27 Ultrastructural organization of the fat body in three species of mayfly (Ephemeroptera)

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*The fat body structure of larvae of three mayfly species (*Ecdyonurus *gr.* venosus, Choroterpes picteti *and* Ephemera danica*), was compared using light and electron microscopy.*

Scanning electron microscopy (SEM), indicated that the fat body of young larvae was constituted of several cell ribbons coiled together. These are turned into flat lobules in mature nymphs, as observed in Ecdyonurus *gr.* venosus. *Fat body was seen to be covered by a continuous sheet, the basal membrane, that kept the cells in place.*

The main function of the fat body as a storage tissue was evident at transmission electron microscopy (TEM) level by the large amount of lipid vacuoles. These appeared first as droplets that coalesced into large globules, leaving little space for the cytoplasmic matrix. Indeed, during lipid accumulation the cytoplasm was reduced to narrow strands interposed among lipid vacuoles. Owing to this storage process, even the nuclei were pressed aside. Storage activity was associated the typical organization of a secretory tissue. Much of the cell cytoplasm consisted of packed free ribosomes, Golgi complexes and variously shaped electron-dense bodies. The rough endoplasmic reticulum (RER) was extensively developed, showing elements of different length that exhibited in E. *gr.* venosus *a characteristic arrangement in rows of concentric circles.*

The great numbers of cytosegregosomes visible when nymphs of Ephemera danica *were near emergence was good evidence for autophagocytic events and supported the interpretation that in Ephemeroptera the fat body may also play an important role in providing an energy reserve for metabolism of adults, which do not feed.*

Introduction

The fat body of insects is an organ of great interest owing to the ability of its cells to perform a wide range of functions. These include several aspects of the synthesis, storage and metabolic regulation of lipids, nitrogenous compounds, sugars and proteins (Wyatt 1980, Cochran 1985, Hyatt and Marshall 1985, Wigglesworth 1987). As a consequence, the fat body cells are able to provide the successive needs related to growth, metamorphosis, dispersal and reproduction. The role of the fat body changes during the life span of the insects (Thomsen and Thomsen 1974), allowing a turnover of components related to uptake from haemolymph, storage inside the cells and subsequent mobilization and release of reserves into it.

Among the various functions performed by this organ, protein secretion represents the central metabolic activity, including the production of larval serum proteins (Wolfe et al. 1977), lipophorin (Gilbert et al. 1977), haemoglobin (Bergstrom et al. 1976) and vitellogenin (Hagedorn and Kunkel 1979). This secretory pathway has been recently reconstructed at the ultrastructural and immunocytochemical level in stick insects (Mazzini and Giorgi 1986, Mazzini et al. 1985, 1989).

In addition, the term "fat body" is restrictive in view of the organ's probable involvement in the acquisition of immunity (Faye et al. 1975).

Although fat body organization and function have been examined in several groups of insects, no papers have been devoted to the investigation of this organ in the Ephemeroptera. The present paper represents a first approach to studying the fine detail of fat body organization in such insects, with an eye to the role that it could play in these animals characterized by a feeding stage limited to the aquatic phases and by a very short adult life.

Materials and Methods

Specimens of Heptageniidae (*Ecdyonurus* group *venosus*), Leptophlebiidae (*Choroterpes picteti*) and Ephemeridae (*Ephemera danica*) were studied for the present paper. After the head was cut off, the fat body was removed from the abdomen of larvae and nymphs.

For scanning electron microscopy (SEM) investigation, selected material was immediately fixed for one hour at 4°C in 0.1 M cacodylate buffer at pH 7.2, containing 5 per cent glutaraldehyde and 4 per cent formaldehyde fixative. Fat body fragments were then washed overnight in the same buffer, dehydrated in a graded series of alcohols and critical point dried by the critical point method in a Bomar apparatus equipped with a liquid $C0_2$ inlet. Material was attached to specimen

holders using a silver conducting paint, coated with gold in a Balzers evaporator, and observed with a Philips SEM 515.

For transmission electron microscopy (TEM), fat body fragments were fixed in glutaraldehyde-formaldehyde as above, washed in cacodylate buffer and post-fixed for one hour in 1 per cent osmium tetroxide in 0.1 M cacodylate buffer at pH 7.2. After dehydration in a graded series of alcohols, fat body tissue was embedded in an Epon-Araldite mixture and polymerized at 60°C for three days. Thin sections were collected on formvar coated grids, stained with uranyl acetate and lead citrate and examined in a Jeol JEM 1200 EX II electron microscope.

Results

Fat body is located in the haemocoel, at each side of the alimentary canal, uniformly bathed by the haemolymph, and closely apposed to the abdominal walls. Scanning electron microscopy (SEM) revealed that in *Ecdyonurus* gr. *venosus* the fat body consists of many cellular ribbons coiled together (Fig. 1) to form a cylindrical structure in young larvae, or organized in more flattened lobules in mature nymphs (Fig. 2). Tracheoles, derived from the tracheal trunk, enter the fat body and in female specimens this organ is closely associated to ovarioles. The most outstanding feature of the fat body is the many bulges that characterize its outermost surface (Fig. 3).

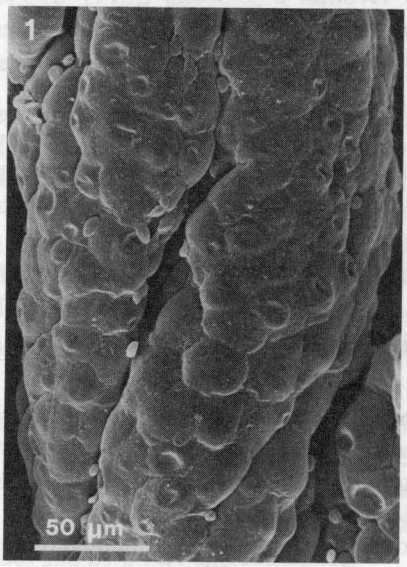
At the light microscopy level the fat body appears to consist of a clump of cells whose most striking morphology is related to the great number of lipid droplets separated from each other by thin cytoplasmic strands (Fig. 4). Cells are morphologically uniform and nearly spherical. Their nuclei, with a large amount of heterochromatin, are reduced in size and, owing to lipid droplet accumulation, are pressed aside.

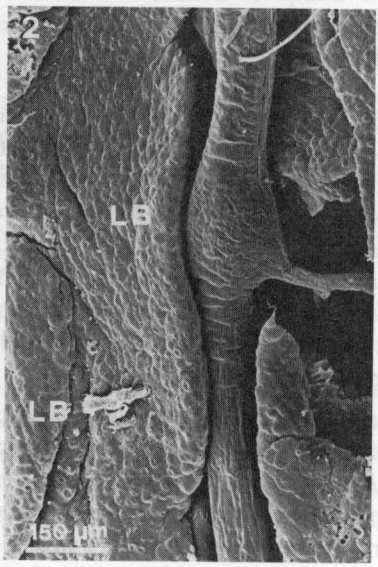
The striking contrast between lipid and cytoplasm volumes is particularly evident in appropriately cut fat body samples observed under SEM, which also reveals the presence of a uniform peripheral envelope covering the fat body (Fig. 5).

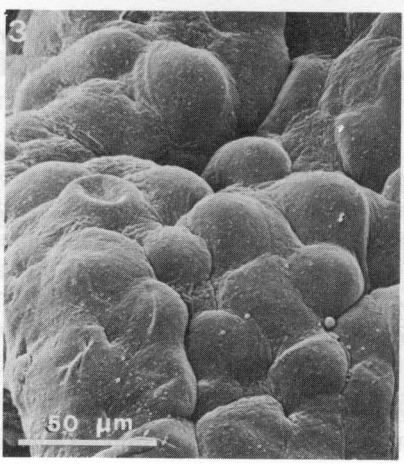
In *Ephemera danica*, transmission electron microscopy (TEM) investigations revealed that the covering is constituted of a basement membrane that keeps fat body cells in place (Fig. 6). The cell surface is increased by plasma membrane invaginations that penetrate inwards forming a reticular system on faces that are exposed to the haemolymph (Fig. 6). Cytoplasm is squeezed between a number of lipid globules (Fig. 7), which present a wide dimensional range as evident in *E.* gr.*venosus* (Fig. 8).

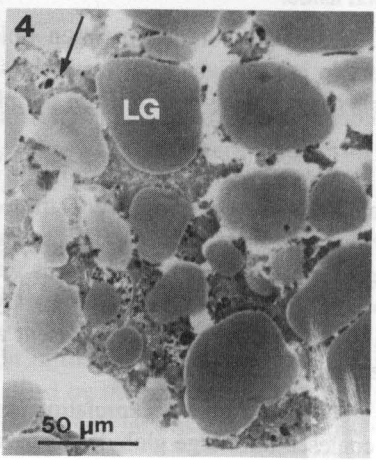
The onset of lipid accumulation is revealed by droplets of a few μm diameter that coalesce into large globules of over 60 μm in diameter with smooth contours

Figures 1-4. **SEM micrographs of the fat body of *Ecdyonurus* gr. *venosus* (1-3) showing: 1. cells organized in ribbons coiled together in a young larva; 2. flattened lobules (LB) of a mature nymph; 3. outermost surface irregularly protruding towards the haemolymph. Light micrograph (4) of an Epon-Araldite embedded semi-thin section of a fat body fragment of a female nymph of *Choroterpes picteti*. Note the large number of lipid globules (LG) of different sizes filling the cytoplasm and a small nucleus pressed aside (arrow).**

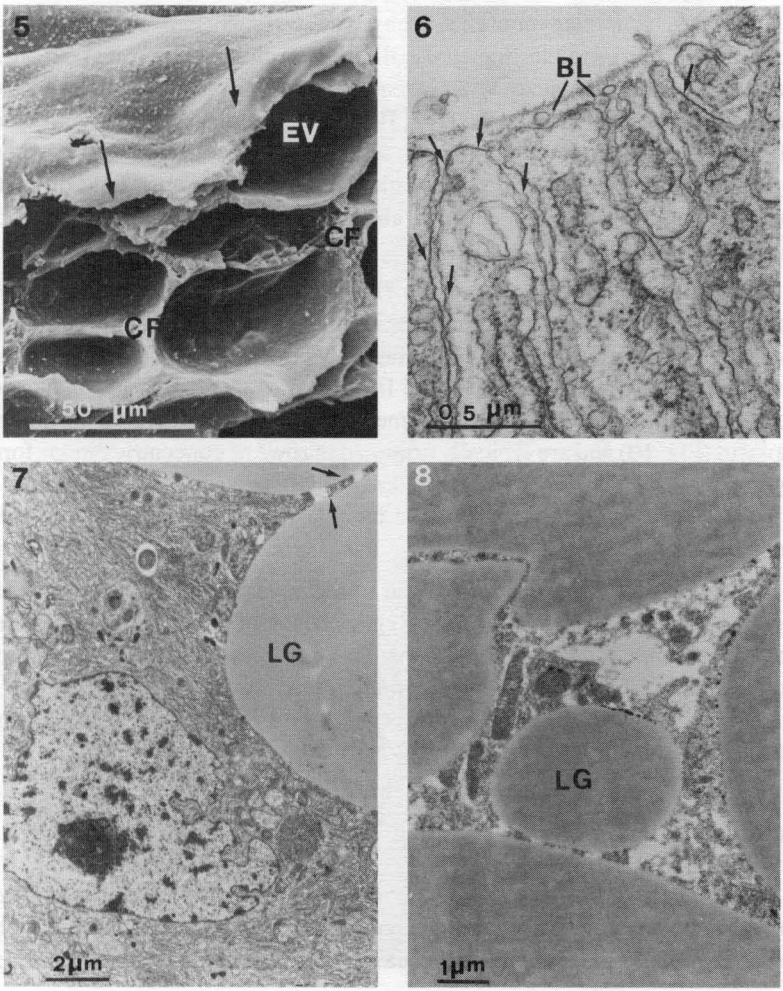








Figures 5-8. **5. SEM view of a fractured fat body of a mature nymph of *Ecdyonurus* gr. *venosus* enwrapped by a uniform covering (arrows). Empty vesicles (EV) indicate the extracted lipids separated by thin cytoplasmic fringes (CF). 6-8. Fat body of a young nymph of *Ephemera danica* (6,7) and of *Ecdyonurus* gr. *venosus* (8) seen at TEM. 6. the apical end of a cell showing the plasma membrane reticular system (arrows) with the superimposed basement lamina (BL). 7. lipid globules (LG) separated by a thin cytoplasm fringe (arrows). Note the irregular peripheral border of the nucleus and the packed chromatin. 8. detail of a cytoplasmic fringe squeezed by lipid globule accumulation (LG).**



outlined by profiles of endoplasmic reticulum. The edge of the lipids may be an interface or a thicker, denser layer, but there is no unit membrane. Lipids are easily extracted during the procedures for ultrastructural investigations and this fact, together with the lack of any affinity of saturated lipids for osmium fixative, gives rise to empty vesicles that may also include some drops of unsaturated lipids (Fig. 9). These latter are concentrated peripherally or variously scattered.

Nuclei are small and present packed chromatin scattered in a homogeneous matrix (Fig. 7). In *E.* gr. *venosus* the nucleolus is scarcely present but in *Choroterpes picteti* and in *Ephemera danica* nuclei are typically nucleolated. In *E. danica*, in particular, the nuclei are larger than in the other species and show an irregular peripheral border with some protrusions towards the cytoplasm (Fig. 7).

The cytoplasm also contains many variously shaped mitochondria and membrane-bounded protein bodies usually dispersed in the cytoplasm (Fig. 10). Occasionally, Golgi complexes with flattened cisternae flanked by secretory vacuoles with electron-dense material occur (Fig. 10). Golgi complexes are generally located in the more peripheral region of the cells, sometimes occupying the cortical cytoplasm just beneath the basement lamina. Indeed, in this area, lipid droplets are reduced in size or only rarely present, allowing mitochondria and electron-dense granule accumulation.

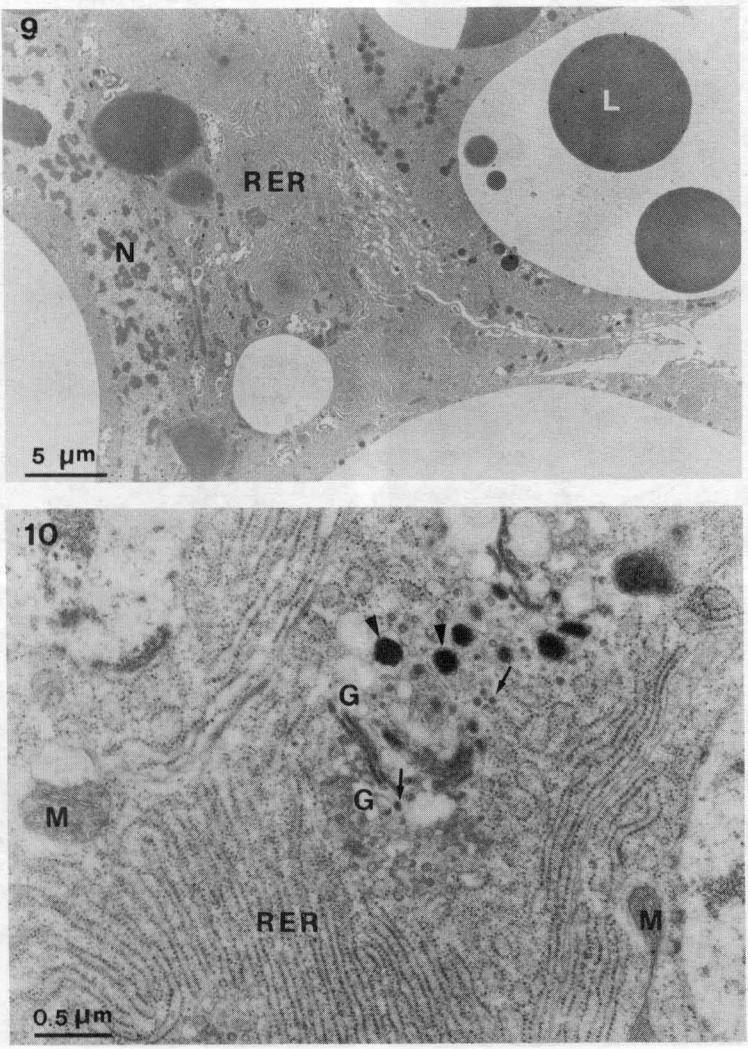
The rough endoplasmic reticulum (RER) is highly developed (Figs. 9,10), forming a discrete structure between the lipid vacuoles and protein bodies. Cells delimiting the outermost surface of the fat body present a more linear arrangement of the RER that develops as sequential linear arrays located perpendicular to the cell surface. In *E.* gr. *venosus*, RER elements occupy a considerable portion of the cytoplasm (Fig. 10) and are typically arranged in rows of concentric circles forming "finger print" islands (Fig. 11), occasionally associated with lipid droplets (Fig. 12). In this species a major part of the cytoplasm contains densely packed free ribosomes.

In *E. danica*, in particular, at the end of the aquatic phase of its life cycle, the cytoplasm is greatly enriched by cytosegregosomes, visible as vesicles with cisternae of the endoplasmic reticulum trapped inside. Vesicles consist of an electron-dense granular component associated with a membrane-like system, recalling the RER (Fig. 13), or show a more composite organization resulting in a heterogeneous matrix and membrane accumulation (Fig. 14).

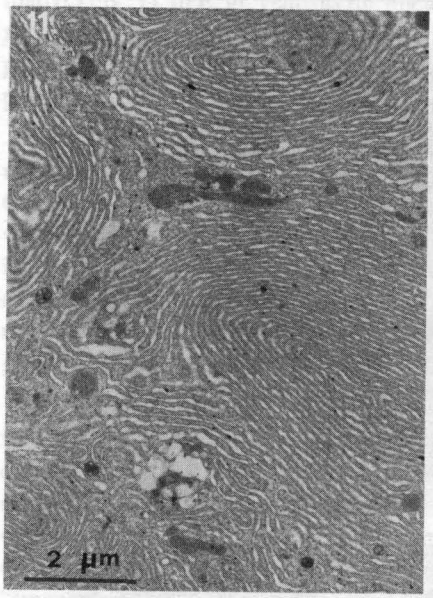
Discussion

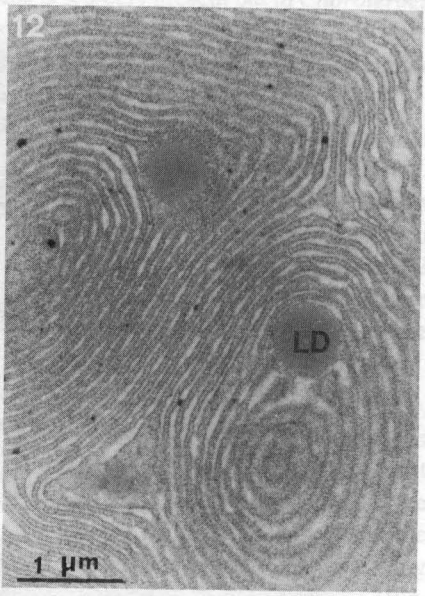
The current state of knowledge demonstrates that fat body cells are mainly involved in lipid accumulation and this process leads to an increase in cell volume. Lipid storage takes place during the aquatic stages of the life-span, the only phase

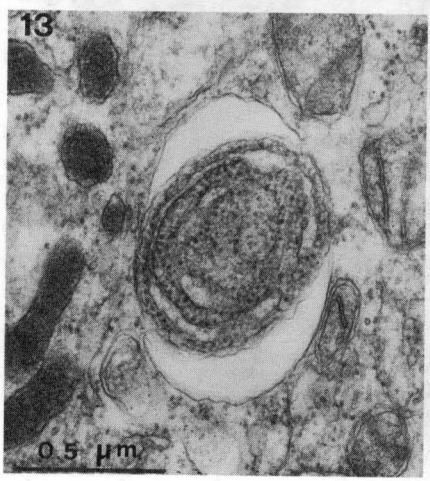
Figures 9-10. 9. Fat body cell organization in a mature nymph of *Ecdyonurus* gr. *venosus*. Note the widespread development of the rough endoplasmic reticulum (RER) located close to the nucleus (N) and among partially extracted lipid components (L). 10. Nymph of *Choroterpes picteti*. Note Golgi complexes (G) flanked by electron-dense material (arrows), protein bodies (arrowheads), mitochondria (M) and rough endoplasmic reticulum (RER).

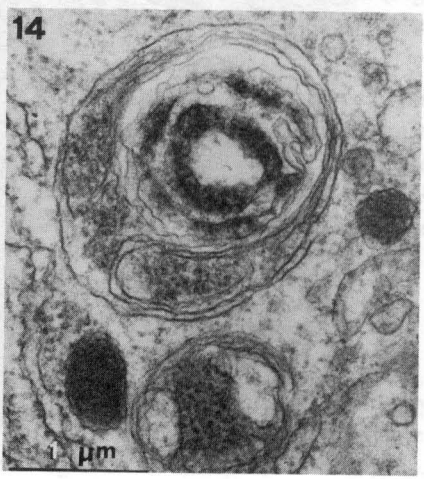


Figures 11-14. 11-12. Detail of rough endoplasmic reticulum (RER) in *Ecdyonurus* gr. *venosus*. 11. arrangement in concentric rows. 12. lipid droplets (LD) associated with RER. 13-14. Cytosegregosomes in a mature *Ephemera danica* nymph near emergence. 13. granular component associated with membrane-like system. 14. co-existence of smooth membranes and electron-dense heterogeneous components.









during which feeding occurs. Lipids are first accumulated within the cytoplasm as droplets that coalesce, giving rise to large lipid globules that permeate the cytoplasm. Lipids are a constant component of the fat body, and even their mobilization does not change the peculiarity of the tissue, whose cells conserve a high volume.

This process is accompanied by the synthesis and accumulation of dense bodies whose electron-density is in agreement with their proteinaceous nature. It is known that in the fat body the metabolism of several secretory proteins involves rough endoplasmic reticulum (Lauverjat 1977, Stoppie et al. 1981, Raikhel and Lea 1983) and post-translational modification in the Golgi apparatus (Thomsen et al. 1980, Minoo and Postlethwait 1985). In this sense, our ultrastructural observations are in line with what might be expected. At the TEM level, the protein components show a typical electron-density. Recently, such electron-dense granules were identified as vitellogenin precursors (Raikhel et al. 1986, Raikhel 1987, Mazzini et al. 1989), thus emphasizing the role of the fat body during egg maturation.

Structural and metabolic modifications have been described in the fat body of several insect groups in connection with the life cycle (Locke 1986, Willott et al. 1988, Maurizii et al. 1992), with reproduction (Wuest 1978) and under experimental conditions (Wigglesworth 1982). The uptake and modification of components from the haemolymph and, conversely, the mobilization and subsequent release of metabolic products into the haemolymph represent the typical turnover of a secretory and storage tissue. As a consequence, the fat body plays a key role in intermediary metabolism controlling molting (Locke 1980, Marx, 1983) and maintaining ion balance during water deprivation by urate crystals (Hyatt and Marshall 1985).

Our preliminary observations have demonstrated some peculiarities in the ultrastructural organization of the fat body cells that allow us to envisage an initial phase of rapid synthesis followed by protein and lipid accumulation. In addition, the morphology of several cytoplasmic vacuoles also suggests an autophagic activity that allows cells to re-use endogenous components as well as lipids. This process is particularly evident in those mature nymphs that are about to emerge, thus strengthening the ability of the fat body to elicit metabolic changes during the mayfly's life-span.

It is worth stressing that the switch in metabolic activity from accumulation to ready availability is associated with the last feeding stage of the life cycle. As a consequence, it seems acceptable to consider that in mayflies the fat body actually represents an important source of energy to satisfy the demands of flying and mating, in the absence of any uptake of nutrients from the environment. In addition, it is well known that lipid storage represents a fundamental system to obtain water through classical metabolic pathways. Therefore, the fat body, thanks to its large amount of lipids, could also reduce the rate of dehydration of these insects after emergence, thereby improving their survival during adult life.

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