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Fine Structural Change of the Follicular Cells during Oosorption in the Starved Lady Beetle, *Epilachna vigintioctomaculata* MOTSCHULSKY, with Special Reference to the Demonstration of Acid Phosphatase*

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Synopsis During oosorption in *Epilachna vigintioctomaculata*, fine structural changes occurred in the egg follicle; significant increase of autophagic vesicles in the apical region of the follicular cells, extrusion of cytoplasmic projections into the oocyte following disappearance of the microvilli, and deformation and liquification of the proteid and lipid yolk spheres attacked by lysosome-like bodies, and formation of residual structures, such as myelin figures, vacuoles, membranous components and others. These changes may indicate that the follicular cells uptake actively the dissolving ooplasm by their phagocytic activities. According to the electron-histochemy, acid phosphatase activity was recognized in the almost all of the lysosome-like structures, microvilli and intercellular spaces between the electron dense and light cells consisting of the follicular epithelium. This enzyme probably acts not only to autolize the microvilli of the follicular cells and oocyte but to dissolve the cortical ooplasm.

Introduction

According to many histological and histochemical investigations on oosorption in insects, it has been generally concluded that the follicular cells cease the supplying function of the precursors of proteid yolk spheres and resorb the ooplasm by their phagocytic activities (DE WILDE, 1964; HOPKINS & KING, 1964; LUSIS, 1963; MAETA & KURIHARA, 1971; WIGGLESWORTH, 1936).

In *Epilachna vigintioctomaculata*, resorption of oocytes occurred when the adults were starved or transferred to the short photoperiodic condition. During the oosorption process, the follicular epithelium became thick and invaginated partially into the oocyte. Furthermore, PAS-positive and sudanophilic masses, probably deformed proteid and lipid yolk spheres, were detected histochemically in the follicular cells. From these results, the author also suggested that the follicular cells might act as phagocytic cells to resorb the ooplasm and yolk as same as in many other insects (KURIHARA, 1967, 1968).

In the allatectomized Colorado potato beetle, *Leptinotarsa decemlineata*, on the contrary, DE LOOF and LAGASSE (1970) clarified electron microscopically that dissolution of proteid yolk spheres was not carried out by a phagocytic activity of the

* Studies on oogenesis of the lady beetle, V.

follicular cells as suggested in the findings on the oosorption of this species (DE WILDE, 1964), but by lysosome-like bodies appeared in the oocyte itself. Thus indeed, there are some doubt about the mechanism of yolk sphere breakdown, and two possibilities have been discussed by BELL and BOHM (1975); lecitholytic, phagocytic activities by invading follicular cells, and lysis by enzymes contained in lysosome-like bodies within the oocyte.

Therefore, the present study was carried out with electron microscope to clarify the functional changes of the follicular cells during oosorption in *Epilachna* and further, with cytochemical demonstration of acid phosphatase activity as one of the specific indicator of lysosomes.

Materials and Methods

Insects were reared under 16 hr-photophase, and 25°C with supply of potato leaves to observe the fine structure of the follicular cells in the normal oogenesis. Thereafter, some of them were transferred to the 8 hr-photophase and starving condition to obtain the degenerating ovaries as same as in the previous studies (KURIHARA, 1967, 1968).

Electron microscopical observation: The normal and degenerating ovaries removed in ice-cold gultalaldehyde solution were fixed with 1% osmium tetroxide buffered with veronal buffer (pH 7.4) for 1.5 hours. After usual dehydration by ethanol, the ovaries were embedded in Epon-812. Thin sections were made by Poter Blum MT-1 ultramicrotome and double-stained with uranyl acetate and lead nitrate. Observation was done by Hitachi HU-125 electron microscope.

Cytochemical demonstration of acid phosphatase: Acid phosphatase in the ovary was demonstrated cytochemically by the deposition of lead phosphate as the reaction product (GOMORI method). For electron microscopy, the ovaries were prefixed with 2% gultalaldehyde in 0.1 M sodium cacodyrate buffer at pH 4.0 which is optimum pH for acid phosphatase activity of the ovaries in *Epilachna* (SUZUKI, unpublished), for 2 hours at 5°C. The fixed ovaries were then washed in the same buffer for 1 hour. For the demonstration of phosphatase, the fixed ovaries were incubated at 30°C for 1 hour in the following incubation medium: 10 ml of 0.1 M tris-malate buffer (pH 4.0), 10 ml of 1.25% disodium β-glycerophosphate (Sigma Chemical Co.), 10 ml of deionized water and 20 ml of 0.2% lead nitrate. The final pH was adjusted to 4.0 with tris-malate buffer. After incubation, the ovaries were washed with chilled deionized water, and were postfixed with 1% osmium tetroxide in 0.1 M sodium cacodyrate buffer at pH 7.4 for 2 hours. The dehydrated and Epon-embedded ovaries were sectioned and stained with uranyl acetate. The preparations which were incubated in the lead-free medium containing 10 mM fluoride sodium were also studied as control.

Results

1. *Fine structure of the normal egg follicle*

The author reinvestigated electron microscopically on oogenesis of *Epilachna* from the previtellogenic to the mid-vitellogenic stages when resorption of oocytes may occur.

The early previtellogenic oocyte situated at the undermost region of the germarium has a large spherical nucleus and its cytoplasmic organelles are very poorly developed. Only few slender mitochondria and several electron light lipid droplets, like the primary lipid droplets observed in the early oocyte of *Bombyx mori* (MIYA *et al.*, 1970), are frequently observed in the oocyte as seen in the nutritive cord (Fig. 1).

At the neck region between the germarium and the vitellarium, the follicular cells migrate around the growing oocyte, but do not form a single-layered epithelium. In the oocyte, there are prominent well-developed golgi apparatus, many round-shaped mitochondria with poorly developed cristae and with dense matrix, several primary lipid droplets, lysosome-like vesicles and dense bodies (Fig. 2).

In the mid-previtellogenic oocytes, the follicular cells become cuboidal and form a mono-layered epithelium surrounding the oocyte but their microvilli do not yet develop (Fig. 3). Many clusters of spherical and spheroidal mitochondria, and primary lipid droplets are particular features in the ooplasm of this stage, perhaps indicating their proliferations. In addition, rough-surfaced endoplasmic reticulum with short thread-like cisternae are widely distributed throughout the oocyte (Fig. 4). Occasionally, dense bodies are found only at the cortical ooplasm. Through the opening of the nutritive cord, numerous mitochondria and some primary lipid droplets inflow actively into the oocyte (Fig. 5).

In the late previtellogenic stage, the follicular cells become more thick in width and their microvilli develop well. Furthermore, mitochondria and rough-surfaced endoplasmic reticulum increase evidently in their cytoplasm surrounding the nucleus. In the oocyte, on the other hand, golgi apparatus, ellipsoidal mitochondria with well-developed cristae and lipid droplets become more abundant. These lipid droplets are divided into following three types. The first type of lipid droplets is electron-light and of rather round in shape (Fig. 6, PL), and they have exhibited already in the young oocytes, and perhaps they have been descended from the nurse cells during the early previtellogenesis. The second type is electron-light and contains some electron-dense materials (Figs. 6 & 7, L1), and the last type is composed of contiguous lamellated materials (Fig. 7, L2). Two latter types increase in number from the late previtellogenic to the early vitellogenic stages. Such polymorphism in the lipid droplets may show the developmental processes of the L2 lipids which will be finally formed at the later oogenesis, although it is uncertain what changes in chemical components may occur. In addition, some annulate lamellae are often observed particularly at the antero-lateral surface of the oocyte.

Inflow of the free ribosomes, mitochondria and lipid droplets *etc.* into the oocyte through the nutritive cord significantly decreases at the early to mid-vitellogenic stages (Fig. 8). Mitotic divisions of the follicular cells are frequently observed near the opening of the nutritive cord (Fig. 8), and such multiplication causes to close the opening site of the nutritive cord by the newly formed follicular cells at this stage. In the follicular cells surrounding the lateral side of the oocyte, on the contrary, well developed rough-surfaced endoplasmic reticulum and numerous mitochondria *etc.* abundantly distribute through the cytoplasm as seen in the previtellogenic stages (Fig. 9). Microvilli of these cells develop well and are filled with dense materials, and moreover, small proteid yolk vesicles deposit actively within the cortical and subcortical cytoplasm due to micropinocytosis (Fig. 9).

2. *Fine structural changes in the oocyte and follicular cells during oosorption*

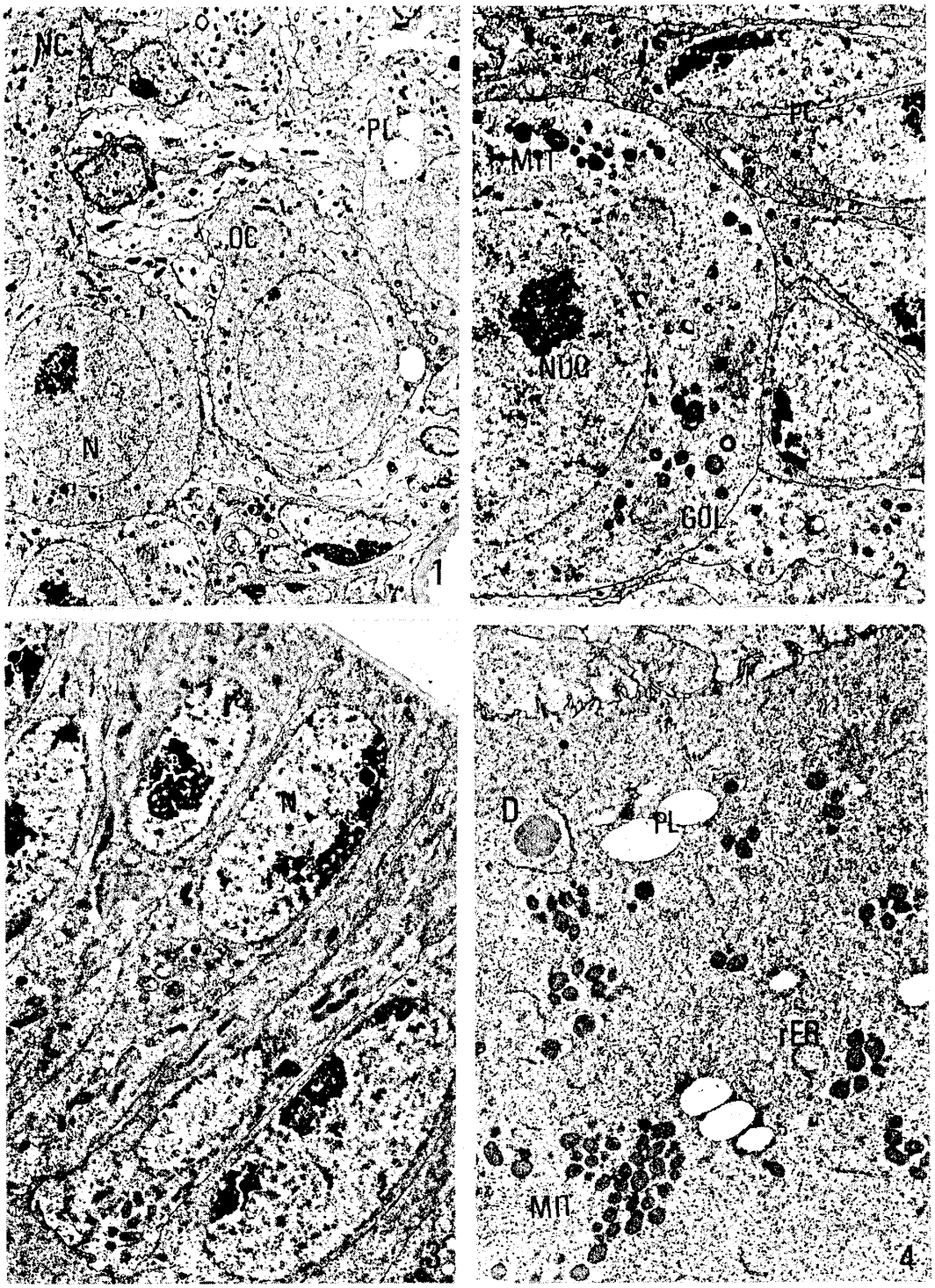
1) Early phase of oosorption

Within 24 hours after starvation, any light microscopical changes could not be observed in the egg follicle, except the nucleolus enlargement like an endobody described first by BIER *et al.* (1967) in the germinal vesicle (KURIHARA, 1975, 1976). Electron microscopically, however, considerable amounts of small vesicles crowded together so closely become easily detectable at the oocyte cortical cytoplasm just beneath the follicular cell microvilli (Fig. 10). No other organelles except glycogen granules, proteid yolk spheres and lipid droplets could be already observed. With progress of oosorption, microvilli of the follicular cells become more and more indistinct and lose the normal arrangement. Substitutionally, some cytoplasmic projections of the follicular cells begin to extrude into the ooplasm (Fig. 11, arrows).

2) Mid-phase of oosorption

In this phase, a drastic change proceeds in the cytoplasm of the oocyte and the follicular cells. Thickening and folding of the follicular epithelium, partial coagulation and liquification of the haematoxyline-positive ooplasm, and moreover, many PAS-positive and sudanophilic masses in various sizes were easily detectable light microscopically (KURIHARA, 1967, 1968). These changes were also recognized with electron microscopy. With the progress of oosorption, microvilli of the follicular cells and oocytes entirely disappear and well elongated cytoplasmic projections of the follicular cells extrude more deeply and more irregularly into the liquified ooplasm (Fig. 14). Frequently, the liquified ooplasm appears to be surrounded by the projections. However, the mono-layered arrangement of the follicular cells is main-

Figs. 1–4. —— 1. Early previtellogenic oocytes at the undermost region of germarium. OC, Oocyte; NC, nutritive cord; N, nucleus; PL, primary lipid droplet. ×2,400. —— 2. Previtellogenic oocyte at the neck region between germarium and the top of vitellarium. MIT, Mitochondria; GOL, golgi apparatus; NUC, nucleolus; FC, follicular cell. ×3,000. —— 3. Follicular epithelium in the mid-previtellogenic stage. ×4,400. —— 4. Clusters of mitochondria and primary lipid droplets in the mid-previtellogenic oocyte. D, Dense body; rER, rough-surfaced endoplasmic reticulum. ×4,800.



tained normally and the cell membranes remain distinctly until the more advanced stage of oosorption. In the apical region of the follicular cells, many lysosome-like vesicles in various sizes increase significantly as a particular feature in this phase, while the basal region of the cells just inside the tunica propria is filled with well-developed rough-surfaced endoplasmic reticulum, many mitochondria and golgi apparatus *etc.*, as in the normal (Fig. 12). In the cortical and subcortical region of the vitellogenic oocytes, on the other hand, pinocytotic vesicles, proteid yolk spheres and other cytoplasmic organelles are entirely absent. Therefore, the oocyte is filled with only electron dense liquified substances (Fig. 12). Sometimes, large electron dense masses, probably denatured proteid yolk spheres can be discriminated in the regions. The lipid yolk droplets in the vitellogenic and also previtellogenic oocytes, on the contrary, remain for a long time in the normal state, being surrounded by some small lysosome-like vesicles.

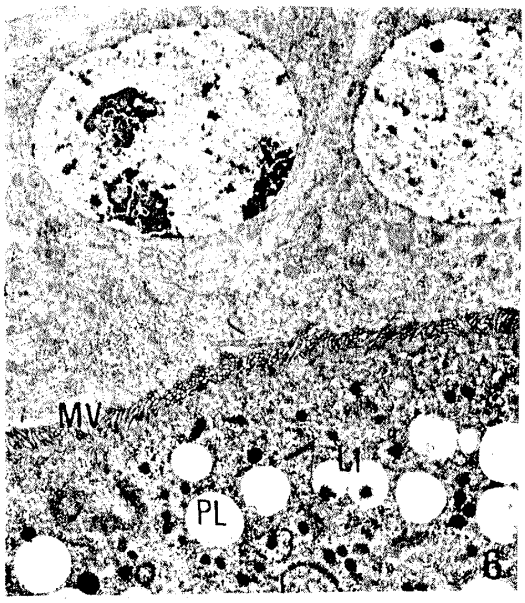
3) Late phase of oosorption

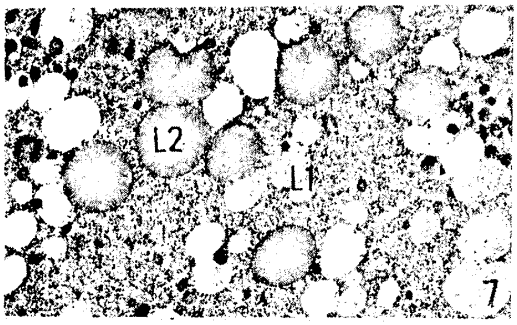
The nucleus of the follicular cell becomes more or less irregular in shape and situates at the basal region of the cell. The nucleolus and the nuclear membrane, however, can be observed distinctly. Throughout the cytoplasm, there are observed numerous residual structures which are large vacuoles, myelin figures, unknown membranous components, and also deformed proteid and lipid yolk spheres attacked by some small lysosome-like vesicles (Fig. 13). Figure 15 shows the detail of dissolution of proteid and lipid yolk spheres. Evidently, large proteid yolk spheres lost the spherical form and invaginate at the site where the lysosome-like vesicles attached (arrows). The dissolution of the lipid yolk droplets proceeds more slowly than that of the proteid yolk spheres. At the later phase of oosorption, they intermingle to each other and large electron dense masses are formed (Fig. 13). Finally, ooplasmic contents almost disappear, and many pycnotic nuclei of the follicular cells and residual bodies are filled within the more contracted oocyte, and thereby corpus luteum is formed.

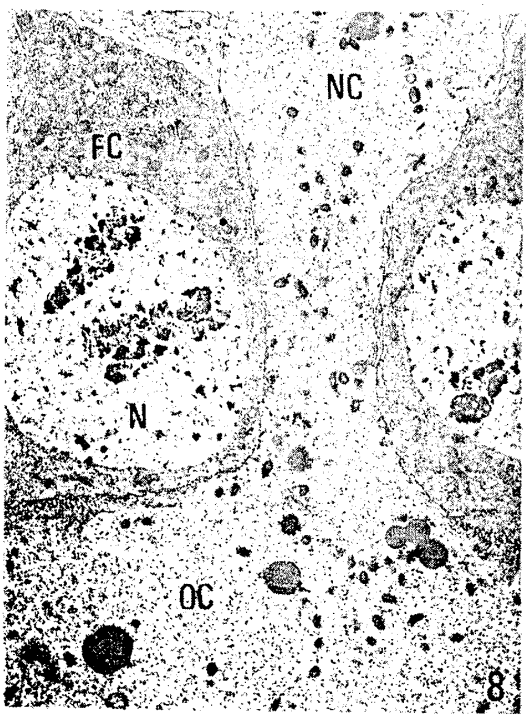
3. *Cytochemical demonstration of acid phosphatase in the lysosome-like structures*

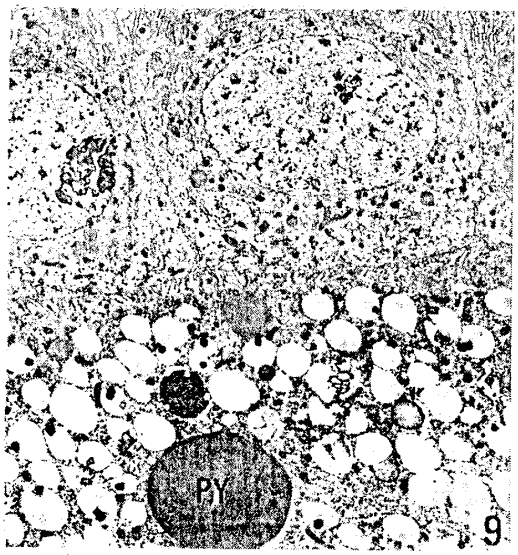
Lysosome-like structures in the degenerating follicular cells and oocytes were demonstrated by the cytochemical technique which detect acid phosphatase activity as one of the typical enzymes in the lysosome. The results are shown in Figures 16–24. Almost all of the lysosome-like vesicles in the apical cytoplasm of the follicular

Figs. 5–9. —— 5. Opening site of nutritive cord in the previtellogenic oocyte. Numerous slender mitochondria and some primary lipid droplets migrate into the oocyte through the nutritive cord. ×3,000. —— 6. Late previtellogenic oocyte. Well developed microvilli (MV) of the follicular cells. L1, Lipid droplet including electron dense materials. ×2,400. —— 7. Development of lipid droplets in the previtellogenic oocyte. L2, Completed lipid droplets consisting of intricated lamellate component. ×5,000. —— 8. Opening site of nutritive cord in the vitellogenic oocyte. Cytoplasmic organelles in the cord become very poor.. ×3,000 —— 9. Early vitellogenic oocyte with proteid yolk spheres (PY). ×3,000.









cells at the early phase of oosorption showed strict lead deposition, and only several large lysosome-like structures formed at the later phase showed the same reaction. Besides, on the surface of the small vesicles that appeared at the cortical ooplasm less reaction of lead was also observed, although inner contents were entirely negative (Fig. 24).

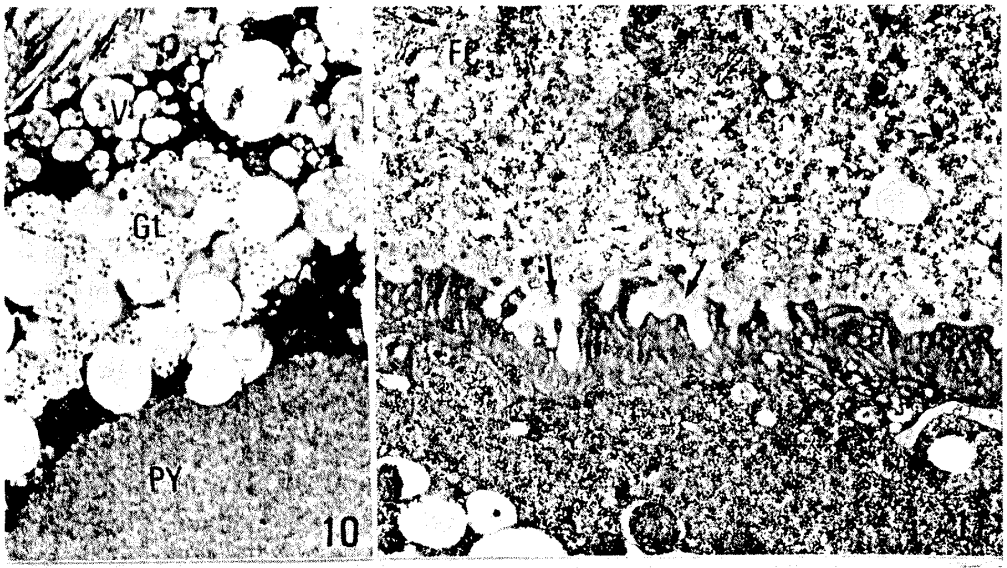
In *Epilachna*, the follicular epithelium consists of two different cell types, electron-light and dense cells. The former cells are a principal constituent of the follicular epithelium, while the latter cells are few, usually triangular in shape and intervene between the light cells here and there. These dense cells are stained intensively with protein- and RNA-staining procedures, because they are rich in free ribosomes. Although these cells are always observed in the normal oogenesis, nothing is known as to the stage of their appearance and their function (Fig. 16). Further, it is noticeable that these dense cells carry out a characteristic change on the acid phosphatase reaction during oosorption.

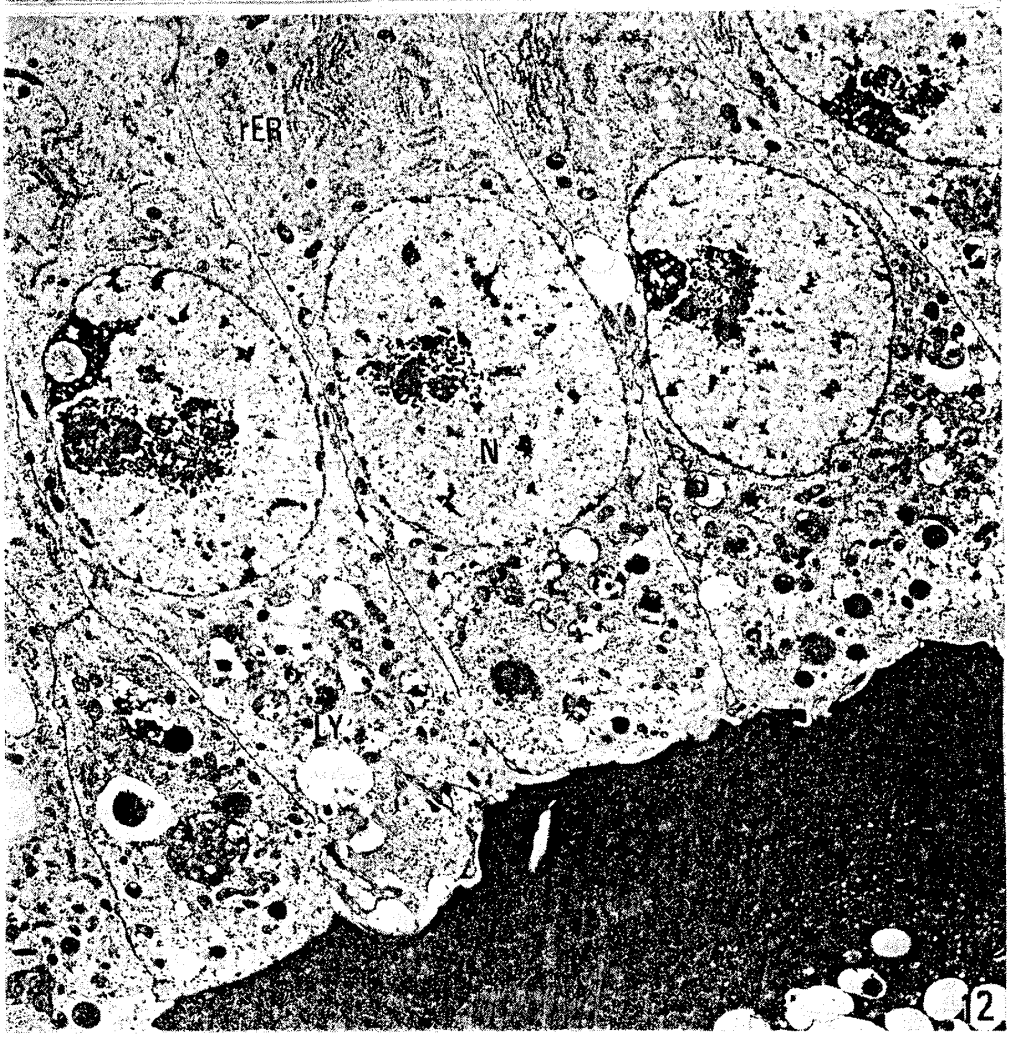
At the onset of oosorption, deposition of free lead granules becomes visible throughout the cytoplasm of the electron-dense cells. The same lead granules are also deposited at the intercellular spaces between the electron-dense cell and the adjacent electron-light cells (Figs. 17 & 18). Furthermore, the lead granules are accumulated at the undermost region of the apical cytoplasm of the electron-dense cells. In the microvilli of the cells, particularly strong lead reaction is detected just after the onset of oosorption (Fig. 17). Figures 18–20 show active deposition of lead granules in the microvilli of the dense cells. In the intercellular spaces between light cells, however, the lead granules could not observe through all stages of the oosorption, but the microvilli of these cells gradually deposite the lead granules as same as in the dense cells.

With disappearance of the microvilli, numerous fine lead granules are discharged into the cortical ooplasm and become hardly observable except the surface of lysosome-like vesicles. Electron-dense cells never form the cytoplasmic projections and never show phagocytic activities but soon degenerate at the earlier phase of oosorption.

In the control treated with the medium containing 10 mM fluoride sodium, the reaction of lead could not detect in any lysosome-like vesicles in the cytoplasm of the follicular cells, intercellular spaces and microvilli (Figs. 20 & 22).

Figs. 10–12. —— 10. Lysosome-like vesicles (V) in the oocyte cortical cytoplasm at the early phase of oosorption. GL, Glycogen granules. ×9,000. —— 11. Early phase of oosorption. Some cytoplasmic projections of the follicular cells begin to elongate (arrows). ×7,600. —— 12. Egg follicle at the early phase of oosorption. Liquified ooplasm, disappearance of microvilli, and increase of lysosome-like vesicles (LY). ×4,300.





Discussion

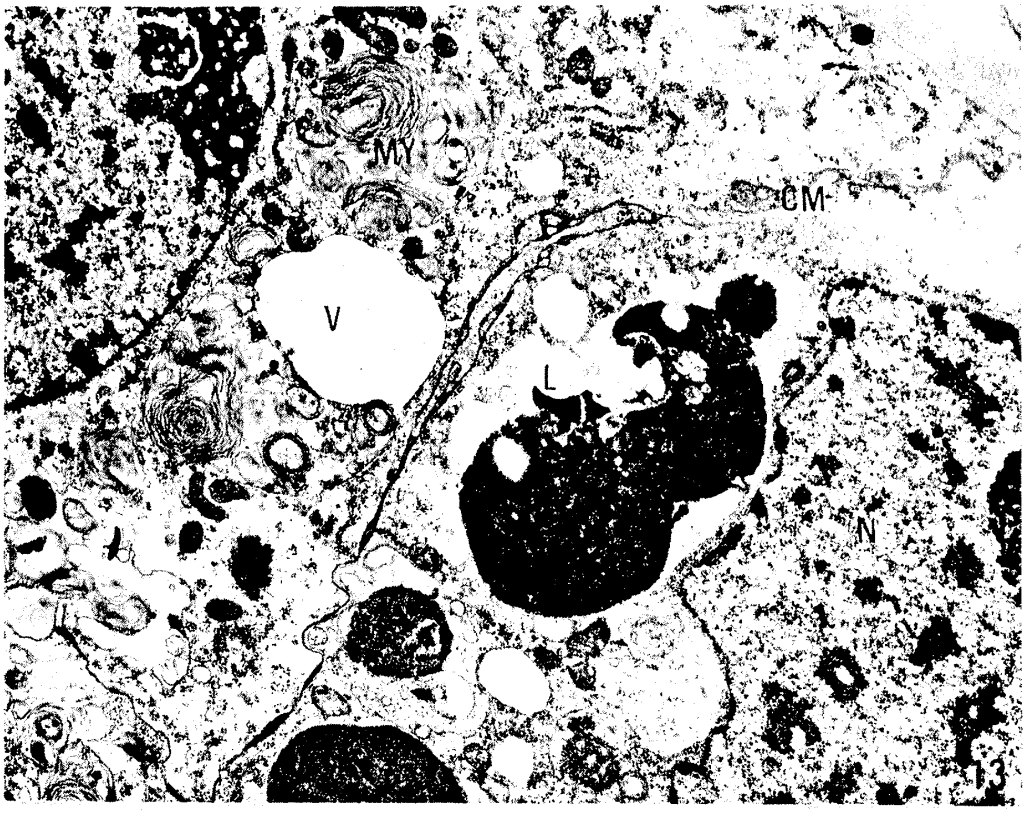
In insects, as to the mechanism of yolk sphere breakdown during oosorption process, following two possibilities have been discussed by BELL and BOHM (1975); lecitholytic, phagocytic activity by the invading follicular cells, and lysis by enzymes contained in lysosome-like bodies within the oocyte.

In *Epilachna*, the following changes were observed in the follicular cells during oosorption. The autophagic vesicles increased significantly in the cytoplasm of the apical region at first, the microvilli disappeared rapidly followed with high accumulation of acid phosphatase and subsequent extrusion of cytoplasmic projections, and finally the cytoplasm was filled with large amount of residual structures, such as deformed proteid yolk spheres, fused lipid yolk droplets attacked by lysosome-like bodies, myelin figures, large vacuoles, membranous structures and others. These phenomena may indicate that the follicular cells not only autolize themselves but also uptake and resorb actively the ooplasm and yolk by their phagocytic activities.

In the allatectomized *Leptinotarsa*, on the contrary, DE LOOF and LAGASSE (1970) clarified electron microscopically that proteid yolk spheres were dissolved by the lysosome-like bodies appeared in the ooplasm. Dissolving yolk spheres may inflow passively into the follicular cells as the result of a shrinking of oocyte. During oosorption process, however, electron dense materials or precursors of lysosomes were accumulated in the cortical region of the oocyte. These materials presumably originated in the follicular cells. Thereby, they suggested that supplying the precursor of lysosome into the oocyte was an role of the follicular cells in the resorption of this species. Although it may be overhasty to conclude, because the studies on oosorption have scarcely been done, such different results in *Epilachna* and *Leptinotarsa* may indicate species-specific function of the follicular cells in the both species.

The first step of oosorption in *Epilachna* is characterized by disappearance of the microvilli of the follicular cells and the oocyte like in *Leptinotarsa*. Prior to the morphological changes, large amount of acid phosphatase begins to accumulate rapidly in the microvilli, as soon as oosorption occurs. This enzyme may be related to both, the breakdown of the microvilli and also rapid dissolution of the cortical ooplasm. The increment of the same enzyme has been observed in the peripheral region of oocytes in *Nasonia vitripennis* deprived of hosts (HOPKINS & KING, 1964). They suggested that it might be concerned with the change in the 'A' yolk particles (proteinous) with onset of oosorption. Besides, in *Leptinotarsa* (DE LOOF & LAGASSE, 1970) the long and irregular offshoots appeared in some microvilli containing much more electron-dense materials in comparison with a healthy oocyte. Although the

Figs. 13–15. —— 13. Follicular cell in the late phase of oosorption. Myelin figures (MY), vacuoles in various sizes (V), and deformed lipid droplets (L) in the cytoplasm. CM, Follicular cell membrane. ×7,500. —— 14. Elongated cytoplasmic projections of the follicular cell (arrows). ×9,000. —— 15. Proteid yolk sphere attacked by lysosome-like bodies (arrows). ×3,400.



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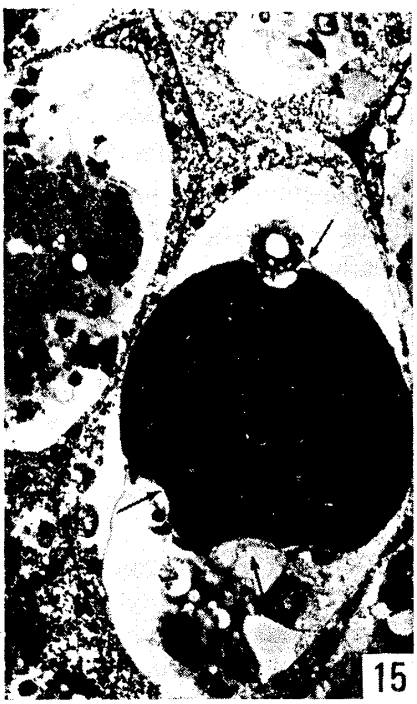

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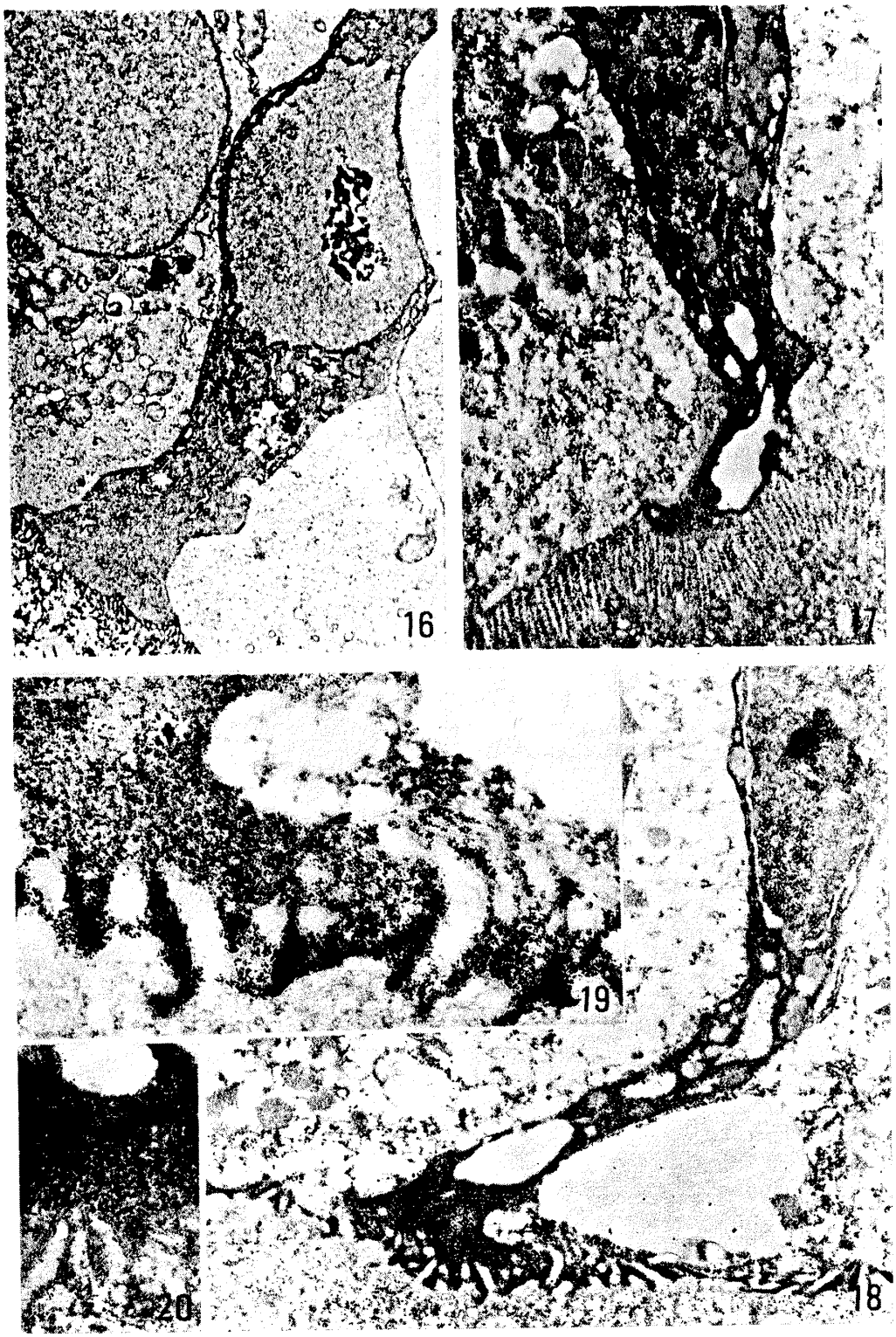
nature of the dense materials did not provided histochemically, it may be probable that the increased catabolic activity relating to the disappearance of microvilli and dissolution of ooplasm occurs, at first, in the oocyte-follicular cell interspaces at the early phase of oosorption in *Leptinotarsa* as in *Epilachna* and *Nasonia*.

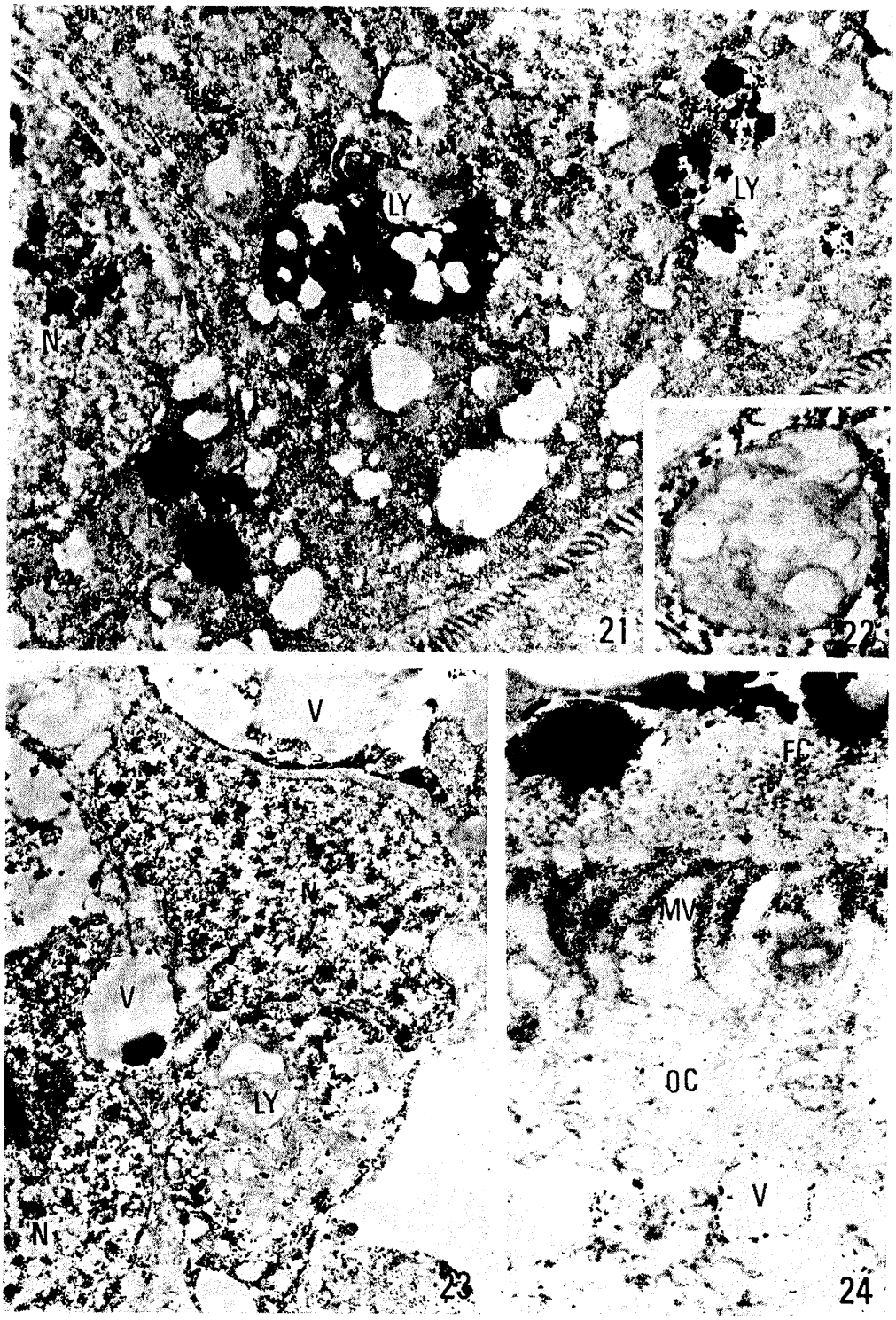
The follicular epithelium of *Epilachna* consists of two cell types, numerous electron-light cells and few triangular electron-dense cells, and the latter cells show characteristic structural changes during oosorption. The same cell types in the epithelium have been observed in *Anagasta* (*Ephestia*) *kuhniella* (CRUICKSHANK, 1964) and *Bombus terristris* (HOPKINS & KING, 1966). In *Bombus*, common follicular cells and darker stained narrower cells appear at the previtellogenic stage in 3: 1 proportion. The cytoplasm surrounding the nucleus in the latter cells contains high concentration of free ribosomes, and in more apical regions of the cells numerous proteinous particles are present. Following the completion of their activities, these cells break down during the late vitellogenic stage, resulting in the wider intercellular spaces between adjacent follicular cells (HOPKINS & KING, 1966).

In *Epilachna*, however, electron-dense cells become visible from the early previtellogenic stages at which do not yet arrange themselves to a single epithelial layer and exist through the more advanced stages of oogenesis. The site of appearance and proportion of two cell types are changeable in different individuals. Although their function is yet unknown during normal oogenesis in this species, they may discharge ribonucleoprotein granules into the ooplasm as suggested in *Anagasta* and *Bombus*.

During oosorption, the autophagic vesicles increased more remarkably and quickly in the electron-dense cells rather than those in the light cells, and afterward, numerous acid phosphatase began to accumulate throughout the cytoplasm. The same enzymatic activity had been frequently observed in tissue undergoing autolysis, such as in the degenerating areas of the salivary gland cells of *Chironomus* at the end of the larval-pupal ecdysis (SCHIN & CLEVER, 1965), in the peripheral giant glial cells of the nerve cord by 12 to 24 hours after pupal ecdysis of the moth, *Galleria mellonella* (BUSER, 1972), and also in the silk glands as the larvae pupate (AIDELLS *et al.*, 1971). The acid phosphatase in the dense cells of *Epilachna* not only accumulated into the microvilli but also discharged actively into the intercellular spaces between the neighbouring light cells. The electron-dense cells did not extrude the cytoplasmic projections and soon degenerated at the early phase of oosorption. Therefore, they

Figs. 16–20. —— 16. Electron dense follicular cells in the normal oogenesis. ×12,000. —— 17. Electron dense cell in the early phase of oosorption demonstrating by acid phosphatase reaction. Free lead granules begin to accumulate in the microvilli. ×10,200. —— 18. Acid phosphatase reaction in the electron dense cell in the early phase of oosorption. Intercellular spaces between electron dense and light cells, and microvilli filled with free lead granules. Microvilli lost their normal arrangement. ×10,500. —— 19. Enlarger aspect of Fig. 18. ×41,000. —— 20. Control treated by lead-free medium. Lead granules in the microvilli disappear. ×29,000.





may have only important function to supplying the enzyme which dissolve both, the microvilli of the follicular cells and oocyte, and thereafter the cortical ooplasm and yolk.

At the onset of oosorption, trypan blue was uptaken by the follicular cells at first, as stipple pattern and then as large patches in *Epilachna* (KURIHARA, unpublished) like in *Blatta orientalis* (SAMS, 1975). SAMS concluded that the initial loci of trypan blue uptake might be first site of breakdown of the membrane in the follicular cells and oocyte. In *Epilachna*, it may be thought that the site of direct passing of the dye reflects the wider intercellular spaces formed by destroying of the electron-dense cells. Through the spaces, a rapid resorption of the liquified ooplasm may proceed with progress of the more advanced phase of oosorption.

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Figs. 21–24. —— 21. Acid phosphatase reaction in the autophagic vesicles (LY) in the follicular cell. ×10,500. —— 22. Control of Fig. 21, treated by lead free medium. ×18,000. —— 23. Acid phosphatase reaction in the follicular cell cytoplasm at the late phase of oosorption. A part of large lysosome-like structure (LY) and vacuoles (V) show intense reaction. ×6,000. —— 24. Acid phosphatase reaction in the microvilli and cortical ooplasm. Small lysosome-like vesicles (V) show lead reaction only on the surface. ×36,000.

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