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Anatomical and Histological Studies on the Germinal Vesicle in Degenerating Oocyte of Starved Females of the Lady Beetle, *Epilachna vigintioctomaculata* MOTSCHULSKY (Coleoptera, Coccinellidae)*

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Synopsis In the oocyte of *Epilachna vigintioctomaculata* the normal development of germinal vesicles was characterized by the existence and the disintegration of the primary nucleolus and the DNA-containing body in early diplotene stage, in addition, by the karyosome formation and the development of the endobody in the advancing growth of oocytes. In the germinal vesicle of the starved females, no particular change was recognized until the stage of karyosome formation. Subsequently, two degenerating structures, namely, a large ring shaped body and alveolate structures, appeared in the germinal vesicle prior to the ooplasmic degeneration. Origin and formative process of both structures are discussed.

Introduction

As to the change during the oocyte resorption, many investigations have been reported, particularly on the relation between the inactivation of corpus allatum and the failure of proteid yolk formation (BELL and BARTH, 1971; ENGELMANN, 1970; JOHANSSON, 1958; WIGGLESWORTH, 1936; DE WILDE, 1964), on the changes of ooplasm (HOPKINS and KING, 1964; LŪSIS, 1963), and on the functional changes of follicular cells to resorb ooplasm (LŪSIS, 1963; DE WILDE, 1964).

Recently, DE LOOF and LAGASSE (1970) reported an interesting fact in the allatectomized Colorado beetle that the dissolution of proteid yolk is not performed by a phagocytic activity of the follicular cells, as already known in the previous investigations, but by lysosome-like bodies which appear abundantly at the periphery of the oocyte.

Up to this time, however, no observations have been made on the abnormality of germinal vesicle in the degenerating oocyte of insect species. The author mentioned in the previous papers that a considerable change occurred in the germinal vesicle during the degeneration of oocyte and suggested an abnormality of the nuclear function (KURIHARA, 1967, '68). The aim of this study is to clarify anatomically and histologically the changes in the germinal vesicle of degenerating oocyte, in comparison with those of the normal one.

* Studies on oogenesis of the lady beetle III.

Materials and Methods

Newly emerged females were reared at 25°C and under 16 hours of light per day. Some of the mature females were vivisected immediately after the first oviposition, and the removed ovaries were used to the study of the normal oogenesis. Almost all remnant females oviposited were transferred to 8 hours of light and starving condition with only supplying water. Subsequently, they were vivisected at the intervals of 24 hours to observe the process of degeneration in the oocytes.

Anatomical observations:— The ovaries were fixed with CARNOY's fluid (6: 3: 1) overnight and stained with carbol-thionine, dehydrated and mounted in ceder oil.

Histological and histochemical observations:— The following staining techniques were used. MALLORY's staining after fixation with GILSON's fluid and haematoxylin-eosin staining were mainly used to observe the morphology of germinal vesicle. To detect the nucleoli, ALTMANN's staining with CHAMPY's fixative and toluidine blue staining with 1% osmium tetroxide fixative were also effective. For detection of the nucleic acids, KURNICK's methylgreen pyronine staining, azure B staining by FLAX and HIMES' method, and Feulgen reaction were applied. To detect RNA, a 1/5,000 dilution of crystalline ribonuclease (Worthington Biochemical Corp.) in distilled water was used at 55°C for one hour.

Results

Normal oogenesis

Developmental stages of oocytes

To describe the development of oocyte nuclei during the normal oogenesis, the oocytes were conveniently divided into the following five stages according to their morphological peculiarities.

Stage I:— The youngest oocyte restricted at the posterior region of the germarium. The oocyte has a large spherical diplotene nucleus, but poor in ooplasm.

Stage II:— The younger oocyte at the anterior zone of the vitellarium. Prefollicular nuclei crowd around the oocyte but do not arrange themselves to form a single epithelial layer. Ooplasm more or less increases.

Stage III:— The previtellogenic oocyte at the middle of the vitellarium. Ooplasm largely increases because of the supply of nutrient substances through the nutritive cord from the nurse cells which are restricted to the germarium. Follicular cells form a single epithelium.

Stage IV:— The vitellogenic oocyte at the terminal region of the vitellarium. The proteid yolk begins to deposit from the periphery of the oocyte and fills in the ooplasm at the end of this stage when the oocyte comes to maturity.

Stage V:— The completed eggs with chorion. Follicular cells degenerate.

Development of the germinal vesicle

As is evident from Table 1, the growth of germinal vesicles is very conspicuous at stage III, during previtellogenesis, while the oocyte growth is rather rapid at stage IV, when the yolk deposition begins. At the middle of stage IV, the germinal vesicle loses the spherical form and becomes crescent. The nuclear membrane becomes partially discontinuous. Finally, the germinal vesicle disintegrates and disappears.

Table 1. Development of oocyte and germinal vesicle of *Epilachna vigintioctomaculata* during normal oogenesis.

Stage of oocyte	Oocyte dimension (μ) Length	Width	Germinal vesicle Diameter (μ)	Morphology
I	10.0–32.5	10.0–15.0	7.5–12.5	spherical
II	40.0–142.5	25.0–85.0	17.4–37.5	—
III	190.0–520.0	130.0–270.0	45.0–107.5	—
IV	520.0–950.0	260.0–330.0	80.0–132.5*	spherical or crescent
V	620.0–1370.0	320.0–500.0	86.0–162.5*	crescent

The data are shown by the average length of every three sections from serially sectioned oocytes.
* Length of longitudinal axis.

Histology of the germinal vesicle

In the ovarioles of the mature larvae, the cylindrical germarium is filled with numerous round nuclei, and the longitudinal proliferation zone is often observed, which runs from the anterior to the posterior region of the germarium. In pupae, the germarium slightly elongates and the protoplasmic nutritive chamber containing only fine RNA rich granules, as described BONHAG (1958), is formed in the posterior region of the germarium. At this area the nuclei are considerably smaller than those in the upper zone of the germarium, and have delicate chromatin threads which attach to a large round pyronine positive nucleolus at one side. These nuclei may be of the oocytes at leptotene or so-called bouquet stage (Fig. 1). All nuclei existing at the upper or lower zone of the germarium are embedded in a common cytoplasm and any cell boundaries could not be observed.

In the ovarioles of adult females, the germarium grows to form a structure peculiar to this insect, that is, each nurse cell packed tightly in the greater part of the germarium has a large spherical nucleus (ca. 26 microns in diameter) and is delimited 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 apparently and connected with younger oocytes. When the oocyte nuclei reach the diplotene stage, the chromatin threads become more or less shorter and thicker than those at the leptotene stage (Stage I, Fig. 2). Histochemically, one or two Feulgen positive bodies and a pyronine positive mass are detected near the central position of the nucleus (Fig. 3).

At stage II, the oocyte migrates into the anterior zone of the vitellarium, and the Feulgen positive bodies and pyronine positive mass in the large spherical nucleus begin to disperse throughout the nucleoplasm (Fig. 3, OC 2).

At the beginning of stage III, when the disintegration of DNA bodies and pyronine positive mass already finished, the chromosomes become detectable as a long thread-like structure (Fig. 4, CH). During the subsequent progress of previtellogenesis, the chromosomes begin to contract around a round pyronine negative body (Fig. 5, CH). This body rapidly increases in size and reaches maximum volume, 4 microns in diameter, at the middle of stage III (Figs. 4–6, EB). The chromosomes condensed, on the other hand, form a complete karyosome in close contact with the well developing body (Fig. 6, KA). After the middle of stage III, when the oocyte reaches ca. 272 microns in length, a number of small spheres are formed on the surface of a complex which consists of a karyosome and a large pyronine negative body (Fig. 7). Some of the small spheres have a thin cortex with many fine spherules, ca. 2 microns, and others appear to have knobby bodies consisting of many fine spherules. These spheres considerably increase in number in the nucleoplasm through the early phase of vitellogenesis (Fig. 8). Subsequently, they become undetectable, when vitellogenesis has almost finished (Fig. 9). Both the complex and the budding spheres are red by MALLORY or ALTMANN's staining, but show no sign of RNA with azure B or methylgreen pyronine staining. Under careful observation, on the other hand, Feulgen positive mass stained more weakly is found among the RNA negative round bodies consisting of the complex.

At the late stage V, the germinal vesicle becomes crescent, and the nuclear membrane turns partially discontinuous and finally disintegrated.

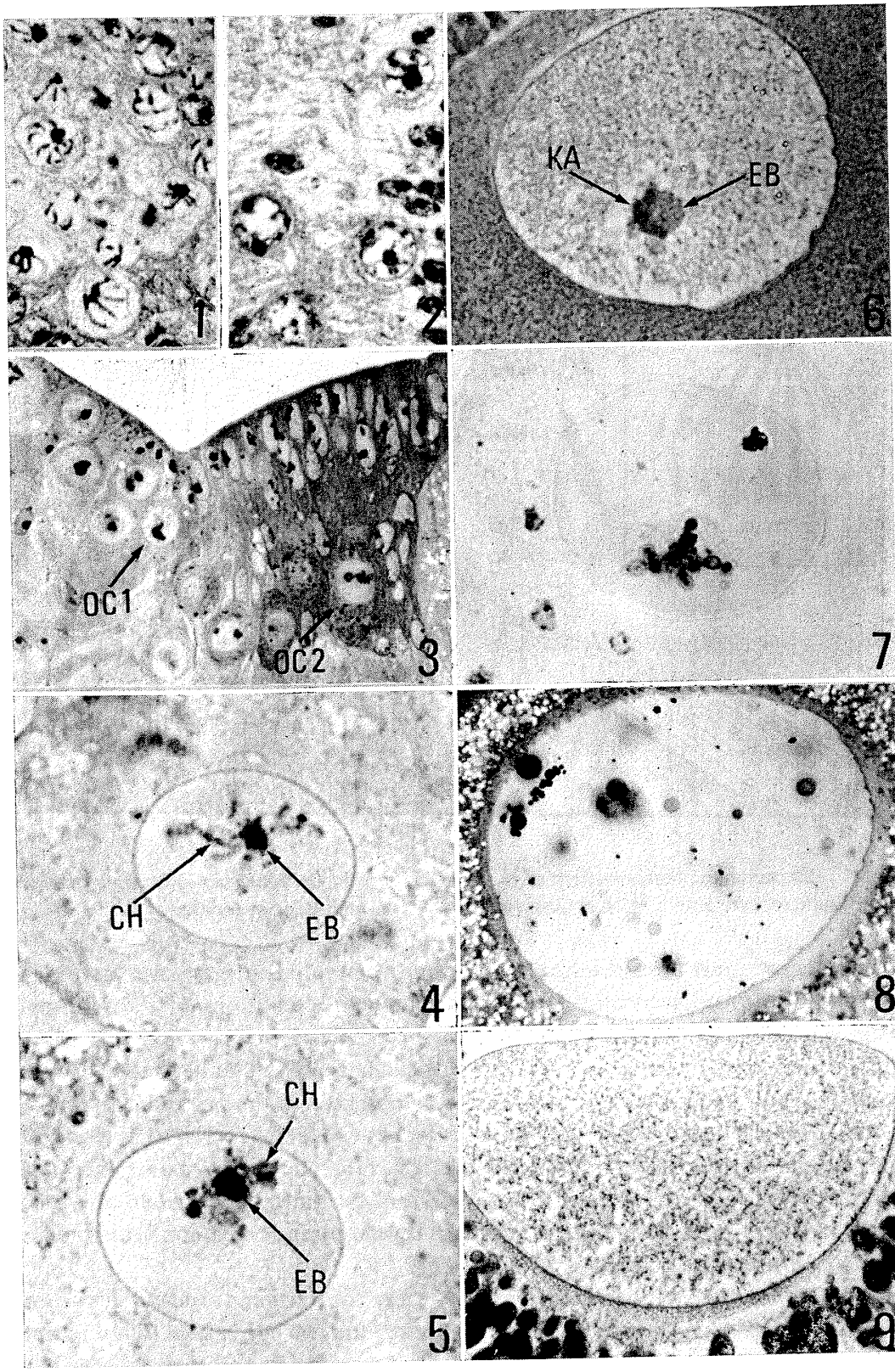
Degeneration of the germinal vesicle

Anatomy of the germinal vesicle during the degeneration of oocyte

1) Ovarioles of the females just after the first oviposition.

Each ovary of this insect consists of about 35 ovarioles, which attaches to the

Fig. 1. Leptotene nucleus of oocytes in posterior region of germarium of pupal ovariole; haematoxylin, ×1,380. —— 2. Diplotene nucleus of oocytes in posterior region of germarium of newly emerged females; haematoxylin, ×1,380.—— 3. Longitudinal section through posterior region of germarium and anterior part of vitellarium showing successive development of young oocytes; OC1 and OC2, stage I and II oocytes, respectively; toluidine blue, ×830. —— 4. Long thread-like chromosomes (CH) and endobody (EB) in germinal vesicle at stage III; ALTMANN staining, ×1,230.—— 5. Germinal vesicle at beginning of stage III showing chromosomal condensation; ALTMANN staining, ×1,230. —— 6. Well developed endobody and complete karyosome (KA) in germinal vesicle at middle of stage III; MALLORY staining, ×630. —— 7. Budding nucleoli at surface of endobody; ALTMANN staining, ×760. —— 8. Budding nucleoli scattered in nucleoplasm at the end of stage III; toluidine blue, ×580. —— 9. Germinal vesicle at stage IV; MALLORY staining, ×480.



egg calyx with its short pedicel. At the outer portion of the calyx, there exist about 15 ovarioles surrounding the inner remaining ovarioles as a ring-like layer. Development of oocytes begins, at first, in the terminal oocytes in the outer ovarioles and the chorion is formed. When the first matured oocytes have descended to the egg calyx, the terminal oocytes in the inner ovarioles rapidly grow and mature. In such a way, descending of mature oocytes to the egg calyx occurs alternatively in the outer and the inner ovarioles (KURIHARA, 1967, and Fig. 10).

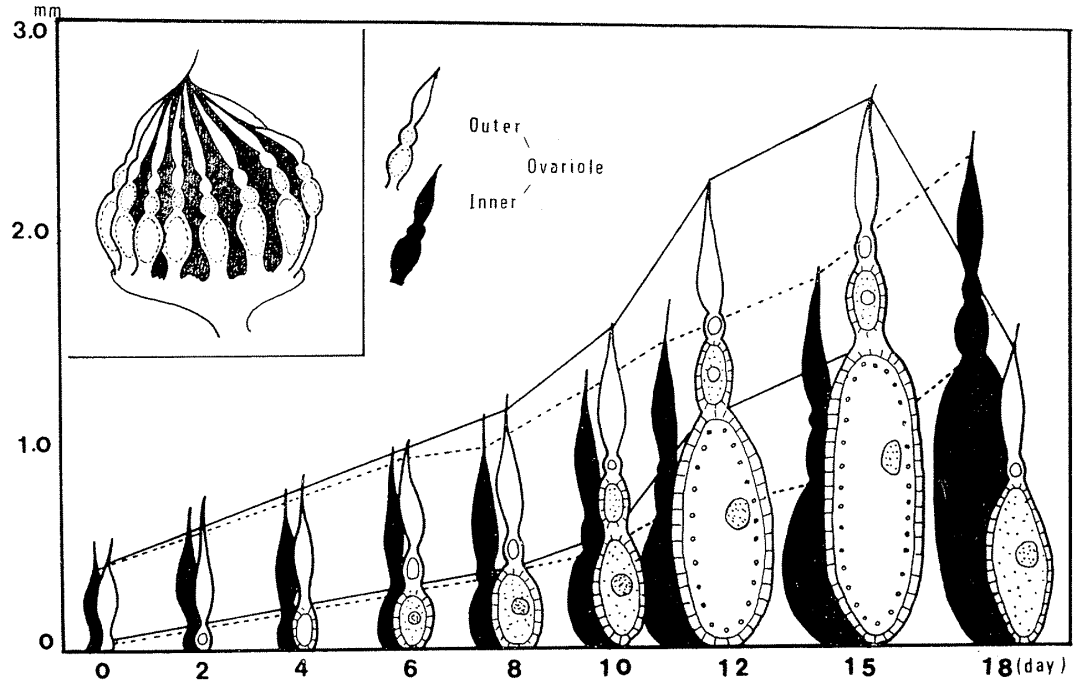


Fig. 10. Diagrammatic representation of normal oogenesis in *Epilachna vigintioctomaculata*. Ordinate, oocyte length in mm; abscissa, day at 25°C and 16 hr-photophase.

In the females just after the first oviposition, therefore, the ovarioles are easily divided into two types according to the developmental conditions of the terminal oocyte in each ovariole.

In the first type (outer ovariole), the elongated corpus luteum that has been oviposited already under the long photophase, is observed in the posterior region of ovariole. The terminal oocyte (E1) nearly reaches stage IV, when it is ca. 650 microns in length and its germinal vesicle is ca. 116 microns in diameter. The second oocyte (E2), ca. 203 microns in length, and the third (E3), ca. 94 microns, reach stage III and stage II, and their germinal vesicles are ca. 36 and 15 microns in diameter, respectively (Fig. 11, A).

In the second type (inner ovariole), however, the terminal oocytes come to maturity and a half of them have already descended into the egg calyx. The second (E2′) and the third oocytes (E3′) reach stage III and stage II and their lengths are

ca. 395 and ca. 101 microns and their germinal vesicles are ca. 79 and ca. 27 microns in diameter, respectively (Fig. 11, B; terminal oocyte is omitted).

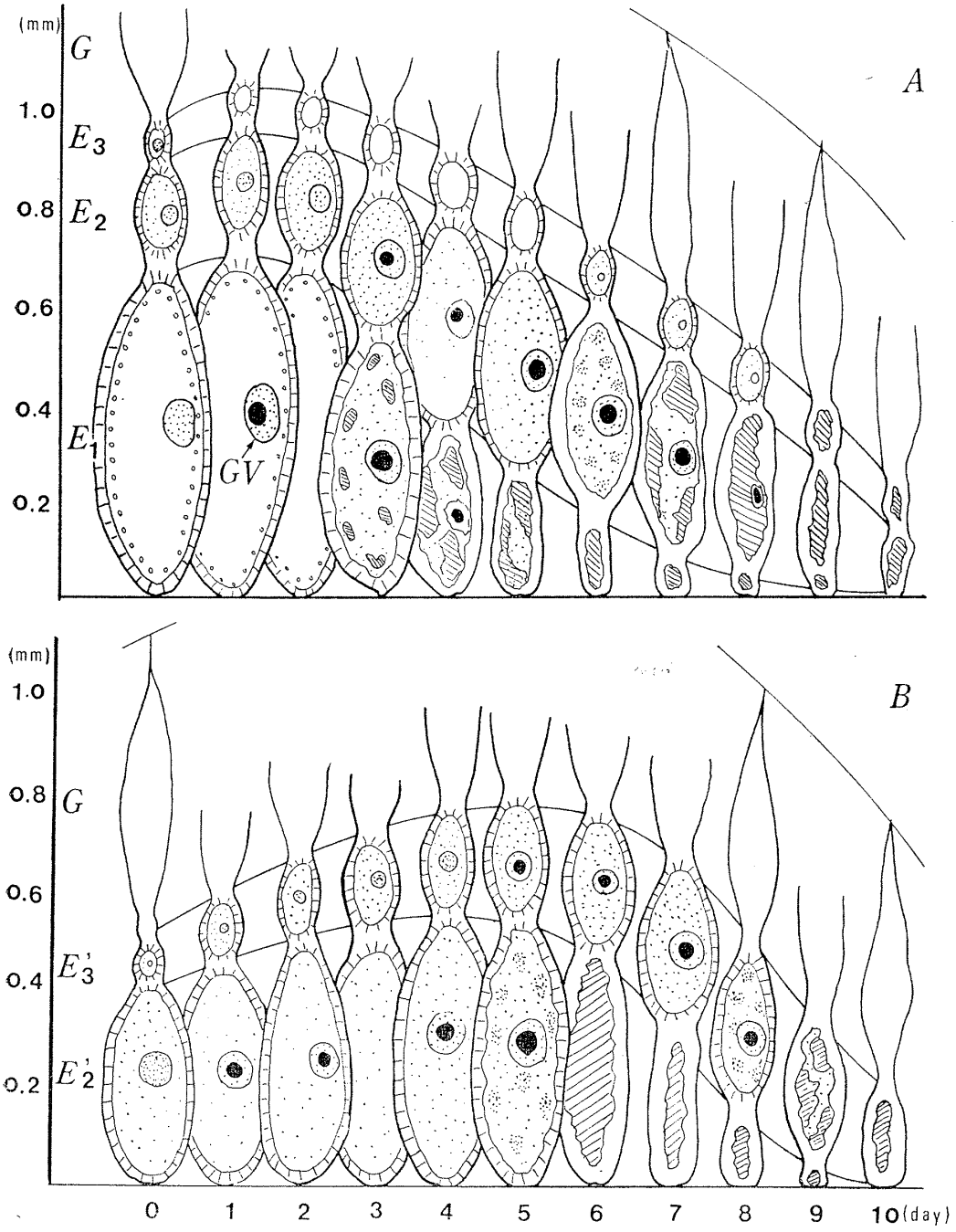


Fig. 11. Diagrammatic representation of degeneration process of oocyte in *Epilachna vigintioctomaculata*. —— A and B, outer and inner ovariole. Ordinate, oocyte length in mm; abscissa, day of starvation at 25°C and 8 hr-photophase; GV, germinal vesicle; dark sphere in GV shows ring-shaped body.

2) Changes occurring in the germinal vesicle.

The females were transferred to the short photophase and the starvation immediately after the first oviposition. The successive changes in the germinal vesicle during the oocyte degeneration were observed anatomically and histologically. The results obtained were illustrated in Fig. 11, A and B.

(a) Changes in the ovariole of type 1 (Fig. 11, A)

About a half of the terminal oocytes (E1) develop normally and descend to the egg calyx during the first day of starvation. The remaining oocytes seem to be almost normal until the second day of starvation, with the exception of some typical changes appearing already in the germinal vesicle one day after starvation. Namely, a large spherical or the ring-shaped body, which is ca. 36.5 microns in diameter and consisting of numerous fine granules stained dirty brown with carbol-thionine, is observed grossly in the germinal vesicle. The ring-shaped body gradually develops until the third day, when abnormality of the oocyte, *viz.*, coagulation of the fine cytoplasmic granules, vacuolation, diffusion and deformation of the proteid yolk, etc. (refer to the previous papers; KURIHARA, 1967, '68), becomes obvious. After the fourth day, the ooplasm becomes almost absent in any degenerating oocyte and the empty follicle forms the corpus luteum. With rapid construction of the corpus luteum, the second oocyte (E2) develops with a remarkable rate until fifth day, and consequently its length becomes twice as long as the initial one. Thereafter, the oocyte begins to degenerate without vitellogenesis and finally its cytoplasm becomes almost empty after ten days. The germinal vesicle of the second oocyte gradually grows with the progress of the development, and reaches a maximum volume at the fourth day (ca. 80 microns). The ring-shaped body, ca. 20 microns in diameter, appears in the germinal vesicle at the third day prior to the ooplasmic degeneration which becomes obvious at the sixth day. Subsequently, the volume of this body rapidly increases and reaches a maximum of ca. 45 microns in diameter, which equals that in the terminal oocyte (E1). As to the changes of the germinal vesicle of the third oocyte (E3), it is difficult to observe anatomically due to its very small size, although degeneration may occur in the slightly elongated oocyte. In this case, the ring-shaped body does not appear. Any fourth oocytes do not migrate to the vitellarium.

(b) Changes in the ovariole of type 2 (Fig. 11, B)

Nearly matured oocytes in the terminal region of the vitellarium (E1′) continue their growth and descend to the egg calyx within a day of starvation (E1′ is omitted in Fig. 11, B). In the previtellogenic growth of the second oocyte (E2′), any abnormality cannot be recognized until the fourth day of starvation, while the ring-shaped body appears already in the germinal vesicle at the first day. After the fourth day, degeneration occurs without vitellogenesis, as same as in the second oocyte in the ovariole of type 1. The third oocyte (E3′) at stage II develops normally and reaches

stage III at the fifth day. When its length increases from ca. 101 to 310 microns, the ring-shaped body, ca. 51 microns in diameter, appears in the germinal vesicle. Further growth of this oocyte continues normally until the seventh day, while the degeneration of ooplasm becomes detectable at the eighth day. Any fourth oocytes do not appear in the vitellarium.

Histology of the germinal vesicle during degeneration

The germinal vesicle of the degenerating oocyte has two distinct morphological structures which can easily be distinguished by MALLORY's staining, that is, one is the large ring-shaped body with reddish color mentioned above, and the other, which cannot be recognized anatomically, is a blue alveolate structure. The alveolate structure, so termed by the author for convenience, appears considerably later than the ring-shaped body.

1) Large ring-shaped body.

The ring-shaped body can be detected only in the germinal vesicle of the degenerating oocyte at or after the middle of stage III. In the early stage of degeneration, one reddish sphere, ca. 10 microns in diameter, appears near the center of the germinal vesicle (Fig. 12, arrow). This sphere has numerus small reddish granules on the surface, and is similar to the round pyronine negative body consisting of the complex which appears in the germinal vesicle at the same stage during normal oogenesis. With the progress of degeneration, this sphere usually develops to a huge ring-shaped body enclosed by a reddish envelope with uniform thickness (Fig. 18). Otherwise, two different forms of this body are observed: one is enclosed in a thick envelope which contains many vacuoles (Figs. 14 and 16) and numerous spheres attach to its surface (Fig. 13), and the other is a large complex consisting of several ring-shaped bodies (Fig. 17). The latter is observable only in the degenerating oocyte with proteid yolk. From the observation by serial sections, the ring-shaped body seems to exist as a hollow sphere in intact state. The contents of this body may be the same as the outer nucleoplasm surrounding the body.

When the degeneration proceeds also in the ooplasm, the envelope is broken and the contents are dispersed throughout the nucleoplasm.

2) Alveolate structures.

These structures are easily distinguishable from the large ring-shaped body according not only to their peculiar structures but to the different stainability in which they are stained blue by MALLORY's staining. It is peculiar that the appearance of these structures is limited only in the nucleoplasm in which the large ring-shaped body is well developed. The prebodies of these structures appear, at first, attaching with the inner surface of the nuclear membrane (Fig. 15), or freely in the nucleoplasm (Fig. 16). As degeneration progresses, these prebodies develop into alveolate structures with many vacuoles containing fine reddish granules (Fig. 19).

In the final stage of degeneration, the germinal vesicle more shrinks and the nuclear membrane becomes partially discontinuous. The condensed structures migrate into the ooplasm and remain there for a long time (Fig. 19).

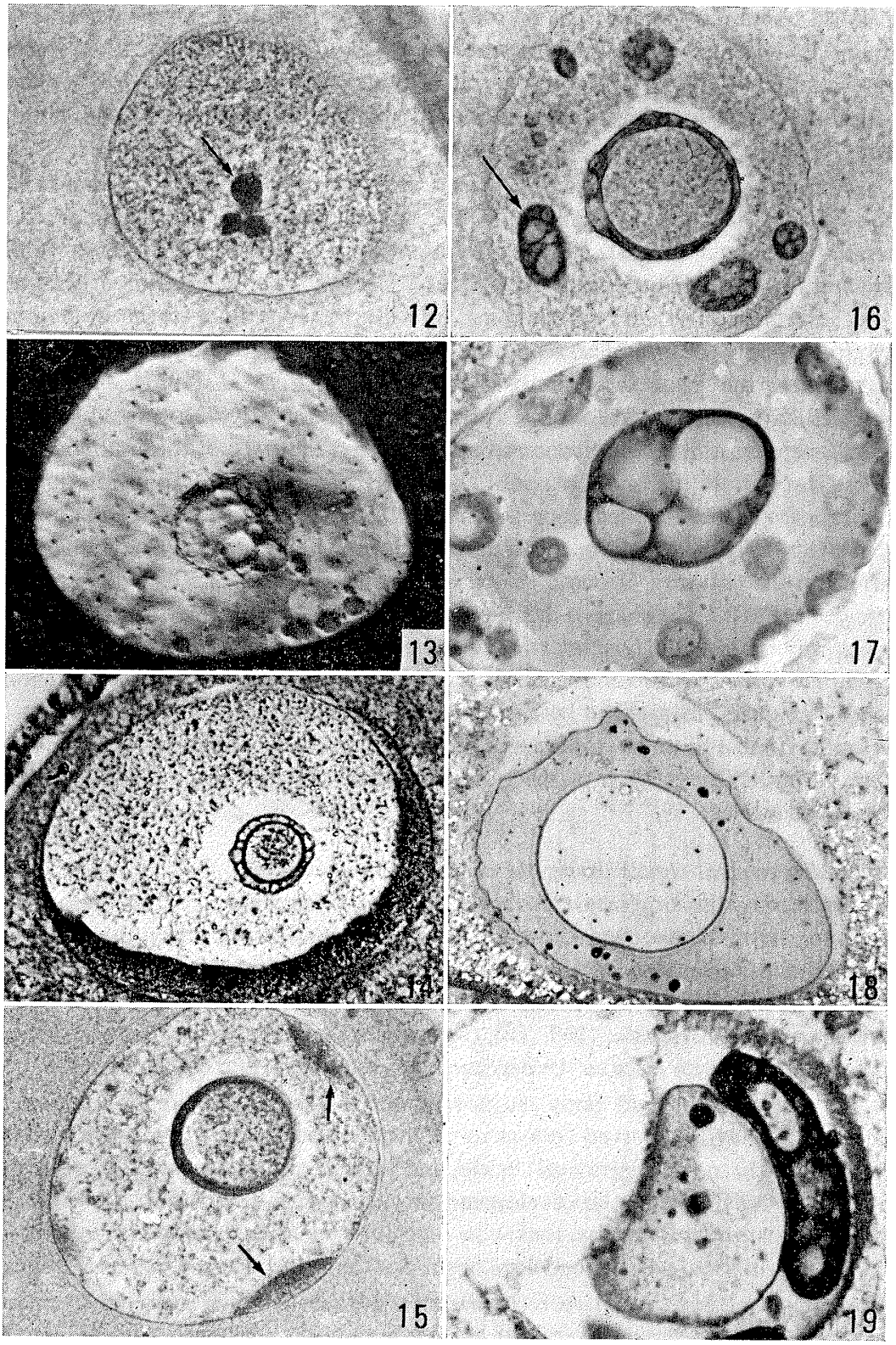
Discussion

Characteristics of the germinal vesicle during normal oogenesis

In *Epilachna*, a diplotene nucleus of the oocyte at the posterior region of the germarium passes through the prophase of the first meiosis during almost all period of oogenesis, as in other insects (SCHLOTTMAN and BONHAG, 1956; SESHACHAR and BUGGA, 1963). Morphologically most remarkable changes in the germinal vesicle are characterized by the appearance and dispersion of primary nucleolus and DNA-containing bodies, karyosome formation, and development of endobody. Such successive changes are commonly observable during the meiotic prophase in some insects such as in *Acheta* (ALLEN and CAVE, 1968, '69), and in carabids (BIER *et al.*, 1967), although some little differences may be noticed morphologically.

In *Epilachna*, a typical RNA-positive nucleolus and DNA-containing bodies exist in the early diplotene nucleus of the oocyte. These two nuclear structures rapidly disappear during the short period of early previtellogenic growth. Generally, the nucleoli break into minute particles and extrude into ooplasm through the nuclear membrane as in *Acheta* and *Chrotogonus* (GUPTA, 1966), in *Blatta* (GRESSON and THREADGOLD, 1962), and in *Periplaneta* (ANDERSON, 1964; GUPTA, 1966). In *Epilachna* although it was difficult to recognize only light microscopically whether the nucleolar materials might pass into ooplasm through the nuclear membrane or not, such phenomenon was suggested indirectly by the fact that the accumulation of fine basophilic granules was induced in ooplasm surrounding the germinal vesicle, as shown in *Acheta, Locusta* and *Chrotogonus* (GUPTA, 1966) and *Periplaneta* (ANDERSON, 1964). This possibility was also enhanced by the electron microscopical findings of the electron dense granules emitted through the nuclear membrane in *Epilachna* (MATSUZAKI, 1964), and by various results in other insects on the nucleolar emission granules (ALLEN and CAVE, 1968, '69; MATSUZAKI, 1971; MULNARD, 1954) or accessory nuclei (HOPKINS, 1964; MAETA and KURIHARA, 1970; PAYNE, 1932). Such morphological phenomenon must be the indication of an important function of the germinal vesicle relating to the ooplasmic storage in the normal previtellogenesis.

Fig. 12. Prebody of ring-shaped body (arrow) in germinal vesicle of starved females; MALLORY staining, ×480. —— 13. Knobby ring-shaped body consisting of many spheres; osmium staining, oblique illumination photograph, ×660. —— 14. Ring-shaped body having vacuolized envelope; MALLORY staining, ×660. —— 15. Prebody of alveolate structures attached to inner surface of nuclear membrane (arrows); MALLORY staining, ×480. —— 16. Alveolate structures formed freely in germinal vesicle (arrow); MALLORY staining, ×480. —— 17. Large complex consisting of several ring-shaped bodies; osmium staining, ×560. —— 18. Ring-shaped body having a thin homogeneous envelope; toluidine blue, ×560. —— 19. Final aspect of degeneration of germinal vesicle; haematoxylin-eosin, ×560.



DNA-containing bodies, on the other hand, are visualized in contact with the primary nucleolus in early diplotene stage as in *Chrysopa* (GRUZOVA, 1972), *Acheta* (ALLEN and CAVE, 1968, '69), and in gryllid crickets (ALLEN and CAVE, 1972). With the disappearance of the primary nucleolus, the bodies also disintegrate rapidly during the short period of previtellogenesis.

After the dispersion of two nuclear structures, the thread-like chromosomes become detectable and they condense to form a karyosome as have been observed in carabids (BIER *et al.*, 1967), *Tenebrio* (ULLMANN, 1973), *Drosophila* (KING *et al.*, 1956) and *Crioceris* (GUPTA and RILEY, 1966). Besides the condensation of chromosomes, several small spheres appear mingling with the thread-like chromosomes and they grow rapidly to form a large body. It is pyronine negative but rich in proteins and resemble the 'endobody' firstly described by BIER *et al.* (1967). Finally, a completed karyosome attaches to the surface of the endobody. During mid- to late previtellogenesis, a number of spheres bud off from the surface of the endobody and disperse throughout the nucleoplasm. Ultimately, they greatly diminish in number and disappear at the late vitellogenesis, while in *Drosophila*, as studied by MAHOWALD and TIEFERT (1970), the endobody disappears prior to vitellogenesis. In the panoistic ovaries, on the contrary, it continues to increase in size until the end of oogenesis (BIER *et al.*, 1967). The changes in the endobody in *Epilachna*, which has telotrophic ovaries, are approximately similar to those of the panoistic ovaries rather than the polytrophic ones. Although the origin and functional significance of the endobody are not yet quite clear either in *Epilachna* or in other insects, such differences in development must be an interesting and important problem relating to the functional difference of RNA synthesis of the germinal vesicle between the panoistic and the meroistic ovarioles.

Changes in germinal vesicle during the degeneration of oocyte

Concerning the ooplasmic changes during the resorption of oocytes, many interesting results have been reported by some investigators. But there are no reports which suggest the causal relation between the abnormality of the function of germinal vesicle and the degeneration of ooplasm. As the author pointed out in previous papers (KURIHARA, 1967, '68), two distinct morphological structures appear in the germinal vesicle prior to the degeneration of ooplasm and follicular cells.

The large ring-shaped body may originate closely relating to the endobody. That is, this body is observed only at or after the same stage as appearance of the endobody in the normal germinal vesicle, and there is often observed the successive changes that may indicate the development of this body. At present, however, the following questions remain unsolved; why only one huge ring-shaped body is formed, and why the ring-shaped envelope develops remarkably with homogeneous thickness.

Although nothing could be recognized on the origin of alveolate structures in this study, a possible formative process of these structures is assumed by the following observations: 1) In the early stage of degeneration, spherical prebodies appear

attaching with budding nucleoli and develop to alveolate structures with the progress of degeneration. 2) Alveolate structures have many vacuoles containing fine granules which show the same stainability as budding nucleoli. 3) These granules may decrease when the alveolate structures well develop at the late stage of degeneration. From these facts, the alveolate structures seem to originate from the budding nucleoli.

These results obtained suggest that some functional abnormalities may occur in the germinal vesicle of degenerating oocyte, *viz.*, the failure of the formation of budding nucleoli on the surface of the endobody or the abnormal condensation of emission granules originating from the budding nucleoli.

Summary

The changes in the germinal vesicle during the degeneration of oocytes of the starved females of the lady beetle, *Epilachna vigintioctomaculata* MOTSCHULSKY were observed anatomically and histologically, in comparison with those during the normal oogenesis.

The early diplotene nucleus of the oocyte contains a primary nucleolus and DNA-containing bodies. Both nuclear structures disintegrate rapidly during the subsequent short period of the previtellogenesis. At the middle of the previtellogenesis, chromosomes begin to condense and form a karyosome. The endobody, on the other hand, appears in contact with the karyosome. With the progress of vitellogenesis, small spheres bud off from the surface of the endobody and disperse throughout the nucleoplasm. Finally, they disappear at the late vitellogenesis, perhaps, by breaking into minute particles.

In the degenerating germinal vesicle, two characteristically distinct structures appear at the time when any degenerative features are not yet observed in the ooplasm or the follicular cells.

1. One large ring-shaped body may be formed by an abnormal enlargement of the endobody.

2. Alveolate structures appear somewhat later than the former body. These structures may originate closely relating with the budding nucleoli.

Both degeneration structures do not react to any cytochemical stainings for detection of the nucleic acids.

From these results, it was emphasized that the abnormality of the nuclear function might occur prior to the degeneration of ooplasm.

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礼文島 *Epilachna* の食草名の訂正

山 崎 柄 根

YAMASAKI, T.: The Correct Name of the Host Plant of *Epilachna* in Rebun Island

本誌 42 巻 2 号 206–207 ページの報文中で，*Epilachna* の食べていた植物名が誤っていたので，ここに訂正する．正しい食草名は，同じキク科のゴボウ *Arctium lappa* である．なお，食草がゴボウだということになると，この葉はオオニジュウヤホシテントウが食ってもきわめてわずかで，葉に小点刻しか残さず，しかも 1 令からこの葉を給与すると全部死亡してしまうという小山の報告 (応用昆虫，**6** (1): 25–35, 1950, および防虫科学，**22** (1): 86–94, 1957) があるだけに，ますます興味深く感じられる．