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Oosorption in the Lady Beetle, *Henosepilachna vigintioctomaculata* (Coleoptera, Coccinellidae)

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Synopsis

Morphological changes in the egg follicle of *Henosepilachna vigintioctomaculata* induced by starvation were observed light and electron microscopically, comparing with those in the normal one. Remarkable structural changes, enlargement of endobody and crystallized proteinous structure, are seen first in the germinal vesicle. Subsequently, the following fine structural changes occur in the follicular cells; 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, deformation and liquefication of the proteinous and lipid yolk spheres by lysosome-like bodies, and formation of residual structures such as myelin figures, vacuoles in various sizes, membranous components and others. According to the electron histochemy, acid phosphatase activity is recognized in the almost all of the lysosome-like bodies, microvilli and intercellular spaces between the follicular cells. These changes in the follicular cells may indicate that the follicular cells uptake actively the dissolving ooplasm by their phagocytotic activities and acid phosphatase probably acts not only to autolize the microvilli of the follicular cells and oocyte but to dissolve the cortical ooplasm.

When the starved females are refed, only the oocytes at the early phase of oosorption in which such morphological changes in the follicular cells do not yet occur are able to develop normally and are oviposited. In these oocytes, two kinds of abnormal structures developed in the germinal vesicle disappear quickly.

From these results, the functional significance of oosorption in *H. vigintioctomaculata* is discussed particularly from the viewpoint of the morphological changes in the germinal vesicle.

Introduction

Oosorption is a special case of cell death (Bell and Bohm, 1975) and is often induced by unfavorable conditions for oocyte growth. Its process, however, varies largely among insects,

perhaps by different patterns of oogenesis and/or mode of oviposition.

Up to this time, many studies have been done on oosorption, especially for researching of the internal and external factors inducing oosorption, and for analysis of the endocrine mechanism concerned to the control of oogenesis. However, cytological and fine structural approaches to clarify the functional changes of the egg follicle during oosorption are insufficient. Particularly, there is no study which suggests the causal relation between abnormality of the germinal vesicle and degeneration of ooplasm.

In *Henosepilachna vigintioctomaculata* Motschulsky, oosorption occurs during the prediapause period in the adults induced by short photoperiod (Maki *et al.*, 1964; Kono, 1980) and temperature (Maki and Kurihara, 1965). Furthermore, oosorption also induced by the deficiency of the nutrition as food quality, especially by starvation (Kurihara, 1975; Kono, 1979). Kono (1980) clarified the endocrine mechanism inducing oosorption in this insect, mainly by the histochemical architecture on the changes of secretory activity of the neurosecretory cells of the brain under the short photoperiodic condition. However, he did not observe the morphological changes of the oocytes.

This review is the summary of the studies on the oosorption of the lady beetle, with special reference to the demonstration of the changes of germinal vesicle, including both the results obtained so far (Kurihara, 1967, '68, '75, '76, '81) and some unpublished data on the recovery of the oocyte development and oviposition in the females refed after varying periods of starvation.

Materials and Methods

1. *Rearing conditions:* Insects were reared under 16hr-photophase and 25°C with supply of potato leaves to observe the normal oogenesis. Thereafter, some of them were transferred to the 8hr-photophase and starving condition to obtain the oosorptive ovaries. Furthermore, the insects were replaced on the 16hr-photophase with food after varying periods of starvation and redevelopment of the oocytes was observed.

2. *Morphological observation*

1) Anatomical observation: The ovaries were fixed with Carnoy's fluid for 2 hr and then stained with carbol-thionine, dehydrated and mounted in ceder oil.

2) Trypan blue injection: For exact determination of vitellogenic or oosorptive oocytes, 1% trypan blue (buffered with an aquatic solution containing 0.02 M KCl, 2×10^{-4}M $CaCl_2$, 2×10^{-4}M $MgCl_2$, 0.25 M tris malate, at pH 6.2) was injected about 0.025 ml to the female abdomen (modified from Anderson and Telfer's method, 1970). At an hour after the injection, the ovaries were dissected and fixed with Bouin's fixative for 3 hr Observation was done by total preparations which were dehydrated and mounted in ceder oil.

3) Histological and histochemical observation: The almost all of the ovaries were fixed in cold gultalaldehyde solution for 2 hr and then embedded in JB-4 or Acrytron-E, and stained with toluidin blue. Histochemically, the following methods were used: PAS staining (Hotchkiss and McManus method) for polysaccharides and glycogen with salivary treatment, Feulgen test and MG-P staining (Kurnick method) for DNA and RNA with ribonuclease treatment control, Sudan B.B or osmium staining for lipids, and Gomori's method for acid phosphatase to observe by electron microscope.

4) Electron microscopic observation: The ovaries removed in ice-cold gultalaldehyde

solution were fixed with 1% osmium tetroxide buffered with veronal buffer (pH 7.4) for 1.5 hr. 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.

Results

I. Normal oogenesis

1. *Mode of oocyte development and oviposition*

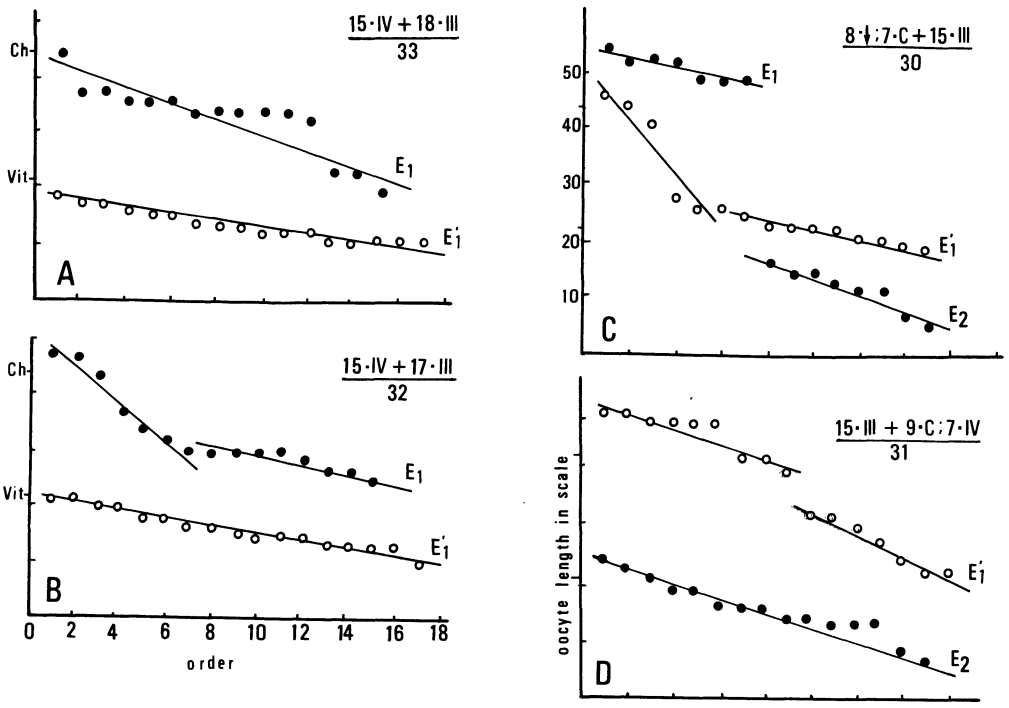
As described in the previous papers (Kurihara, 1967, 1975), each ovary in this insect consists of 32.1 ± 2.7 ovarioles, each of them attaches to the egg calyx with its short pedicel. About a half of the ovarioles exist in outer portion of the egg calyx and surround the remaining ovarioles which arrange themselves in ring like layers.

Under 16hr-photophase, oviposition is continued periodically, every or every other day as an egg mass containing about 30 eggs that is equal to about a half of total ovarioles. In each oviposition cycle, about 15 chorionated eggs did not be oviposited and remain always in the egg calyx. This oviposition cycle well corresponds to the cyclicity of oocyte development as shown in Text figure 1. Until 6 days, the previtellogenic growth of the basal oocytes can not be distinguished in the outer or inner ovarioles. Thereafter, almost all of the basal oocytes in the outer ovarioles begin to deposit the proteinous yolk, while the remaining basal oocytes in the inner ovarioles are staying in the previtellogenic stage (Text fig. 1-A, E1). When the largest basal oocytes in the outer ovarioles reached to maturity and chorionated, no growth of inner basal oocytes can be ever observed as in Text figure 1-A (Text fig. 1-B, E'1). Text figure 1-C shows the state which a half of the mature basal oocytes in the outer ovarioles (E1) had descended to the egg calyx. The remaining half of them are wholly chorionated. Some basal oocytes in the inner ovarioles (E'1) begin yolk deposition. Replacing the first basal oocytes descended to the egg calyx, the penultimate oocytes situate at the basal portion of the ovarioles (Text fig. 1-C, E2). As the result of descending of the remaining half of the basal oocytes (E1), the basal portion of all outer ovarioles is filled with the penultimate oocytes (Text fig. 1-D, E2). All basal oocytes in the inner ovarioles (E'1), on the contrary, reach to the vitellogenic stage and also the half of them chorionate as same as the state of the basal oocytes in the outer ovarioles as shown in Text figure 1-B. In the second or more successive oviposition cycles, the same development of oocytes will be also repeated periodically. Therefore normal oviposition may proceed basically as follows: the mature eggs stored in each egg calyx, number of which correspond to about one fourth of the total ovarioles, will be pushed out into the common oviduct by the subsequent descending of the basal oocytes from the vitellarium and deposited as a mass containing the eggs of about half of the total ovarioles.

2. *Fine structure of normal egg follicle*

1) Morphology of ovariole

Morphological characteristics of the ovariole in *H. vigintioctomaculata* were described by Matsuzaki (1964) and Kurihara (1975). The ovariole of this insect belongs to the telotrophic type like in other coleopteran insects. Namely, each trophocyte packed tightly in the greater part of the germarium has a large spherical nucleus, ca. 26 μm in diameter, and is delimited



Text figure 1. Mode of oocyte development in the 1 oviposition cycle under 16hr photophase.

Figures A–D show the oocytes in an ovary arranged in order of their longitudinal lengths. E1, E2 Basal and penultimate oocytes of outer ovarioles (●), E'1 Basal oocytes of inner ovarioles (○), A Vitellogenesis begins in E1, B Some E1 chorionate, C About a half of E1 descent to the egg calyx and E2 replace them, D Remnant E1 descend to the egg calyx and basal portion of each ovariole is occupied with E2.

Formula inserted in each figure represents;

$$\frac{\text{Number of oocytes in outer ovarioles} \cdot \text{Oocyte stage} + \text{Number of oocytes in inner ovariole} \cdot \text{Oocyte stage}}{\text{Total number of basal oocytes}}$$

III, IV and C Previtellogenic, vitellogenic and chorionated oocytes, ↓ Oocytes descend to the egg calyx, CH beginning of chorionation. Vit Beginning of vitellogenesis.

by a definite cell membrane. The oocytes, on the other hand, are detectable only in the protoplasmic nutritive chamber. They are relatively small and are situated among many prefollicular nuclei. The nutritive cords are formed appearently as the elongation of cytoplasm of both the trophocyte and oocyte (Kurihara, 1975).

2) Fine structure of the oocyte and development of the germinal vesicle

According to the developmental stages of oocytes divided by Kurihara (1975), the fine structure of the oocyte and the germinal vesicle will be described.

Stage I. The youngest oocytes restricted at the protoplasmic nutritive chamber: The cell organelles are very poor. A few slender mitochondria and lipid droplets inflow with numerous free ribosomes into the oocyte through the nutritive cords. In the germinal vesicle a primary nucleolus and DNA-containing bodies intermingle with each other and form a large complex. Both structures can be easily detected with their histochemical and fine structural characteristics, because the primary nucleolus is pyronine positive and consists of only fine fibrilar component with high electron density, while the DNA-containing body is, of course Feulgen or methylgreen positive and is relatively electron-medium compared to the former (Plate I, Fig.1).

Stage II. The younger oocytes at the anterior zone of the vitellarium: Prefollicular cells crowd around the oocyte but do not yet form a single epithelial layer. Ooplasm more or less increases. Well developed Golgi aparates, some lysosome-like bodies and sometimes one large electron-medium and compact sphere, probably an aggregate of the nuclear emission granules, are particular in the ooplasm of this stage. In the germinal vesicle, the primary nucleolus and DNA-containing body begin to get untied. The primary nucleolus aggregates to form an electron dense mass. Attaching to it, the DNA-containing body also grows to the large mass which consists of fine fibrous materials and has usually a single cavity in the center (Plate I, Fig.2). At the late stage II, the primary nucleolus begins to separate to small granules, ca. 900 Å, and disperse throughout the nucleoplasm, following the subsequent granulation and migration of the DNA-containing bodies (Plate I, Figs.3 and 4).

Stage III. The previtellogenic oocytes at the middle of the vitellarium: Ooplasm largely increases. The follicular cells form a single-layered epithelium, but their microvilli do not yet develop. Until the beginning of this stage, disintegration of the DNA-containing body and the primary nucleolus has already finished. Thereafter, at the middle of this stage, formation of the karyosphere begins; the long thread-like chromosomes accumulate loosely around the pyronine negative body which increases rapidly in size, ca. 4 μm in diameter. Electron microscopically this body consists of only fibrous component (Plate II, Fig.6). At the same time, a number of small spheres of three types produce actively around the surface of the karyosphere. The first type of the spheres has ca. 2 μm in diameter and shows high electron density with fine granules on its surface (BN1). The 2nd, the same size of the 1st type, shows a ring-shaped sphere having an electron-dense thin cortex (BN2), and the last is an electron-medium, compact sphere consisting of only fibrous component and closely resemble to the endobody first described by Bier *et al.* (1967) (BN3) (Plate II, Fig.5).

Stage IV. Vitellogenic oocytes: The proteinous yolk spheres begin to deposit from the periphery of the oocyte by pinocytosis and are filled in the ooplasm at the end of this stage. The BN1, BN2 and BN3 spheres increase considerably in number through the early stage of vitellogenesis. Finally, they become undetectable, when the vitellogenesis almost finished.

Stage V. The completed eggs with chorion: Fine structure of the vitelline membrane and the chorion formation was described by Matsuzaki (1965). The follicular cells degenerate.

The germinal vesicle becomes crescent and the nuclear membrane breaks and finally disintegrates.

II. Oosorption

1. *Cessat ion of oviposition and changes of the mature eggs induced by starvation*

Immediately after ovipositon, the female beetles were transferred to the 8hr-photophase under starving condition, and the mature eggs produced daily (oviposited eggs plus chorionated eggs in the egg calyx) were examined for 8 days.

Normally, the starved females deposit a few eggs discontinously during the first 4 days of starvation, while the chorionated eggs in the egg calyx increase progressively, perhaps, as the result of chorion formation of the late vitellogenic oocytes in the basal portion of the vitellarium and subsequent descending to the egg calyx. After 6 to 8 days of starvation, however, the deposited eggs more or less increase but the chorionated eggs in the egg calyx decrease conversely. Consequently the same number of the mature eggs were obtained in all starving periods investigated, although the number of eggs produced were slightly increased in the longer starvation. Beyond the 8 day-starvation, subsequent oviposition is not observed and no remaining eggs can not be ever seen in the egg calyx. This may be indicated that some of the nearly matured oocytes and the chorionated eggs stored in the egg calyx do not degenerate but are deposited, while no further development of the vitellogenic oocytes occurs in the vitellarium.

2. *Changes of immature oocytes during starvation*

When the females are starved, all of the immature oocytes in the vitellarium continue to develop slowly for several days and then oosorption starts. The relation between the developmental stages of the oocyte and occurrence of oosorption is summarized in Text figure 2.

At stage I and II, the oocytes develop slowly until formation of karyosphere (stage III). Dispersion of the primary nucleolus and DNA-containing body in these oocyte nuclei seem to be normal. During such slow growth, the BN3 (endobody) begins to develop enormously, ca. 40 μm in a maximum diameter (Plate V, Fig.12). At stage III and IV, however, the enlargement of the endobody completes within 24 hours. After such slow growth of the oocytes the morphological abnormalities become detectable in the egg follicle; decreasing stainabilities of the trophocytes for nucleic acid-staining, folding and irregular arrangement of the follicular cells, coagulation of ooplasm, fusion and liquification of the proteinous yolk spheres with decreasing PAS-stainability, *etc.*, are typical changes detected by light microscope (Kurihara, 1967, '68).

3. *Fine structural changes of the egg follicle during oosorption with special reference to the change of germinal vesicle*

Fine structural changes of the follicular cells and oocytes during oosorption were described by Kurihara (1976, '81).

At the slow growth period of oosorption, any particular changes can not be observed in the egg follicle, except enlargement of the endobody. Subsequently, first visible changes

occur in the apical region of the follicular cells and the cortical ooplasm. Namely, some lysosome-like vesicles which contain acid phosphatase appear significantly, while in the basal region of the cells just beneath the tunica propria, well-developed rER, many mitochondria and Golgi aparates distribute as in the normal (Plate III, Fig.8). According to the histochemistry with electron microscope, free acid phosphatase granules distribute widely throughout the cytoplasm of the follicular cells and are also detected in their microvilli. With progress of the oosorption, the microvilli of the follicular cells disappear rapidly, perhaps because of resolution by acid phosphatase (Plate III, Figs.7 and 8). Substituting for the microvilli well elongated cytoplasmic projections of the follicular cells extrude more deeply and more irregularly into the liquefied ooplasm (Plate IV, Fig. 11). Frequently, at the mid- to late oosorption large lysosome like structures including various residual bodies, such as deformed proteinous yolk spheres, lipid droplets fused each other in a large mass, myelin figures and some membranous components *etc.*, are included in the follicular cells. In these structures the acid phosphatase can also be detected (Plate IV, Fig. 9). Finally, at the late period of oosorption the oocytes become almost empty, and the nuclei of follicular cells inflow into the liquefied oocyte and autolyze. These processes of destruction of the follicular epithelium can be easily detected by trypan blue injection. Namely, as shown in figure 10a-d (Plate IV), the degenerating follicular cells uptake the dye spottedly in the earlier oosorption (b) and massively in the later oosorption (c), respectively.

Changes in the germinal vesicle: During the early phase of oosorption, prior to the cytoplasmic changes of the egg follicle, two particular structures are formed in the germinal vesicle; crystallized proteinous structure and an enlarged endobody. The fine structure, the origin and formative process of them are clarified electron microscopically (Kurihara, 1976).

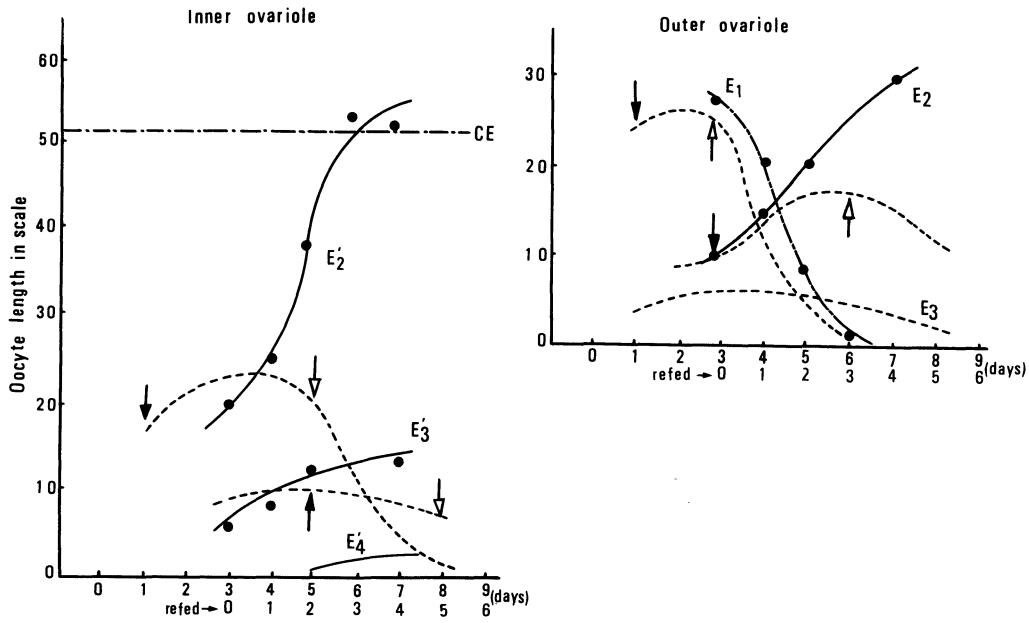
The crystallized proteinous structures are formed by accumulation and crystallization of the small granules that are derived from the primary nucleolus and scattered evenly throughout the nucleoplasm. Namely, at first, the granules aggregate on the surface of the BN3 and/or on the concave portion of the inner surface of the nuclear envelope. Subsequently, the accumulated granules develop to the short threads and finally they form a definite structures (Plate V, Fig.14).

The enlarged endobody is finally formed only one in the germinal vesicle by abnormal development and fusion of the BN3 distributed throughout the nucleoplasm. The enlarged endobody has a thin fibrous envelope consisting of phospholipoid material in nature which is one of the components in the BN3. The contents, on the other hand, do not contain phospholipids and nucleic acids, but are rich in proteins (Plate V, Figs. 12 and 14).

III. Recovery of oocyte development and oviposition in the refed females after varying periods of starvation

1. *Changes of immature oocytes in the vitellarium of the refed females*

To confirm whether the immature oocytes in different degrees of oosorption are able to resume normally their growth or not, the 3 days-starved females were refed and the relationship between redevelopment of the oocytes and changes of the enlarged endobody were examined. In the 3 days-starved females, oosorption proceeds in different extent corresponding to the oocyte development, as follows:



Text figure 2. Development of oocyte in the refed females under 16hr-photophase 25°C, after 3 days-starvation. CE Chorionated eggs, ⬇ Endobody begins to enlarge, ⇩ Anatomically, oosorption becomes visible in the oocyte and follicular cells, —·—·— Oosorption curve, —— Redeveloping curve, ------ Oosorption curve under continuous starvation, E1, E2, E3 Basal, penultimate, and 3rd oocytes in outer ovarioles, E'2, E'3, E'4 Penultimate, 3rd and 4th oocytes in inner ovarioles.

1) Oosorptive stage I (D I). Early previtellogenic oocytes: Anatomically, any abnormality can not be observed in the egg follicle. Probably, oosorption does not initiate.

2) Oosorptive stage II (D II). Mid- to late previtellogenic oocytes with an enlarged endobody in the germinal vesicle: Other abnormalities are not recognized.

3) Oosorptive stage III (D III). Early to mid-vitellogenic oocyte in which oosorption proceeds: Hematoxylin-positive ooplasm begins to coagulate partially and arrangement of the follicular cell becomes irregular. Proteinous yolk spheres are deformed. In the germinal vesicle, an enlarged endobody reaches to the maximum size.

The results are divided conveniently into the outer and inner ovarioles, as shown in Text figure 2

Outer ovarioles: In the outer ovarioles of the 3 days-starved females, the basal oocytes are in D III. Even if the females were refed, the oocytes can not develop and oosorption continues (El in Text Fig. 2, broken line) as in the case of continuous starvation (dotted line). The penultimate oocytes (E2) in D II, on the contrary, begin to grow normally as soon as the starved females are refed.

Inner ovarioles: The ovarioles contain the oocytes in D I and D II. The mature oocytes at the basal portion of the ovarioles descend already to the egg calyx during 3 days of starvation and only corpus luteum remains. Therefore, in the basal part of the vitellarium,

the penultimate oocytes in the late previtellogenic stage (E'2) exist and reach at D II. When the starved females are refed these oocytes begin to grow rapidly, and come to maturity with the chorion after 3 days. The 3rd oocytes (E'3) were in D I, and they also resume the normal development without oosorption.

2. *Changes of the enlarged endobody in the germinal vesicle of the refed females*

Enlargement of the endobody occurs only in the germinal vesicle of the oosorptive oocytes after the mid-previtellogenic stage (Stage III) and is a typical syndrome of the early phase of oosorption.

To observe the change of the enlarged endobody in the redeveloping oocytes, the 24hr-starved females were transferred to the 16hr-photophase with food. The females having the oocytes with enlarged endobody rapidly decrease although they increase temporarily 16 to 20 hours after. Lastly, the enlarged endobody can not be found at 24 hours after refeeding. In the germinal vesicle of the redeveloping oocyte, many fine residual particles (Plate V, Fig.15a) and/or discontinuous envelope of the body (Plate V, Figs.15b, c) are scarecely observed. This may be due to rapid breaking of the envelope and an instantaneous fusion of contents of the body with the surrounding nucleoplasm.

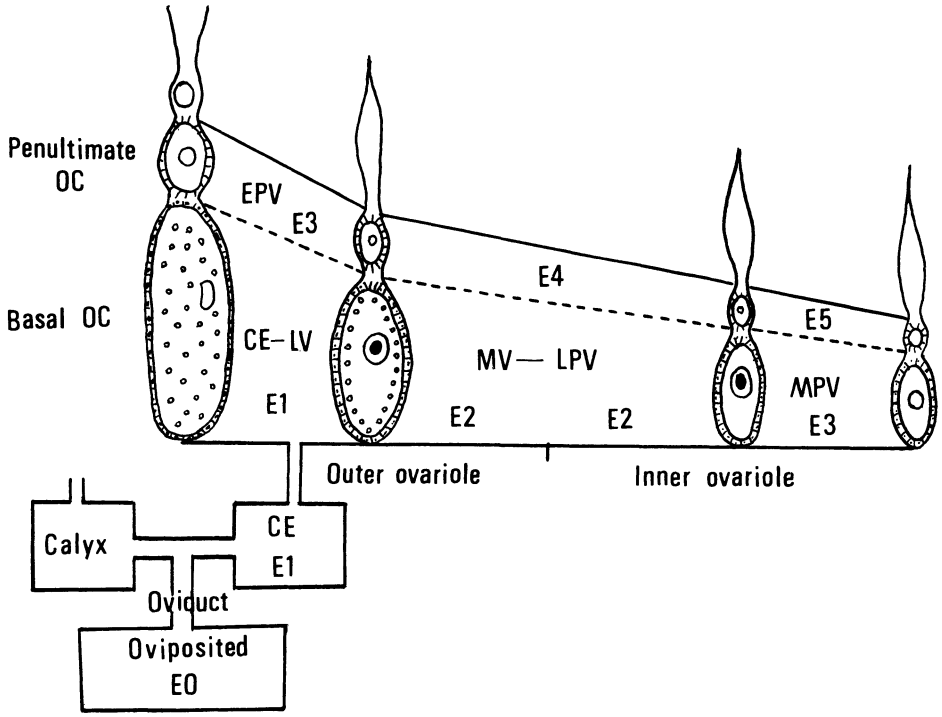
3. *Hatching ability of the eggs deposited by the refed females*

Hatching ability of the eggs deposited in the 1st to 4th oviposition cycles of the refed females after 24hr starvation were investigated. The number of eggs per one egg mass does not differ in each oviposition cycle. Extreme low hatching ability is obtained only in the 2nd oviposition cycle (Table 1). Unhatched eggs are dissected and developmental stages of the embryos are observed anatomically. Many of them (about 60%) had reached at the full grown stage and then die just before or after emergence from the egg shell. Early dead and abnormal embryos and unfertilized or undeveloped eggs are excluded. Almost all of the newly hatched larvae die as soon as they hatch but the survivals grow and pupate normally. Text figure 3 is illustrated the state of oocyte development in the 24hr-starved females. It is clear that the only oocytes corresponding to the 2nd oviposition cycle (E2) are in D II, and about a half of them can not hatch, although they are able to resume development and are laid by refeeding.

Table 1. Hatching ability of eggs oviposited by the refed females after 24hr-starvation.

Oviposition cycles after refeeding	Number of females	Number of eggs per egg mass	Hatching ratio
E0	10	44.5 ± 6.3	95.7 ± 3.8
E1	10	38.6 ± 7.3	89.4 ± 10.6
E2	9	42.2 ± 9.0	564. ± 31.4
E3	7	31.3 ± 10.8	97.2 ± 5.8
E4	6	32.7 ± 5.7	97.3 ± 2.1

E0, eggs oviposited before starvation.



Text figure 3. Oocyte development of the 24hr-starved females and mode of oviposition after refeeding.
EO Eggs oviposited before starvation, E1—E5 Eggs oviposited at the first to the 5th oviposition cycles after refeeding At 24hr-starvation, E2 reaches oosorptive stage II and all of others are in normal (E1, E3—E5). CE Chorionated (Stage V), IV Late vitellogenic, MV Mid-vitellogenic, MPV Mid-previtellogenic, EPV Early previtellogenic oocytes.

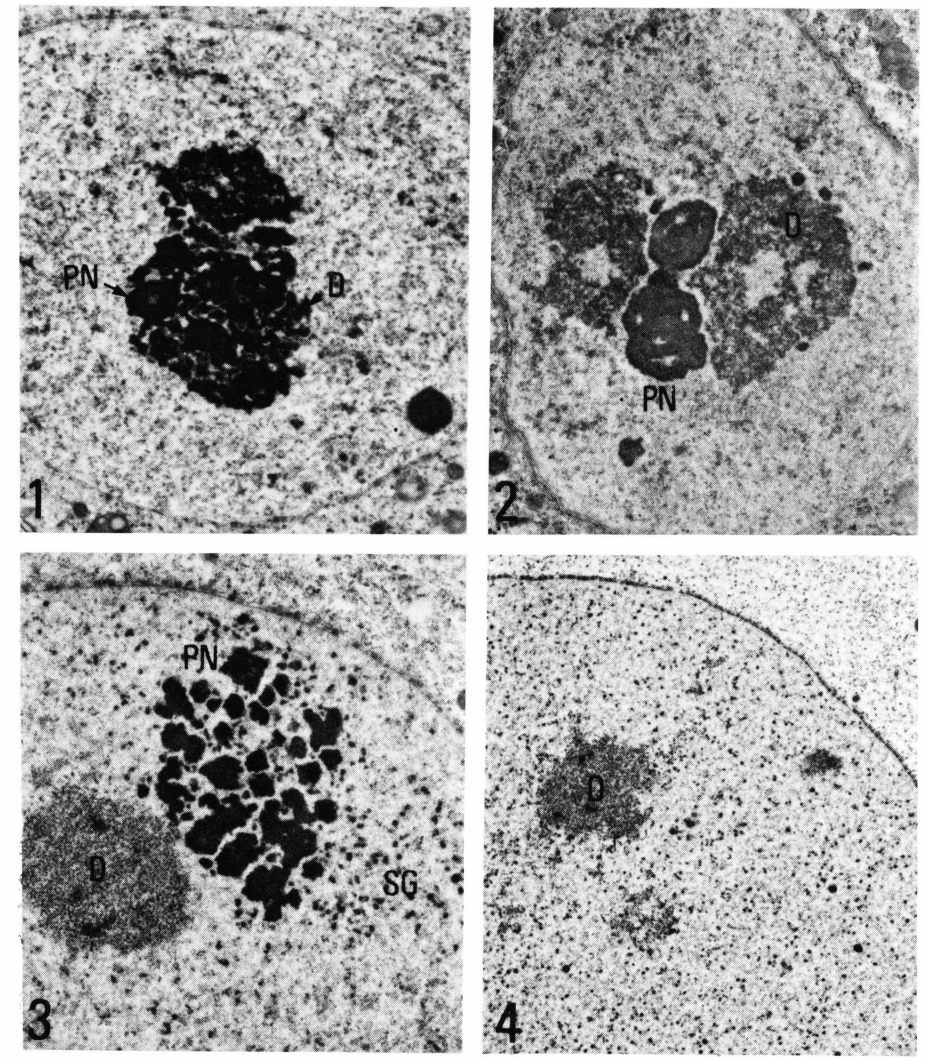


Plate I.

Fig. 1. Oocyte nucleus at stage I. A large complex consisted of DNA-containing body (D) and primary nucleolus mingled with each other. X8,300.

Fig. 2 Oocyte nucleus at stage II. DNA-containing body with a single cavity in its center (D) and primary nucleolus (PN). X7,200.

Fig. 3. Oocyte nucleus at early stage III. Primary nucleolus migrates to the one side of the nucleus and there breaks into small granules (SG). X9,200.

Fig. 4. Oocyte nucleus at mid-stage III. DNA-containing body also breaks into several fascicles and soon disappears. X3,500.

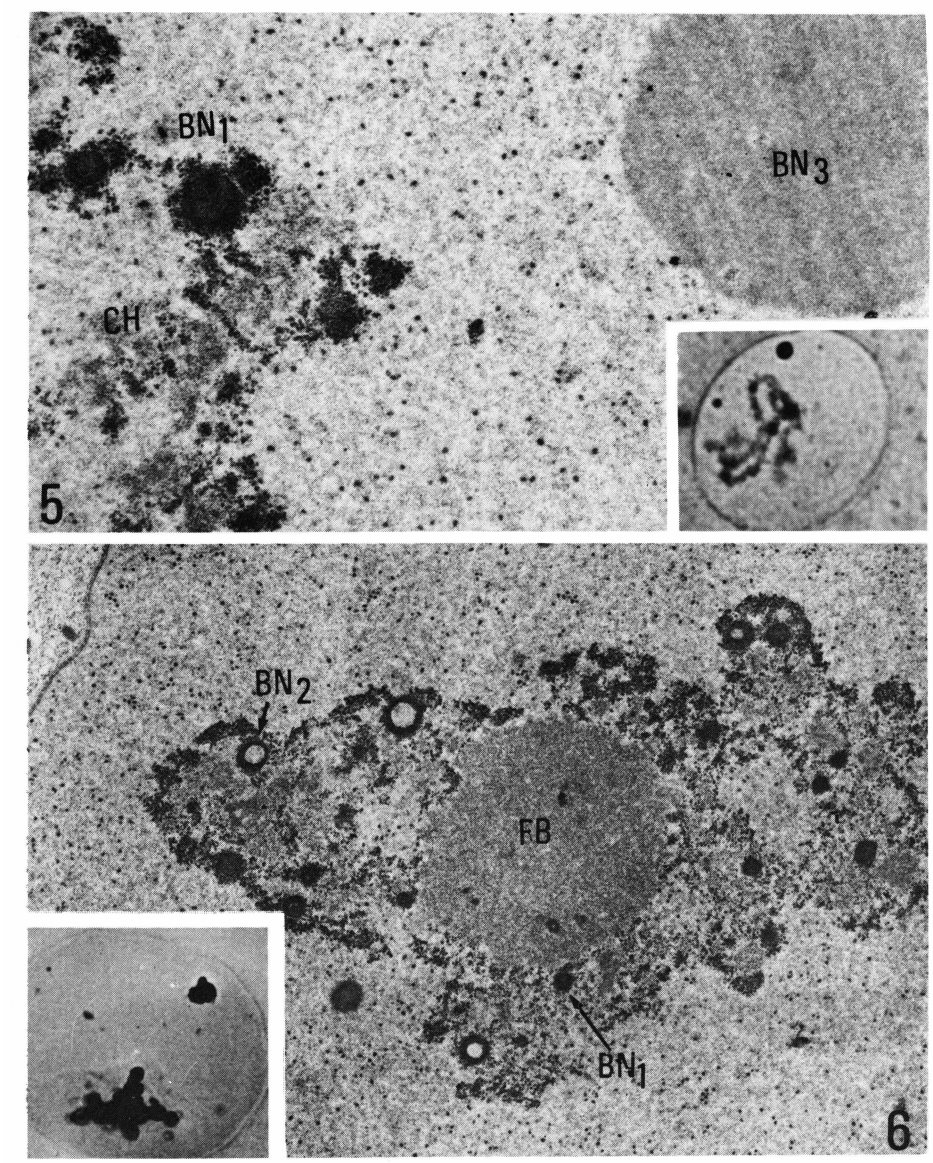


Plate II.

Fig. 5. A part of karyosphere and electron-light sphere (BN3) at stage III. CH condensed chromosome, BN1 electron-dense budding nucleolus. X 9,900. Inset shows total aspect of karyosphere. X760.

Fig. 6. Karyosphere at late stage III. FB endobody-like fibrous body, BN1 and BN2 electron dense and ring shaped budding nucleolus. X 4,500. Inset shows total aspect of karyosphere. X760.

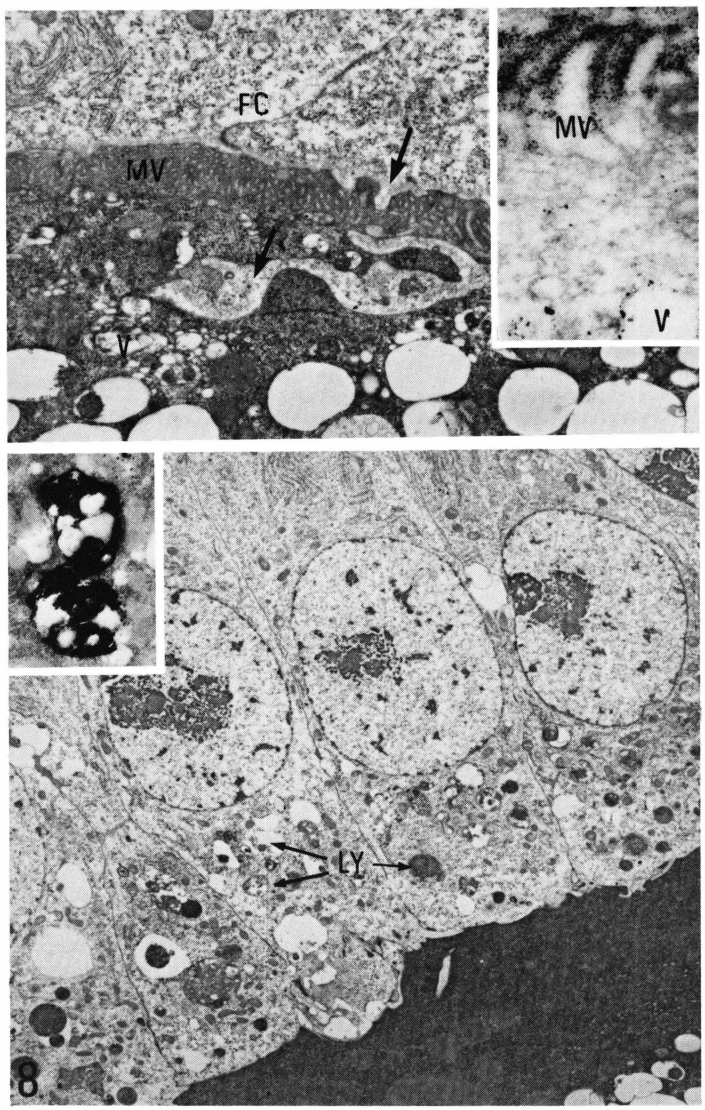


Plate III

Fig. 7. Follicular cells and oocyte at the early phase of oosorption. Microvilli of the follicular cells become unclear and cytoplasmic projection elongate into the oocyte (arrows). The groups of many small vesicles (V) appear at the cortical cytoplasm of the oocyte. X 8,600. Inset shows acid phosphatase in microvilli (MV) and in small vesicle in the oocyte (V). X36,000.

Fig. 8. Follicular cells and oocyte at the early phase of oosorption. Microvilli disappear. In the basal region of the follicular cells many lysosome like vesicles (LY) appear. Cortical cytoplasm of the oocyte is filled with electron dense liquified ooplasm. X 4,300. Inset shows acid phosphatase in lysosome like vesicles. X18,000.

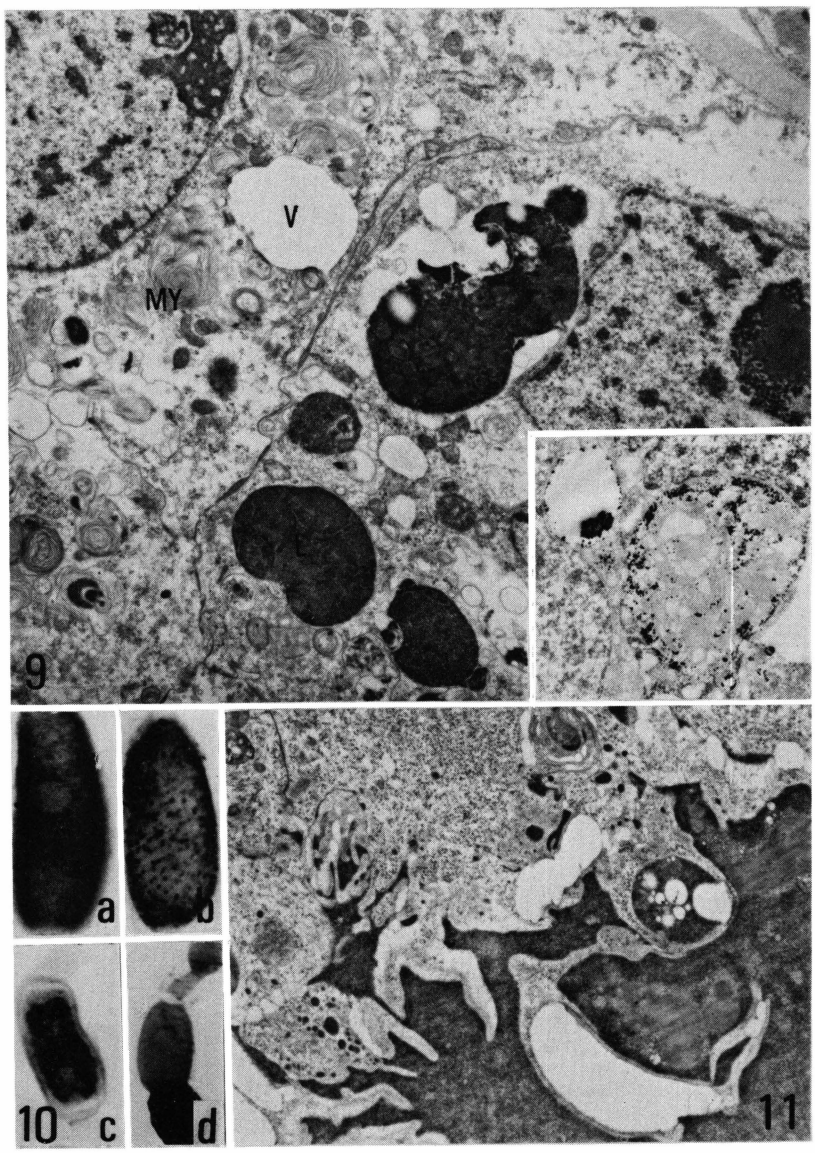


Plate IV.

Fig. 9. Apical region of the follicular cells at the late phase of oosorption. Myelin figures (MY), vacuoles of various sizes (V) and deformed lipid droplets (L) in the cytoplasm. X7,500. Inset shows acid phosphatase in large lysosome-like structure. X6,000.

Fig. 10. Trypan blue uptake in oocytes. a: normal, b–d early, mid-, and late oosorption.

Fig. 11. Basal region of the follicular cells. Well-developed cytoplasmic projections extrude into the liquefied ooplasm. X9,000.

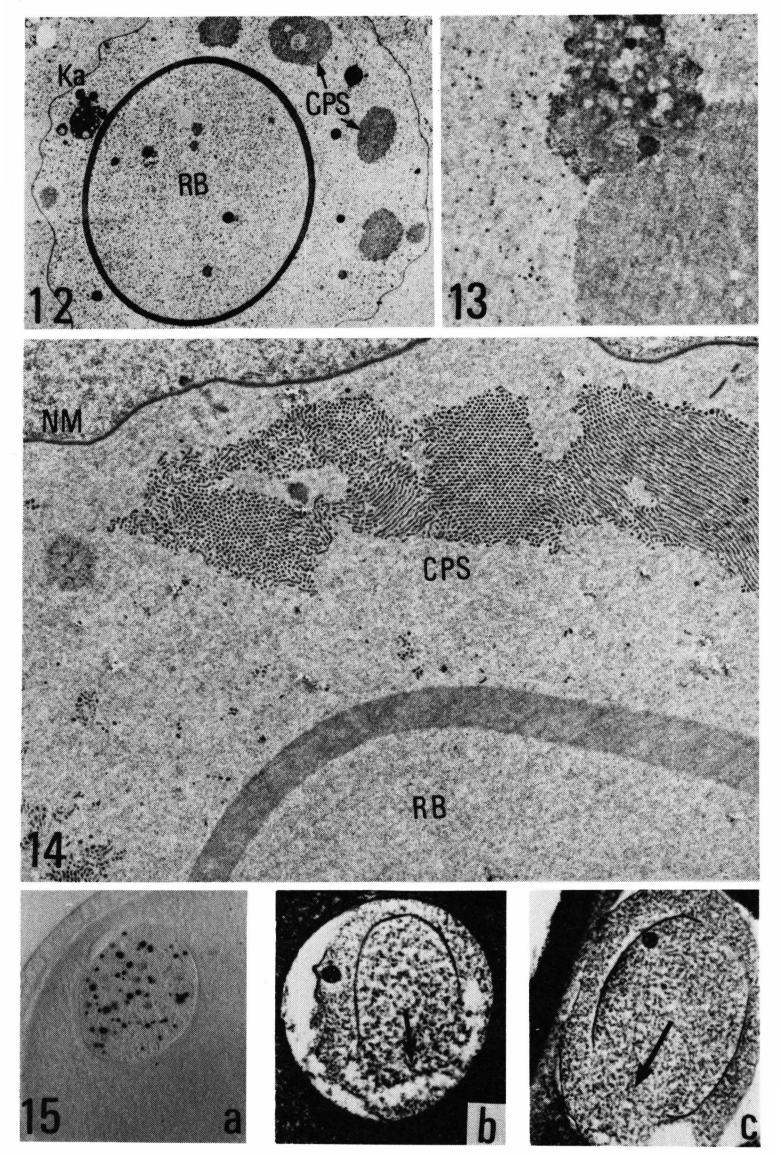


Plate V.

Fig. 12. Oocyte nucleus at the early phase of oosorption. Ka diminished karyosphere, RB ring shaped body (enlarged endobody) with thin envelope, CPS crystallized proteinous structure, NM nuclear membrane. X950.

Fig. 13. Diminished karyosphere at the early phase of oosorption. X5,700.

Fig. 14. Fine structure of ring shaped body (RB), and crystallized proteinous structure (CPS). NM nuclear membrane. X3,700.

Fig. 15. Light micrograph showing disintegration of ring-shaped body. a Fragments of the envelope b and c Discontinous portion of the envelope (arrows). X450.

Discussion

Peculiarity of oosorption process

In *H. vigintioctomaculata,* oosorption process can be divided mainly into two significant phases; the preoosorptive and the oosorptive. During the former phase, oocytes slightly grow and sustain the reversible ability for growth, although a typical abnormality, enlargement of the endobody, appears in the germinal vesicle. In the latter phase following the former one, however, the oocyte growth stops and then oosorption progresses irreversibly even if the starved females would be returned to the adequate conditions for oocyte growth, such as sufficient food supply under the long photophase. This slow growth of the oocyte is indicated well by the low synthetic activity of RNA and proteins by the trophocytes as shown by the decreasing stainability for methylgreen-pyronine in *H. vigintioctomaculata* (Kurihara, 1968) and for Ponsau S or acridine orange in *Rhodnius prolixus* (Pratt and Davey, 1972a–c), and decreasing incorporation of H^3-uridine in *Blatta orientalis* (Sams, 1975). Such transient growth seems to be in general; previtellogenic growth proceeds slowly and then oosorption occurs just before proteinous yolk deposition. For example, in the starved mildweed bug, *Oncopeltus fasciatus,* the previtellogenic oocytes develop until β and γ stages (probably nearly late previtellogenic) before degeneration (Johansson, 1958), and in the allatectomized or the starved *R. prolixus,* the oocytes continue to develop until disappearance of the nutritive cord jointing the oocyte and the trophocytes (Vanderberg, 1963).

Timing for onset of oosorption

As to the timing for onset of oosorption in vitellogenic oocytes, the more delicate differences have been observed not only in the species belonging to the different orders but among the closely relating species. In some starved cockroaches, resorption of the oocytes is characterized by different patterns; it occurs simultaneously with beginning of vitellogenesis in *Brysotria,* while vitellogenic growth does not initiate in *Leucophaea* (Roth and Stay, 1962). Otherwise vitellogenesis continues within 10 days of starvation and then resorption occurs in *Periplaneta* (Bell, 1971), while in *Blatta* vitellogenic arrest continues during 15 days of starvation and then oosorption initiates (Sams, 1975). Therefore, Sams (1975) concluded that, in general, different insects evolve different strategies to cope with period of starvation, the strategy of *Blatta* is to arrest vitellogenesis and to maintain viable oocytes for longest as far as possible without initiating oosorption.

In *H. vigintioctomaculata,* the longer starvation is continued, the less oocytes are able to resume vitellogenesis. At least, within the 2 days-starvation, all of the basal and penultimate oocytes can switch to redevelop, while in all oocytes of the 8 days-starved females oosorption always proceeds. The days required for preoosorptive phase in the basal oocytes seem to be about 2 days in the older oocytes and 3 days in younger ones, respectively. During this phase, the oocytes in the starved females may initiate or resume the vitellogenesis according to the age of oocytes as shown in Text figure 2.

Function of the follicular cells at oosorption

As to the mechanism of breakdown of yolk sphere during oosorption, two possibilities have been discussed; one is phagocytic activity by the follicular cells, and the other lysis by enzymes contained in lysosome-like bodies within the oocytes (Bell and Bohm, 1975).

In normal vitellogenesis, the follicular cells may secret an important protein relating uptake of vitellogenin. Anderson and Telfer (1970) clarified that in cecropia moth, the follicular cells produce H^3-histidine labelled secretion product into the intercellular spaces. In follicles treated with trypan blue, the dye is bound in large amounts in the extracellular spaces and as the result, vitellogenesis stops. Removing the bound dye yolk deposition can be reversed. Therefore they suggested that this protein seems to be a necessary agent in the pinocytotic uptake of blood proteins. Such protein synthesis of the follicular cells may cease at oosorption process. In *B. orientalis*, first sign of the functional changes of the follicular cells is the termination of protein synthesis (Sams, 1975).

Based on electron microscopy of the allatectomized colorado beetle *Leptinotarsa descemlineata*, De Loof and Lagasse (1970) showed that the first sign of oosorption is the accumulation of large quantities of electron-dense materials, lysosome-like bodies, between the microvilli of the follicular cells and the oocyte, and then these materials migrate into the oocyte. Thereby, they suggested that supplying the precursor of lysosomes into the oocyte is a role of the follicular cells in the oosorption of this insect.

The first step of oosorption in *H. vigintioctomaculata* is characterized by the disappearance of the microvilli of the follicular cells and the oocyte like in *L. descemlineata*. Prior to the morphological changes, large amount of acid phosphatase begins to accumulate rapidly in the microvilli, as soon as oosorption initiates. This enzyme may relate to both breakdown of the microvilli and rapid dissolution of the cortical ooplasm. The increment of the same enzyme has been observed in the periphery of the oocytes in *Nasonia vitripennis* taken out from the host (Hopkins and King, 1964) and in *Schistocerca gregaria* (Lusis, 1963).

After the disappearance of the microvilli, the cytoplasmic projections begin to extrude into the oocyte. Finally, the cytoplasm of the follicular cells is filled with large amount of residual structures, such as deformed proteinous yolk spheres and lipid droplets attacked by lysosome-like bodies, myelin figures, large vacuoles in various sizes and membranous structures *etc.* (Kurihara, 1981). These phenomena may indicate that the follicular cells not only autolyze themselves but also supply of the enzyme for dissolution of the cortical ooplasm and yolk spheres and thereafter, they uptake and resorb actively the dissolving ooplasm and yolk spheres by their phagocytic activities.

Function of the endobody and significance of its enlargement at oosorption

The endobody has been first described in several insects by Bier *et al.* (1967). Similar structures were also found in *Drosophila* (Mahowald and Tiefert, 1970; Kinderman and King, 1973), *Chrysopa* (Gruzova *et al.*, 1972; Matsuzaki, 1978). Up to this time, however, the function of the endobody in the normal oogenesis remains unsolved. Normally, the endobody does not synthesize the nucleic acids (Bier *et al.* 1967, Mahowald and Tiefert, 1970). Bier *et al.* (1967) suggested that the endobody may be a storage site of

nucleoproteins because of its active incorporation of H^3-leucin. However, affirmative results could not be obtained in other insects studied thereafter.

In *H. vigintioctomaculata,* the endobody contains no nucleic acids but is rich in acidophilic proteins and phospholipids (Kurihara, 1975). The fine structure closely resembles to that in carabid beetles; it consists of only fibrous component (Kurihara, 1976).

Now, the greatest care must be taken about the difference of growth and disintegration of the endobody among the insect species. During mid- to late previtellogenesis in *H. vigintioctomaculata,* a number of electron light spheres (BN3) bud off from the surface of the endobody-like fibrous body constructing the karyosphere and disperse throughout the nucleoplasm. Ultimately, they diminish in number and disappear at the late vitellogenesis, while in *Drosophila melanogaster* and *D. immigrans,* as studied by Mahowald and Tiefert (1970), the endobody disappears prior to vitellogenesis. Besides, even in the genus *Drosophila,* the endobody of *D. virilis* remains until the beginning of metaphase I in stage 12 (Kinderman and King, 1973). In the panoistic ovary, on the contrary, it continues to increase in size until the end of oogenesis (Bier *et al.,* 1967). The changes of the endobody in *H. vigintioctomaculata* which has the telotrophic ovary are similar to the changes in the panoistic ovary rather than those in the polytrophic one. Although the origin and functional significance of the endobody are not yet quite clear either in *H. vigintioctomaculata* or in other insects such difference in development of the endobody must be an interesting and important problem relating to the functional difference of RNA synthesis of the germinal vesicle between the panoistic and meroistic ovaries. So, as pointed out by Mahowald and Tiefert (1970), the function of the endobody may be not always the same in different insects, even if the fine structure is resemblant.

In *H. vigintioctomaculata,* when the females were starved, enlargement of the endobody in the germinal vesicle is a drastic and first visible sign of the initiation of oosorption. It is a really hollow sphere with a homogeneous thin envelope lipoid in nature and is lastly formed only one in the germinal vesicle. Since the function of the endobody is not known also in any insects, the physiological significance of the enlargement of the endobody in the early phase of oosorption can not be discussed exactly here. However, it is pointed out that such abnormality in the germinal vesicle does not affect the fertilization and the further embryogenesis, because almost all of the redeveloped eggs complete their embryonic development, although many of them can not hatch. Therefore the enlargement of the endobody may indicate that a temporary storage of the nucleoproteins for reversible usage when conditions will become again favorable for vitellogenesis.

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