Ultrastructure of the Pleuropodium in 8-d-old Embryos of *Thermobia domestica* (Packard) (Insecta, Zygentoma)

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ABSTRACT Pleuropodia of the invaginated type were observed on the first abdominal segment in 8-d-old embryos of *Thermobia domestica* (Packard). The pleuropodium is formed by a cytoplasmatic internal part and a mushroom-like cavity. The latter is filled with fluid and is composed of a stem protruding through the epidermis and a vesicle-like copula. The arrangement of membrane folds, mitochondria, and lipid drops was observed on electron micrographs (TEM) of pleuropodium cells. The position and structure of these organelles indicates that the cells of this organ perform transport and secretory functions.

KEY WORDS *Thermobia domestica*, pleuropodium, embryogenesis, active transport, secretion

DURING EMBRYONIC DEVELOPMENT of many insects, paired formations of ectodermal origin appear on pleural walls of the first abdominal segment. They are called pleuropodia and are believed to be homologues of abdominal legs (Wheeler 1890). Rathke (1844) described pleuropodia in *Gryllotalpa vulgaris* L. (Orthoptera) as "mushroom-like bodies." However, their definition, description of structure, and differentiation come from Wheeler (1890), who classified pleuropodia into two types: evaginated, where nuclei are placed above the ectoderm surface, and invaginated, where nuclei of this organ lie below the epidermis line. The first occurs, e.g., in *G. vulgaris* (Rathke 1844, Graber 1888), *Opistoplatia orientalis* Burmeister (Blattaria) (Ando 1971), and *Oligotoma japonica* Okajima (Embioptera) (Ando and Haga 1974), and originates as a protrusion of ectodermal cells from the body or a tightening of apical parts of the first abdominal segment appendage. Invaginated pleuropodia, occurring in *Bactridothrips brevitubus* Takahashi (Thysanoptera) (Ando and Haga 1974), *Epiophlebia superstes* Selys (Odonata), *Gomphus pryeri* Selys (Odonata) (Ando 1953), *Belostoma flumineum* Say (Hemiptera), *Ranatra fusca* Palisot de Beauvois (Hemiptera) (Hussey 1927), and *Carausius morosus* Brunner (Phasmidae) (Louvet and Marcel 1969), develop as a result of the insertion of elongating ectodermal cells into the body, while cytoplasmic projections of these cells form a mushroom-like structure protruding to the outside. Thus, in both evaginated and invaginated pleuropodia, cytoplasm of the pleuropodium cells fills the external copula of the organ formed from embryonic cuticle. However, in many insect embryos, such as *Panorpa pryeri* MachLachlan (Mecoptera), *Bittacus mastrillii* Navas (Mecoptera) (Ando and Haga 1974), and *Apis mellifica* L. (Hymenoptera) (Grassi 1884, Wheeler 1890), no pleuropodia are found. In some insects (e.g., Dermaptera), pleuropodia disappear just after their formation. In others (e.g., Odonata and Plecoptera), they invaginate into the body and develop into a secretory organ that is resorbed at the end of embryogenesis (Schwalm 1988). Using light microscopy, Woodland (1957) described the pleuropodia in *Thermobia domestica* Packard (Zygentoma) and *Ctenolepisma lineata* F. (Zygentoma) as paired, spherical vesicles surrounded by cells of mesodermal origin. The vesicles protrude above the ectodermal cells of the organ. Pleuropodia of *T. domestica* appear at stage J of embryogenesis. They become cylindric organs that lay perpendicular to the body wall. Apical parts of pleuropodium cells, forming long processes, protrude into the epidermis. In that way, the mushroom-like structure is formed. At stage L, the pleuropodium cells withdraw, and the organ degenerates so that at the end of stage M (6.5–10 d of embryogenesis), the pleuropodia are completely resorbed (Woodland 1957).

Here we present evidence that contradicts the above results (Woodland 1957) on the structure of the pleuropodium in 8-d-old embryos of *T. domestica*, because the organ is fully formed at this developmental stage. The function of pleuropodia in embryonic development of insects is not known. However, detailed data indicating the probable function of this organ in *T. domestica* were obtained through analysis of electron micrographs and by performing histochemical assays.

Materials and Methods

Adult specimens of *T. domestica* were kept under laboratory conditions at 37°C and 80% RH (Klag 1971). Eggs were incubated at 37°C. Eight-day-old embryos

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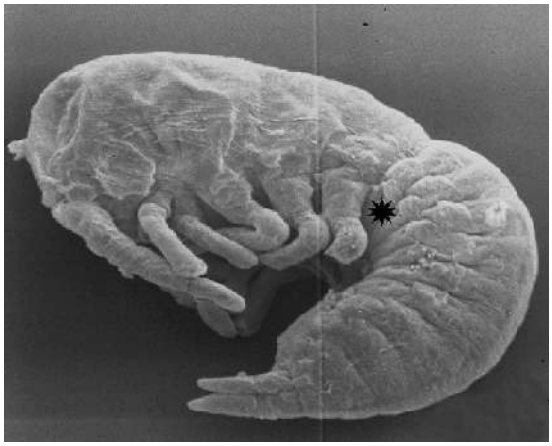

Fig. 1. Eight-day-old embryo of *T. domestica.* *Location of pleuropodium on the first abdominal segment. SEM, bar = 16.5 μm.

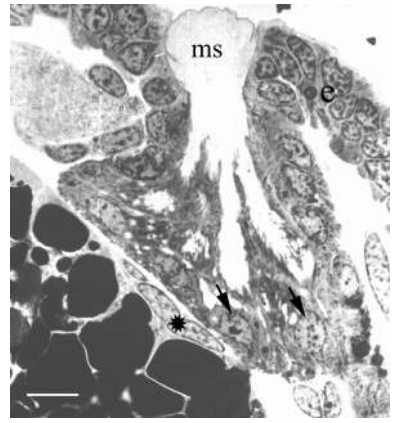

Fig. 2. Longitudinal section through the pleuropodium: mushroom-like cavity (ms), nuclei of pleuropodium cells (arrows), epidermis (e), mesodermal cells (*). Light microscope, bar = 11.5 μm.

of *T. domestica* isolated from the egg capsule were used as the experimental material. Embryos were fixed with 2.5% glutaraldehyde (2 h) and postfixed with 2% OsO_4 (1.5 h). Fixed specimens were dehydrated in a graded series of alcohols (50, 70%, 90, 95, and 100%, each for 15 min) and acetone (15 min) and embedded in Epon 812 (SERVA, Warsaw, Poland). Semi- and ultrathin sections were cut with a Leica Ultracut UCT25 ultramicrotome (Reichert Service, Poznan, Poland). Semithin sections were stained with 1% methylene blue in 0.5% borax (Dykstra 1992) and observed with an Olympus BX60 light microscope (Olympus, Warsaw, Poland). They were also treated with a 2% solution of periodic acid, to remove osmium, and were stained with periodic acid-Schiff (PAS) to localize polysaccharides (Litwin 1985).

Ultrathin sections were stained with uranyl acetate and lead citrate (Reynolds 1963) and examined with a Hitachi H500 transmission electron microscope (Comef, Katowice, Poland) at 75 kV. Some embryos, after fixing with 2.5% glutaraldehyde (2 h), postfixing with 2% OsO_4 (1.5 h), and dehydration in a graded series of alcohols (30, 50, 70, 80, 90, 95, and 100%, each for 10 min) and acetone (10 min), were dried at critical point Pelco CPD2 (Reichert Service, Poznan, Poland) and coated with gold in a Pelco SC-6 duster (Reichert Service, Poznan, Poland). These preparations were examined with a Tesla BS340 scanning electron microscope (SEM; Comef, Katowice, Poland).

Results

Embryonic development of *T. domestica* lasts 14 d at 37°C. Pleuropodia of embryos in the eighth day of embryogenesis (stage M, following Woodland 1957) form small vesicles located symmetrically on side surfaces of the first abdominal segment sternite (Fig. 1). They are formed from elongated cells of ectodermal origin surrounded by a sheath of mesodermal cells on the side of the body cavity (Figs. 2–4). The pleuropodium in this stage of embryogenesis has dimensions of 70 μm in height and 80 μm of width in the basal part of the organ.

The pleuropodium of *T. domestica* is of the invaginated type and is formed by an epithelial internal part and a mushroom-like cavity filled with fluid protruding to the outside (Figs. 2, 4, and 5). The cavity is composed of a stem, protruding through the epidermis, and a vesicle-like external copula. The stem and external vesicle are surrounded by embryonic cuticle (Figs. 5 and 6). The substance filling the cavity is PAS-positive. Polysaccharides are released by exocytosis into the mushroom-like cavity. In the stem area, copula cuticle joins the cuticle covering the epidermis and pushes under it as far as the mesodermal cells. The latter separate the apical parts of pleuropodium cells from the epidermal collar surrounding the vesicle stem (Fig. 4).

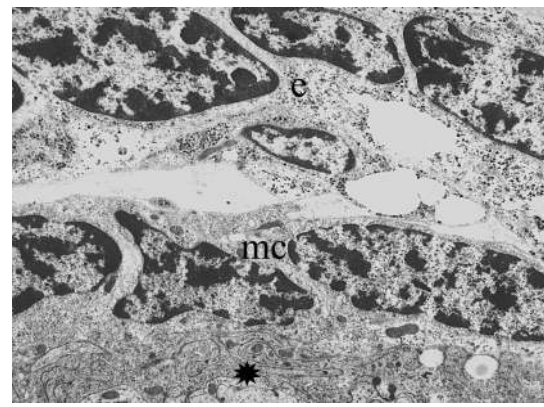

Fig. 3. A fragment of transverse section through the 8-d-old embryo of *T. domestica.* Epidermis (e) and mesodermal cells (mc) surround the pleuropodium (*). TEM, bar = 1.5 μm.

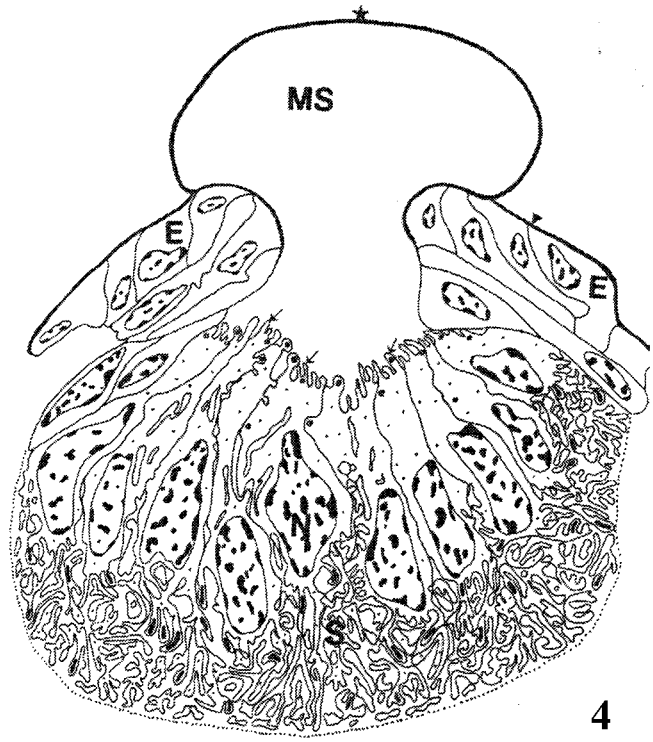

Fig. 4. A diagrammatic representation of pleuropodium: epidermal cells (e), mushroom-like cavity (ms), nuclei of pleuropodium cells (n), "spongy cytoplasm" (s), microvilli (arrows), embryonic cuticle of epidermis (arrowhead), and cuticle of mushroom-like cavity (*).

The internal epithelial part of the pleuropodium is limited by the basal lamina supporting cells of this organ. On the side of the body cavity, the pleuropodium (with basal lamina) is enclosed by a sheath formed by a layer of flat mesodermal cells (Figs. 2 and 3). Pleuropodium cells are elongated and have lobular nuclei located at their mid-height. The nuclei contain condensed chromatin near the nuclear envelope (Figs. 7 and 8). In the basal parts of these cells the cellular membrane invaginates into the cytoplasm, forming many folds and pockets. The invaginations reach as high as the middle of the nuclei or even higher. In the basal part of pleuropodium cells, the cytoplasm forms the so-called "spongy cytoplasm" (Figs. 4 and 7). Cell membrane folds adjacent to the basal lamina form numerous pockets, usually containing long mitochondria (Figs. 7 and 8). Thus, these membrane pockets appear as elongated, finger-like structures. Outside the area of "spongy cytoplasm," where membrane folds are small, mitochondria are most often oval, and single membrane pockets have a bulbous shape. In apical parts of the cells, invaginations of membranes are less extensive (Fig. 9). Mitochondria are most often oval shaped and occur sporadically as in the central part of each cell (Fig. 8). However, in the apical parts of these cells, a large number of mitochondria are sometimes observed (Fig. 9), as in the basal region (Fig. 7). Apical parts of pleuropodium cells have short, sparsely distributed microvilli protruding into the noncellular cavity of the organ (Fig. 4).

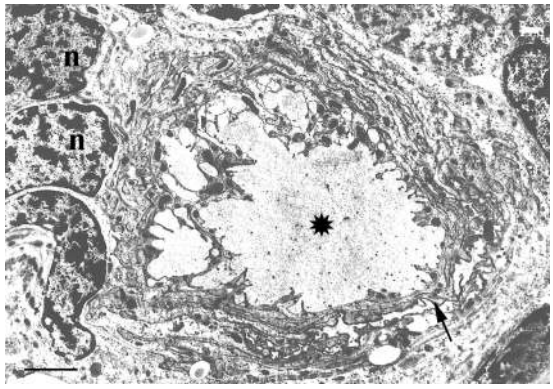

Fig. 5. A transverse section through the pleuropodium with nuclei (n) in the basal cells, apical part of pleuropodium surrounded by cuticle (arrow), and mushroom-like cavity (*). TEM, bar = 2.0 μm.

Ribosomes and glycogen show polarization in their spatial arrangement between basal and apical parts of pleuropodium cells. Most ribosomes are observed near the nucleus in the area of "spongy cytoplasm," where they occur singularly or as polyribosomes attached to rough endoplasmic reticulum (RER). RER is present throughout the cell from the basal lamina to

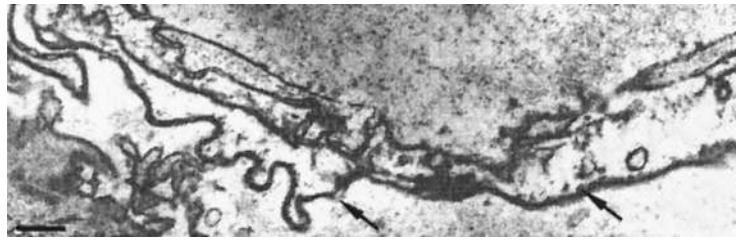

Fig. 6. Longitudinal section through the apical part of pleuropodium—vesicle-like external copula covered by cuticle (arrows). TEM, bar = 0.5 μm.

the membrane contacting the organ cavity. RER is also accompanied by scarce Golgi complexes and smooth ER. Glycogen granules are distributed irregularly. Most are accumulated in the "spongy cytoplasm" (Figs. 7 and 8), while their number decreases toward apical ends of the cells (Fig. 9). In the latter part single, irregularly spaced lipid droplets, as well as secretory vesicles of globular shape and granular structure, are observed. Lipid droplets are also distributed in the basal part of pleuropodium cells (Fig. 7). Single vesicles coming close to the surface of the cell membrane are visible (Fig. 9). Such structures are not observed in the "spongy cytoplasm." No vacuoles, suggesting the degeneration of the organ, are found in the cytoplasm. The entire length of pleuropodium cells is occupied by microtubules, accompanied by thinner filaments (Fig. 9). The latter are most numerous between cell membrane folds of the "spongy cytoplasm." Pleuropodium cells are connected through gap junctions or belt desmosomes (zonula adherens), whereas membranes of adhering cells are extensively folded.

Discussion

In all described cases of pleuropodia of the invaginated type (Hussey 1927, Ando 1953, Louvet and Marcel 1969, Ando and Haga 1974) during the stage of secretory activity of this organ, nuclei were located in the basal parts of cells under the ectoderm surface. Above the ectoderm, the copula, formed by cuticle covering the apical parts of cells, protrudes through the epidermis. Pleuropodium cells in these species are much elongated and reach from the basal lamina to the cuticular copula covering the pleuropodium. Ultrastructural differences between various species are quite small. Basal parts have a "spongy structure," a term that was introduced by Louvet and Marcel (1969) in their description of the basal cytoplasm of pleuropodium cells in *C. morosus.* This term refers to the area penetrated by numerous branched folds of cell membranes. Because of this cytoplasm, the pleuropodium cells contact the basal lamina by narrow cytoplasmic strands containing numerous mitochondria. The area of "spongy cytoplasm" reaches the line of nuclei, above which other organelles are present, while mitochondria are less numerous. In the elongated cell parts, reaching the cuticle covering the external copula, organelles are scarce, and the cytoplasm is mainly filled with microtubule bands (Louvet and Marcel 1969).

Comparing the structure of the pleuropodium in *T. domestica* to those described above, we observed some similarities in ultrastructure. Of note is the different character of this organ in the area under the

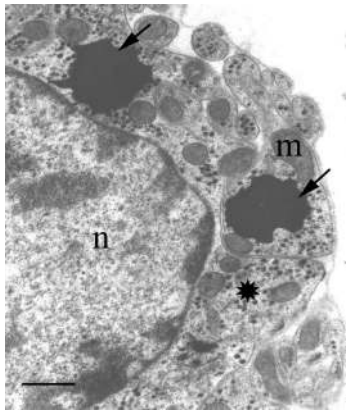

Fig. 7. A fragment of a transverse section through the embryo of *T. domestica.* Longitudinal section through the basal part of pleuropodium cell—region of "spongy cytoplasm" with nucleus (n), mitochondria (m), glycogen (*), and large lipid droplets (arrows). TEM, bar = 1.0 μm.

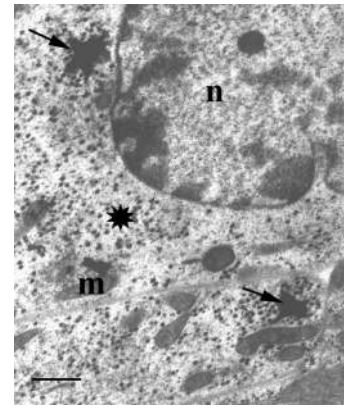

Fig. 8. A fragment of transverse section through the embryo. Longitudinal section through the central part of the cell. Nucleus (n), glycogen (asterisk), mitochondria (m), and lipid droplets (arrows) are smaller than in the basal part of the cell. TEM, bar = 1.0 μm.

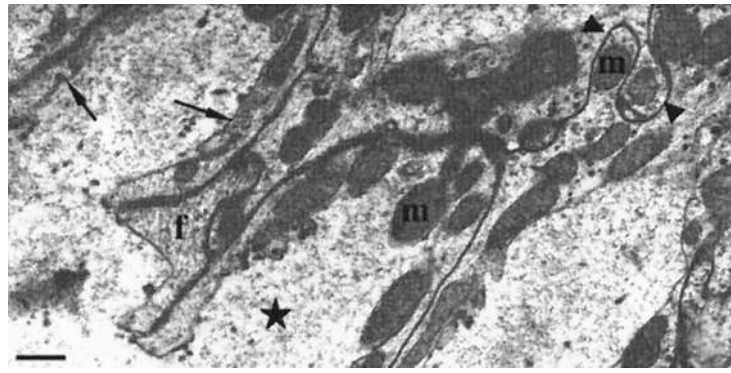

Fig. 9. Longitudinal section through the apical parts of pleuropodium cells. Mitochondria (m), secretory vesicles (arrows), filaments (f), and membrane folds (arrowhead). Pleuropodium cells adjoin to the mushroom-like cavity (*). TEM, bar = 0.8 μm.

cuticular copula. In contrast to the above-described species, as well as the description provided by Woodland (1957), cells of this organ in *T. domestica* do not protrude out of the body. Rather, they reach only the base of the cavity that is composed of the stem exiting through the epidermis and the vesicle-like external copula covered with embryonic cuticle. This latter cuticle is produced by pleuropodium cells (unpublished data) in an early stage of differentiation. Subsequent secretion of a substance between the apical cell membrane and the embryonic cuticle leads to its evagination outside of the body.

The origin of cavities in the cytoplasm of pleuropodia in some insects is explained by the appearance of small vacuoles that merge to form larger ones. This is a sign of organ degeneration (Ando 1953, Ando and Haga 1974). Vacuoles forming cavities in the cytoplasm of pleuropodium cells in *T. domestica* appear much later (11th day of embryogenesis) than was previously described (Woodland 1957). There is no information on how the external vesicle copula of the pleuropodium that we observed in *T. domestica* is formed.

Similar to *C. morosus*, *T. domestica* pleuropodium cells lie on the basal lamina, which does not penetrate into the membrane folds. Basal lamina of epithelial cells in the insect midgut also does not penetrate invaginations of membranes (Anderson and Harvey 1966). In *Locusta migratoria* L. (Orthoptera) the appearance of much folded membranes in both basal and apical parts was observed (Louvet 1975). Extensive membrane folds are characteristic of organ cells taking part in the transport of water, while the additional presence of mitochondria in membrane folds may be evidence of participation in active transport (Beams et al. 1955, Pease 1955, Beams and Anderson 1957, Lehninger 1964, Anderson and Harvey 1966, Fawcett 1967, Louvet and Marcel 1969, Louvet 1973, Louvet 1983, Motta 1990). Anderson and Harvey (1966) also suggested the importance of mitochondria in intercellular transport of ions. The presence of large numbers of mitochondria in pockets formed by membrane folds in pleuropodia of *T. domestica* suggests that intensive processes of active transport occur in this organ. The largest accumulation of mitochondria was observed in pockets of "spongy cytoplasm" and in apical parts of cells. In *C. morosus*, accumulation of mitochondria in pleuropodium cells is visible on both cellular poles (Louvet 1973), whereas in *L. migratoria*, it was observed on the apical pole only (Louvet 1975). Foldings of the basal cell membrane, penetrating the cytoplasm, and the accumulation of mitochondria were found in pleuropodium cells in *Rhizotrogus majalis* Razoum (Coleoptera) (Louvet 1983), salivary gland cells of *Calliphora erythrocephala* Meigen (Oschman and Berridge 1970, Chapman 1985), epithelial cells of the midgut of *Hyalophora cecropia* L. (Lepidoptera) (Anderson and Harvey 1966), *Chrysolina pardalina* F. (Heler 2002), and *Melasoma vigintipunctata* Scopoli (Coleoptera) (Sulik 2002) (JK, unpublished data). Epithelial cells of the insect midgut show their secretory abilities. Mitochondria are localized on both cellular poles, whereas their accumulation is sporadic in the central part of each cell (Chapman 1985, Heler 2002, Nowak et al. 2002, Sulik 2002). We observed the same arrangement of mitochondria and membrane folds in pleuropodium cells of 8-d-old embryos of *T. domestica*.

Individual vesicles coming close to the apical organ membranes in *T. domestica* release, by exocytosis into the mushroom-like cavity, are substances containing PAS-positive elements. Apical cell membranes with numerous microvilli and secretory vesicles are characteristic of organs that fulfill secretory functions (Schmitz and Komnick 1976, Oschman and Berridge 1979, Louvet 1983, Chapman 1985, Heler 2002, Nowak et al. 2002, Sulik 2002). Secretion of enzymes, e.g., in *Petrobius maritimus* Leach (Thysanura), *Stomoxys calcitrans* L. (Diptera), *L. migratoria*, *Calliphora*, *H. cecropia*, or components of egg capsules often appears by the process of exocytosis (Chapman 1985, Wêglarska 1982, Poprawa et al. 2002). Louvet (1975) suggested that the appearance of secretory vesicles present in pleuropodium cells of *L. migratoria* indicates homology with exuviate drops. Secretory vesi-

cles, glycogen, and lipid drops are also found in secretory cells in insects (Louvet 1983, Chapman 1985).

The entire length of pleuropodium cells in *T. domestica* is occupied by many microtubules and filaments connected with microtubules. Their main function is to maintain mechanical stability of the cells. Microtubules also take part in cytoplasmic motion and the resulting transport of substances from the basal to the apical pole of the cell (Motta 1990). The transport function of microtubules for secretion of the liquid fraction in pleuropodium cells of *C. morosus*, as well as a mechanical one has been suggested (Louvet and Marcel 1969, Louvet 1973).

Judging by observation of the spatial distribution and structure of organelles in pleuropodium cells of *T. domestica*, we conclude that this organ serves both transport and secretory functions. It is presumed that it secretes the substance responsible for dissolution of embryonic cuticle (Slifer 1937, Jones 1956, Woodland 1957, Locke 1960, Locke 1961, Wigglesworth 1965, Klag 1978). Experiments with ligatures of embryos of *Melanoplus differentialis* Thomas (Orthoptera) (Slifer 1937), *L. migratoria*, and *Locusta pardalina* Walker (Orthoptera) (Jones 1956) suggest that pleuropodia are responsible for the secretion of an enzyme that digests embryonic cuticle during the synthesis of larval cuticle, but other data exist suggesting that this is less probable (Locke 1960, Locke 1961, Wigglesworth 1965, Klag 1978). We suggest that the function of this secretion is to weaken the chorion before larval hatch. The presence of pleuropodia at the end of *T. domestica* embryogenesis supports this hypothesis.

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