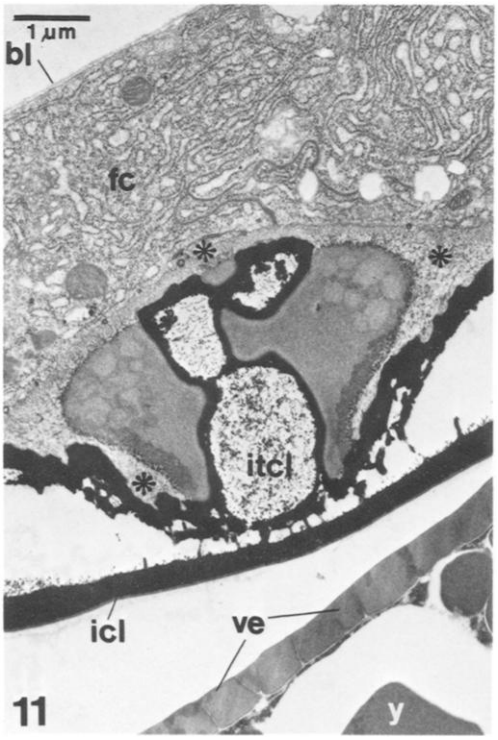
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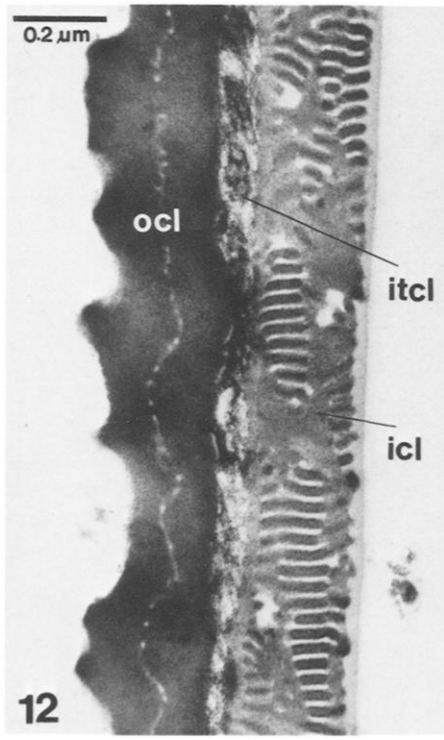
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layer may suggest a crystalline component. The superimposed intermediate chorion layer is derived from granules that accumulate and fuse in the follicle cell-oocyte space. During this process, the cortical cytoplasm of the follicle cells also is characterized by a microgranular matrix similar to that of the space surrounding the egg surface. This matrix becomes compact and is the primordium of the electron-dense mass close to the egg ridges. Consequently, the viscous material of *H. eldae,* seen to adhere to chorionic ridges and to fill their cavities (Mazzini & Gaino, 1985) arises from an active synthesis of follicle cells and represents, therefore, a true chorionic component. Mucous envelopes typically cover the eggshell surface in Ephemeroptera as well as in other insect groups (Fehrenbach et al., 1987). However, mayflies lack accessory glands, and our data provide evidence for the role of the follicle cells in adhesive layer synthesis as was supposed by Koss (1970).

Even though egg envelope secretion takes place gradually within each follicle, the organization of the egg envelopes probably continues after ovulation. In fact, within the follicle, when the chorionic layers already are arranged around the egg surface, the units of the vitelline envelope are not yet fused completely. Comparing these eggs with those dissected from oviducts (Mazzini & Gaino, 1985, 1988), morphological and structural changes evidently occur in the fine texture of the egg envelopes during egg passage down the oviducts; i.e., the vitelline membrane forms a continuous band and the intermediate chorionic layer is less electron-dense, showing a more homogeneous aspect.

Structural changes seem consistent with the protein nature of the chorion (Furneaux, 1970; Furneaux & Mackay, 1972; Margaritis et al., 1980; Rotheram, 1973). By contrast, Margaritis et al. (1980) considered that the fine organization of vitelline plates is suggestive of a hydrophobic nature.

The peculiar feature of the fully developed egg membranes is consistent with their different roles; i.e., the vitelline envelope seems to be involved with controlling egg-shell permeability (Telfer & Smith, 1970), whereas the chorion performs mostly a protective function (Hartley, 1961; King et al., 1968). The presence of interstices within the chorionic envelopes perhaps facilitates respiratory exchange during embryogenesis.

Follicle cells also are able to synthesize complex chorionic sculptures whose specific patterns may be utilized for taxonomic purposes, as emphasized in several studies of chorionic organization among many insect orders (see review

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FIG. 9. Follicle cell (fc) surrounding a chorionic columnar projection. Note microvilli (mv) protruding from cell border and secretory granules (arrows). The intermediate chorionic layer (itcl) forms the central core of the column. Closely applied to both sides of the column is densely packed microgranular material (asterisks). FIG. 10. Follicle cells (fc) showing the release of secretory granules (arrows) that coalesce to form the intermediate chorionic layer. Asterisk indicates the densely packed granular material. FIG. 11. Before ovulation, the space between egg surface and follicle cell (fc) is filled by an amorphous granular matrix (asterisks). FIG. 12. Higher magnification of the egg surface in a region without columns, showing the three-layered structure of the chorion. bl, basement lamina; icl, inner chorionic layer; itcl, intermediate chorionic layer; m, mitochondria; mv, microvilli; ocl, outer bistratified chorionic layer; ve, vitelline envelope; y, yolk.

by Hinton, 1981). The taxonomic value of chorionic sculpturing has been indicated for Ephemeroptera by scanning electron microscopical observations (Alba-Tercedor & Sowa, 1987; Flowers, 1986; Gaino & Mazzini, 1984; Gaino et al., 1987, 1989; Malzacher, 1982, 1986; Sowa & Soldán, 1986). Ultrastructural analyses also demonstrated the adhesive function of peculiar chorionic attachment structures that prevent eggs from being swept away following their deposition in water (Gaino & Mazzini, 1987, 1988). By contrast, *H. eldae* lacks differentiated chorionic attachment structures, but mucous material stored within the ridges might accomplish the same function owing to its hydrophilic nature. Perhaps highly specialized egg envelopes, then, found mostly among species ovipositing in water, constitute a significant device for attaching eggs following oviposition.

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From the President: A Crisis and an Appeal

I forego the traditional "From the President" in order to alert you to a crisis in a sister institution, Britain's Natural History Museum, London (formerly British Museum, Natural History). Major alterations of the Museum's scientific activities and elimination of major areas of research are proposed. Externally, the Museum faces a lack of government funding. Internally, it faces a failure to recognize the importance of collection-based research. Research will be concentrated in the areas of biodiversity, environmental quality, living resources, mineral resources, human health, and human origins. Fundamental taxonomic research will be radically reduced.

In an institution that will study biodiversity, it seems contradictory that research will be (or already has been) discontinued on a diverse spectrum of biota: recent and fossil mammals, testate amoebae, sponges, diatoms, bryophytes, fossil plants and birds, modern bees and wasps, hemipterans, modern birds, spiders, coelenterates, bryozoa, echinoderms, and annelids. Such severe cutbacks will have a significant impact upon the research activities of many members of the American Microscopical Society. However, this scenario remains to be reviewed by the Office of Arts and Libraries. A thoughtful letter from you will help the Museum staff in their efforts to provide continued excellent care of collections and to continue research for which the collections form a requisite basis. Please write to: The Rt. Hon. Richard Luce, M.P., Office of Arts and Libraries, Horse Guards Road, London SW1P 3AL, United Kingdom.—FREDERICK W. HARRISON, President, American Microscopical Society.