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Histological Study on the Ovarioles of *Diplonichus esakii* Miyamoto et Lee (Heteroptera)

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각시물자라(*Diplonichus esakii*)의 卵巢小管에 對한 組織學的 硏究

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적 요

각시물자라의 卵巢小管의 관찰결과 두 개의 卵巢 중 各各 다섯개의 端榮養室型의 卵巢小管을 가졌으며, 各 齡마다의 生殖室의 發生狀態는 다음과 같았다.

第4齡에 있어서 生殖室의 上端部位는 미분화된 細胞로 되어 있었으며, 中央部에는 球形에 가까운 榮養束이 있었다. 그리고 下端部位에는 卵細胞가 있었고 그 다음은 鍾을 거꾸로 세운 모양의 prefollicular tissue가 있었다. 그리고 榮養索은 아주 미발달된 狀態였다.

第5齡에 있어서 榮養束은 방추형이며 榮養束 아래에 위치하고 있는 卵細胞는 prefoliicular tissue 內에서 榮養索으로 榮養束과 연결되어 있었다.

成虫에 있어서는 生殖室 다음에 榮養室이 계속되었으며 榮養室內에는 發達과정에 있는 卵細胞로 가득차 있었다. 그리고 yolk granule은 濾胞上皮細胞層으로부터 卵細胞의 內部로 축적되어졌다. 그리고 나중에는 卵細胞質은 완전히 同質性이 되었다.

그러므로 齡이 進行함에 따라 榮養索이 卵細胞에서 卵殼이 생길 때까지 발달되었으며 濾胞細胞는 두개의 核을 가진 狀態로 되었다. 그리고 成虫의 卵巢小管에는 卵殼을 갖지않는 部分과 卵殼을 갖는 部分이 있었다.

INTRODUCTION

There are three types of ovarioles recognized in the world of insects. They are panoistic type, polytrophic type and telotrophic type.

Anderson (1964) reported oocyte differentiation and vitellogenesis in the roach *Periplaneta americana*, as being the ovariole in panoistic type. And Kulshrestha (1970 a, b) reported morpho-histological changes in the ovarioles of *Musca domestica nebulo* Fabr., and histology of the ovarioles and the role of nurse cells in corpus luteum formation in *Philosamia cythia ricini* (Boisd.). Also, Maeta and Kurihara (1971) reported anatomical and histological studies on the oogenesis about *Osmia*. These ovarioles belong to polytrophic type.

However, in Heteroptera, Brunt (1971) reported about the ovarian histology of

Dysdercus fasciatus. And Masner and Landa (1971) reported the formation of compound egg chamber in a bug. These scientists studied ovarioles of the adult insect. But none of these scientists paid any systematic attention to the detailed histological study of the ovarioles in postembryonic development. In the present paper the authors attempt to find morphological and histological changes occurring in the ovarioles during the postembryonic development of a few later instar larvae in *Diplonichus esakii*.

MATERIAL AND TECHNIQUE

The materials, *Diplonichus esakii*, were collected in Baeja Mott pond near Kyungpook National University during July and August, 1971.

Each instar larva was dissected in Ringer's solution, and the fresh ovarioles were fixed in Gendre's fluid for three hours. The tissues were embedded in paraffin (melting point 56-58°C) and cut by the longitudinal axis of the ovarioles 6-8 μ in thickness The sections were stained with Ehrlich's Hematoxylin and Eosin and Pas-PTAH, after McManus and Mowry (1964) and Luna (1968).

OBSERVATION OF THE OVARIOLES

In Heteroptera female there are one pair of ovaries, each comprising from four to seventeen ovarioles (Miyamoto 1957, 1959). Therefore, in *Diplonichus esakii* the profusely tracheated ovaries lie ventro-laterally at the posterior part in the abdominal cavity, each comprising five telotrophic ovarioles and surrounded by fat bodies. The ovaries are milk-like in color, and have two distinct zones: The anterior zone and the posterior zone. In anterior zone there is terminal filament, followed by germarium, and in the posterior zone there is the vitellarium, followed by the pedicel.

The ovarioles through the pedicel open into the lateral oviduct that is united to the median oviduct. Eventually, it is opened to the exterior. And the wall of the ovariole is constituted by two layers at the longitudinal section, the outer layer is entirely surrounded by external ovariole sheath and inner layer is tunica propria.

The Ovarioles of the Fourth Instar Larva

In the fourth instar larva the germarium is measured approximately 1.176μ in length excluding the prefollicular tissue. And in the anterior part it is seen to contain undifferentiated cells of the trophocytes(or nurse cells) and the oogonia (Plate 1, figs. 1, 2). In middle part there is the trophic core that is measured approximately 0.293μ in length. Also, the trophocytes are found near to the core, and each contains large granular nucleus (Plate 1, Figs. 3, 4). Later, membrane of the trophocytes disappears and the nuclear membrane finally breaks down, and it appears that this material moves into the trophic core (Brunt, 1971). The longitudinal section of the trophic core is of a similar form to a sphere (Plate 1, fig. 4).

Observing the posterior part there are oocytes, followed by the upside down-bell shape prefollicular tissue. This form is characteristic of the fourth instar in this species, and the tissue is measured approximately 0.490 μ in length (Plate 1, fig. 1). On both sides and at the base of the trophic

core, there are young oocytes that have large amount of cytoplasm (Plate 1, fig. 4). But the oocyte is not found in the prefollicular tissue (Plate 1, fig. 2). It is connected with the trophic core by the nutritive cord that is almost invisible at this instar larva of development of the ovariole.

The pedicel is a very long tube (Plate 1, fig. 1) and opens into the lateral oviduct, and in this tube nothing else is observed.

The Ovarioles of the Fifth Instar Larva

In the ovarioles of the fifth instar larva, the germarium is measured approximately five to eight μ in length and the anterior part is seen to contain undifferentiated cells as in the former instar larva (Plate 2, fig. 3; Plate 3, fig. 1). In middle part of the germarium there are trophocytes, and trophic core is enlarged and shaped elliptically, measuring approximately 1.764 μ in length. In the posterior part, the developing oocytes arrange themselves irregularly (Plate 2, fig. 3; Plate 3, fig. 1). Also, in the prefollicular tissue, there appear developing oocytes. They are observed as elliptical, spherical and trigonal form (Plate 2, fig. 3). Each oocyte is connected with the trophic core by the nutritive cord found very distinctly, being fibrillar in appearance and originating from the central trophic core. In the prefollicular tissue is found the developing oocyte, but is not observed in the long-tubed pedicel and oviduct (Plate 2, fig. 1).

The Ovarioles of the Adult

In the ovarioles of the adult, the germarium is measured approximately 7-8 μ in length, similar to the fifth instar larva. The anterior part of the germarium is seen to contain undifferentiated cells (Plate 3, fig. 3). While in the middle part there are trophocytes and an enlarged mature trophic core, measuring approximately 3.626 μ in length.

In the transverse section, the core is seen to be situated in the middle of germarium (Plate 3, fig. 4). In the prefollicular tissue of the posterior part there are oocytes, followed by the vitellarium. Observing the adult the oocytes are surrounded by the cells of the prefollicular tissue. As the oocytes grow the prefollicular tissue gradually becomes a thin layer (Plate 4, fig. 1). Eventually, the tissue around the oocyte will transform into the true follicular tissue and interfollicular plug. The latter gradually becoming thinner in the lower part of the vitellarium. In the vitellarium, the oocyte is connected with the trophic core by the nutritive cord that penetrates the layer of the prefollicular tissue and interfollicular masses. The cord seems to be fibrillar in appearance (Plate 3, fig. 3) and is attached to each young and nearly mature oocyte. After the oocyte matures to the chorionated stage, it no longer is dependent upon the nutritive cord (Plate 4, fig. 1).

In the upper part of the vitellarium, the oocytes tend to become wider and become somewhat brick-like in form (Plat 4, fig. 1). As the oocytes come down from the upper part to the proximal part, the brick-form like oocytes gradually change to a spherical form by their position. Finally, they change to an oval-form in the proximal part of the vitellarium (Plate 3, fig. 2).

The pas-positive yolk granules initially originate at the base of the follicular epithelial layer and proceed from the peripheral round into the inside of oocyte (Plate 4, figs. 2, 3, 4). Finally, the ooplasm of the mature oocyte become completely homogeneous(Plate 5, figs. 1, 2). The follicle cells are squamous and binucleated (Plate 5, fig. 3). At this time the oocyte has a chorion, and then vitellogenesis stops, and the follicle cells begin to break down (Plate 5, fig. 4).

Therefore, in the upper part of the ovariole (excluding the germarium) the unchorionated oocytes line up consecutively and the chorionated oocytes follow in the proximal part.

DISCUSSION

From the study of the histology of the ovarioles of *Diplonichus esakii*, each developing oocyte is connected to the base of the trophic core by a nutritive cord. According to the advancing of instars, the trophocytes increase through continual division, that cause the trophic core to enlarge in size. The cord is found in the fourth instar larva, and also in the fifth instar larva and the adult. The cord is observed distinctly and has a fibrillar appearance as seen under the light microscope.

The cocyte of the fourth instar larva is approximately the same size as the trophocytes, and contains large amounts of cytoplasm. In the fifth instar larva, the oocyte is much larger than the other cells and comes down into only the prefollicular tissue. There are filled by the oocytes in the vitellarium of the adult.

In conclusion, the nutritive cord seems to be the mode of transport of the nutritive substances from the trophic core to the oocytes. The nutritive system, the yolk producing cells, and the comparative histology of the ovarioles of each instar larva have to be investigated by histological and histochemical methods.

SUMMARY

In *Diplonichus esakii* each ovary comprises five telotrophic ovarioles. In the fourth instar, the anterior part of the germarium consists of undifferentiated cells. The middle part contains a spherical trophic and the posterior part comprises young oocytes, followed by the upside down-bell shape prefollicular tissue. The bell form is the standard characteristic in this instar larva, and the nutritive cord is found although somewhat indistinctly.

In the fifth instar larva, the trophic core is elliptical form, and the oocyte is found at the base of the core. The oocytes are connected with the core by the nutritive cord. In the prefollicular tissue are also found some oocytes.

In the adult, the vitellarium is filled by the developmental oocytes. The yolk granules inside each cocyte migrate from the base of the follicular epithelial cells to the center of the oocyte. Finally, the ooplasm of the oocyte becomes completely homogeneous. Therefore, according to the advancing of instars, the nutritive cord developes completely before the cocyte has chorion and the follicular epithelial cell binucleates. The upper part of the ovariole consists of unchorionated oocytes, and the proximal part comprises chorionated oocytes in the adult.

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EXPLANATION OF PLATES

Plate I

fig. 1. The Ovarioles of the Fourth Instar Larva.
fig. 2. The Germarium of the Fourth Instar Larva.
fig. 3. The Oocytes finished Meiotic Division in the Fourth Instar Larve.
fig. 4. The Trophic Core and Trophocytes of the Fourth Instar Larva.

Plate II

fig. 1. The Ovarioles of the Fifth Instar Larva.
fig. 2. Longitudinal Section of the Trophic Core in the Germarium of the Instar Larva.
fig. 3. The Oocytes in the Prefollicular Tissue of the Fifth Instar Larva.

Plate III

fig. 1. The Oocyte is Connected with the Germarium by the Nutritive Cord in the Fifth Instar Larva.
fig. 2. The Ovarioles of the Adult.
fig. 3. Longitudinal Section of the Trophic Core in the Germarium of the Adult. The Nutritive Cord is Fibrillar.
fig. 4. Transverse Section of the Germarium of the Adult. The Trophic Core Occupied Center in Germarium.

Plate IV

fig. 1. The Oocytes are Connected with the Trophic Core by the Nutritive Cord in the Adult.
figs. 2-4. The Pas-Positive Yolk Granules Increase in the Oocytes. (fig. 4. The Nucleus of the Oocyte is Surrounded by Yolk Granules).

Plate V

figs. 1-2. The Pas-positive Yolk Granules Increase in the Oocyte, While the Ooplasm of the Larger One is Completely Homogeneous.
fig. 3. Cuboidal Binucleated Follicle Cell of the Oocyte.
fig. 4. The Mature Oocyte of the Adult.

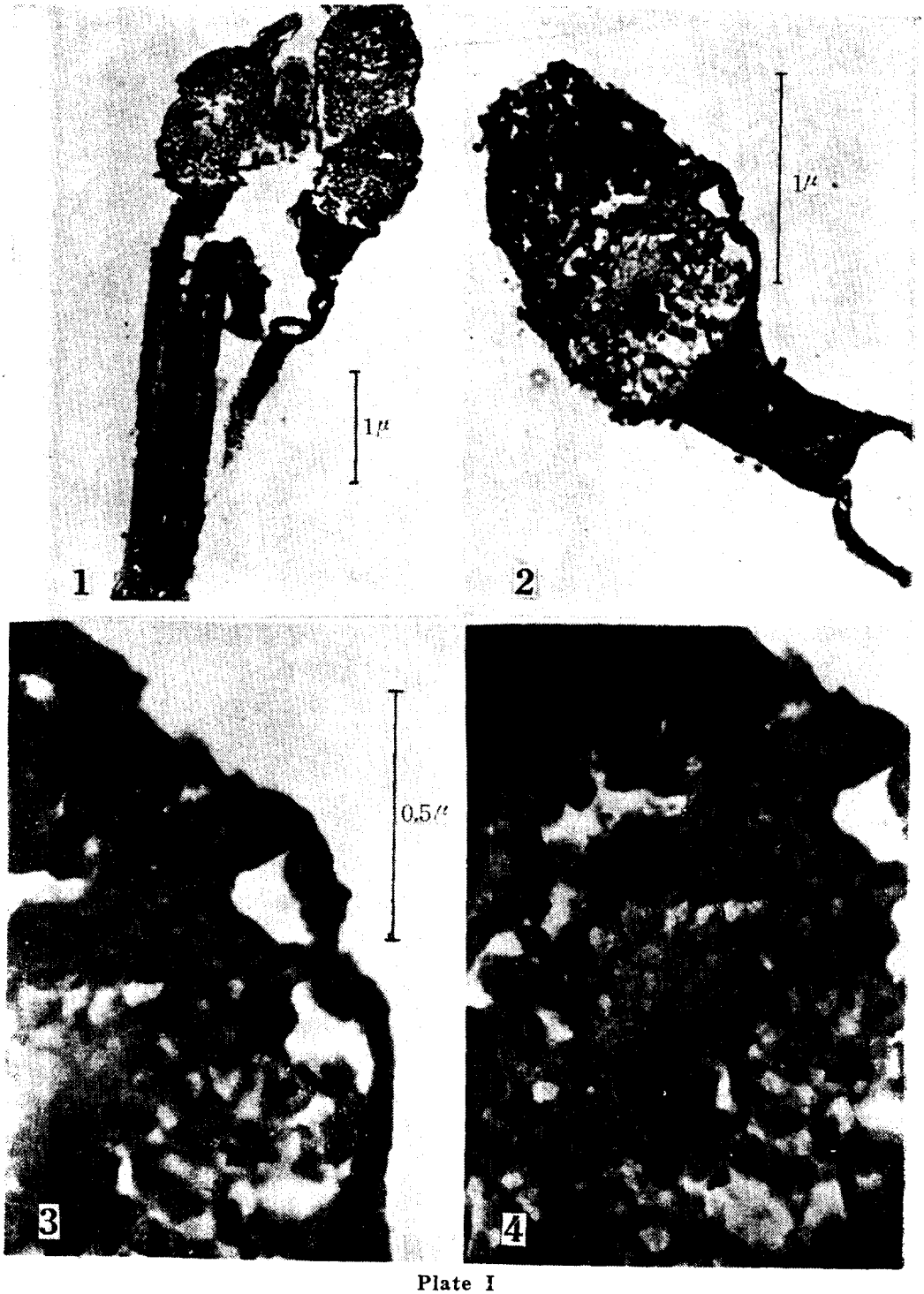

Plate I

Plate II

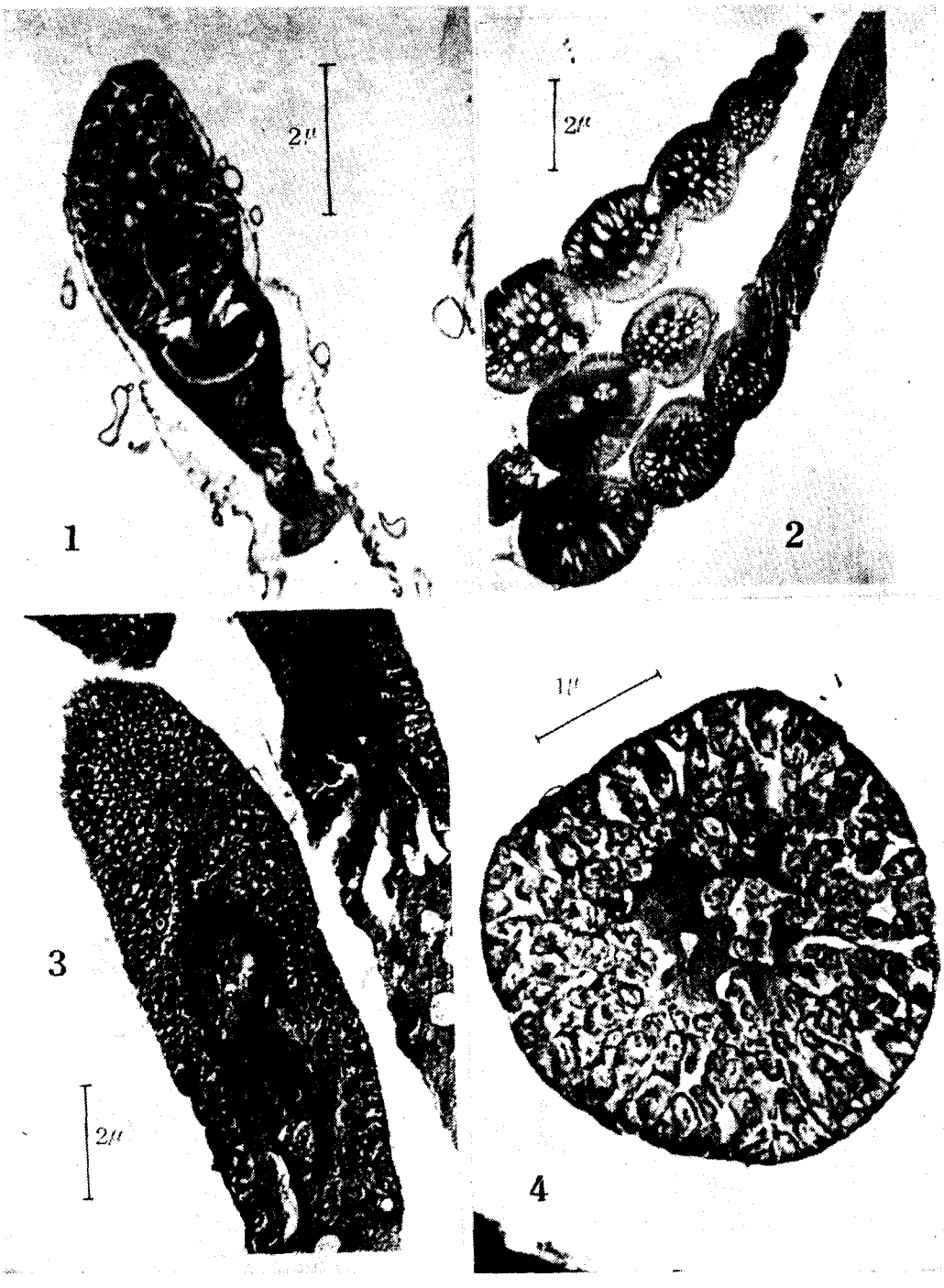

Plate III

Plate IV

Plate V