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A STUDY ON THE FINE STRUCTURE OF THE OVARIUM OF THE LAST INSTAR LARVAE OF AGROTIS IPSILON (HUFNAGEL) (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT:

A pair of ovaries of *A. ipsilon*, at the beginning of the last instar larvae, are spindle shaped. Each of them includes four ovarioles. Ovarioles are covered by some coats. Although profollicular cells and germ cells exist in the ovarioles, the oocytes and the nurse cells are not still distinguishable. Among the germ cells that are being divided successively to produce the oocytes and the nurse cells, there are intercellular bridges which are full of fusome material. The fine structure of ovaries is given with the electronmicrographs.

INTRODUCTION

Agrotis ipsilon is one of the best known species of the family Noctuidae as a pest on the agricultural plants such as sugar beets, grapes, cotton, corn fields in our country as well as in the foreign countries (İren and Ahmed, 1973; Zaazou et al., 1973; Öngören et al., 1974; Tokmakoğlu, 1974; Bushing and Turpin, 1976; İren, 1976; Luckmann et al., 1976; Archer and Musick, 1977 a,b; Jorgensen, 1978; Altınayar, 1981; Dinçer and Pala 1981).

Therefore, this species and other closely related species of the genus *Agrotis* have been the subject of research in wide-scope.

In order to control the insects, it is necessary to have the knowledge of the histology and the fine structure of ovarium and the way of reproduction. After then, the effects of insecticides and chemosterilants on the ovarian structure can be understood.

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The normal structure of ovaries and the events of oogenesis and vitellogenesis of many species of Collembola, Dermaptera, Mecoptera, Megaloptera, Mallophaga, Lepidoptera, Hemiptera, Heteroptera, Coleoptera, Diptera have been studied for years by light microscopy as well as electron microscopy with respect to their cytology, autoradiography, and histochemistry (Perrot, 1934; Bonhag and Wick, 1953; Bonhag, 1956; Bonhag and Arnold, 1961; Bier, 1962; King and Koch, 1963; Ramamurty, 1964; Roth and Porter, 1964; Stay, 1965; Hophins and King, 1966; Pollack and Telfer, 1969; Brunt, 1971; Salama et al., 1971; Cruicshank, 1972; Mahowald, 1972; Matsuzaki, 1973; Theunissen, 1973; Ullmann, 1973; Wightman, 1973; Zinsmeister and Zinsmeister, 1976; Matsuzaki and Ando, 1977; Mandelbaum, 1980).

As to my knowledge, although some researches on the biology of *A. ipsilon* have already been done, the histology and the fine structure of the ovarium of this pest have not yet been studied (Mansour and Dimetry, 1972; Reese et al., 1972; Fahmy et al., 1973; Zaazou et al., 1973; Archer and Musick, 1976, 1977 a, b; Mansour and Salem, 1977).

The aim of this study is to determine the fine structure of the ovarium of *A. ipsilon*. The last instar larvae have been selected for this study since this is the instar larvae just before the differentiations of nurse cells and oocytes occure.

MATERIAL and METHODS

The larvae of *A. ipsilon* have been collected from the fields of sugar beets near Polatlı (Ankara).

During the process of growing of larvae (each contained 50 specimens) it was observed that there was a severe cannibalism as it was mentioned by Reese et al. (1972) and Mansour and Dimetry (1972). Therefore, this method was left. The first two larval instars were kept in Petri dishes, but after moulting to third instar each of the larvae was kept separately. During the first two instars the larvae were exposed to day light for 10 hours and kept in dark for 14 hours. Later, except the time of cleaning and feeding, all the larvae, pupae

and mature specimens were bred in complete darkness at 26 ∓ 1 °C (Luckmann et al., 1976). The humidity was 60 ∓ 10 % (Fahmy et al., 1973).

During the larval period, all specimens were fed on the leaves and rhizomes of sugar beets. Synthetic diet has not been used. When the sugar beets were grown in the greenhouse of the University, extensive care was taken in order not to let them to be contaminated by insectisides or herbicides. Always fresh and clean-washed food was used and the jars were kept clean.

The moths were placed in jars by 5 to 6 pairs together. Some filter paper bands were dropped down in the jars so that the females could lay eggs. The jars were covered with gauze. As a source of food, cotton pieces soaked in 10 % honey solution were given to the moths. The eggs were transferred to the Petri dishes and kept in the same condition.

A. ipsilon larvae have seven instars. After hatching, they reach to the last larval instar in 15 days. So, in the experiments of all the larvae were 15 days old. The eggs of *A. ipsilon* hatched in 4–5 days. The development of moths from the hatching stage requires approximately a month.

Larvae were dissected and ovaries were removed and then washed in natrium phosphate buffer (0.02 M and pH 7.2; Hayat, 1970). The first fixation in 5 % Gluter aldehyde in natrium phosophate buffer (0.02 M and pH 7.2) took an hour. The ovaries were washed in 7.5 % sucrose in the same buffer for 10–12 h., and then transferred to 1 % OsO_4 in the same buffer for an hour for the second fixation. After several changes of sucrose buffer, saturated uranyl acetate (in 50 % ethanol) was used for the third fixation and block staining for an hour. During all treatments the temperature was at + 4 °C. After dehydration in aceton, materials were embedded in araldite (Glauert and Glauert, 1958).

Sections were cut with a glass knife on a Reichert OmU 3 ultramicrotome. Thick sections, 0.5–1 μ, were put on slides (on a drop of aceton 20 %) and dried at 70 °C for two minutes. Lipid staining was made with saturated Sudan Black B (in 70 % ethanol) for 48 hours in room temperature (Mcgee-Russel and Smale, 1963). Methy-

len blue (2 %) was used for two minutes in order to distinguish several tissues. Silver thin sections were mounted on Formvar-coated copper grids and were stained with 2 % uranyl acetate (in 50 % methanol) for 15 minutes at 40 °C. Post staining was made in room temperature for 5 minutes with lead citrate (Reynolds, 1963). Thin sections were examined with a Hitachi H9 transmission electron microscope at The Electron Microscope Laboratory, Faculty of Science, University of Ankara.

RESULTS

The spindle shaped ovaries of the early phase of the last instar larvae are situated on the either side of the alimentary canal. They are whitish in color and 1–1.5 mm in lenght. Each ovary contains four ovarioles embedded in connective tissue. These ovarioles do not yet have the characteristics of polytrophic type as it is seen in Lepidoptera. The different zones of ovarioles are not yet distinguished at this phase (Fig. 1).

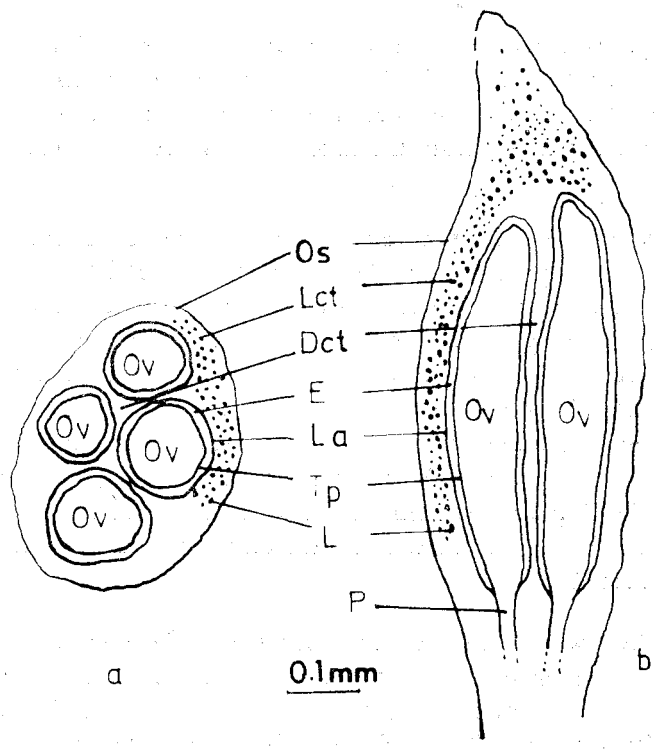

Figure 1. The cross (a) and the longitudinal (b) sections of ovarium made from thick sections for light microscopy.

An amorphous coat covers the ovary from outside as a continuous sheet. Just under this sheet, there is a loose connective tissue made up of 2–3 layered cells. These cells contain lots of lipid granules in different sizes (Fig. 2). Glycogen is also found as a big quantity

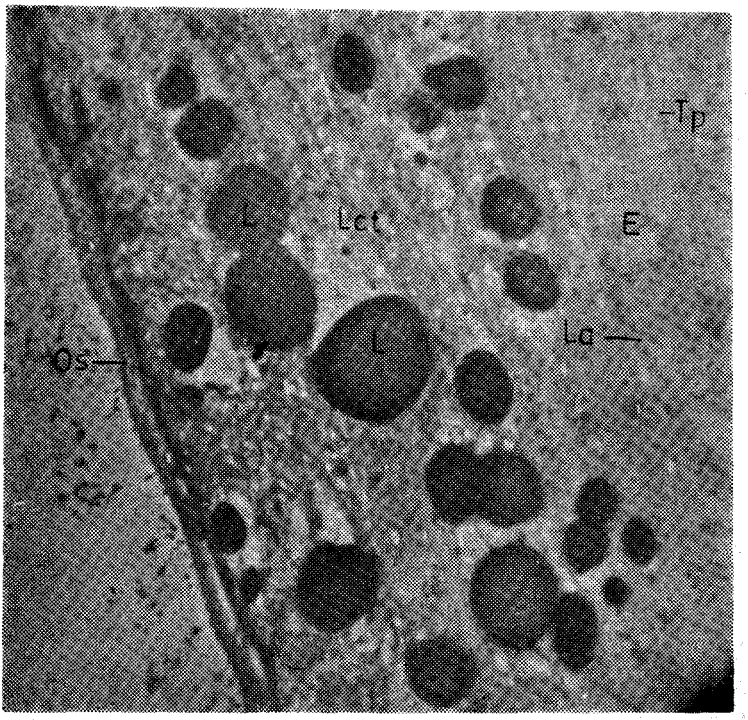

Figure 2. Cross section of ovary. Some parts of the ovariole and the ovarien coat are seen. 1000X

(Fig. 3). Chromatin materials of these cells are relatively homogeneous. Nuclear envelope is not much ondulated. Trachea or tracheole sections might be seen among these cells and just under the coat of ovary.

A dense connective tissue exists among the ovarioles (Fig. 4). The cell membranes of this connective tissue are hardly visible and the nuclear envelopes have deep folds toward the centers of nuclei. The chromation material is rather scanty and is situated near the

Figure 3. Some part of the cells of the loose connective tissue. Glycogen and lipid spherules are seen. 10000X

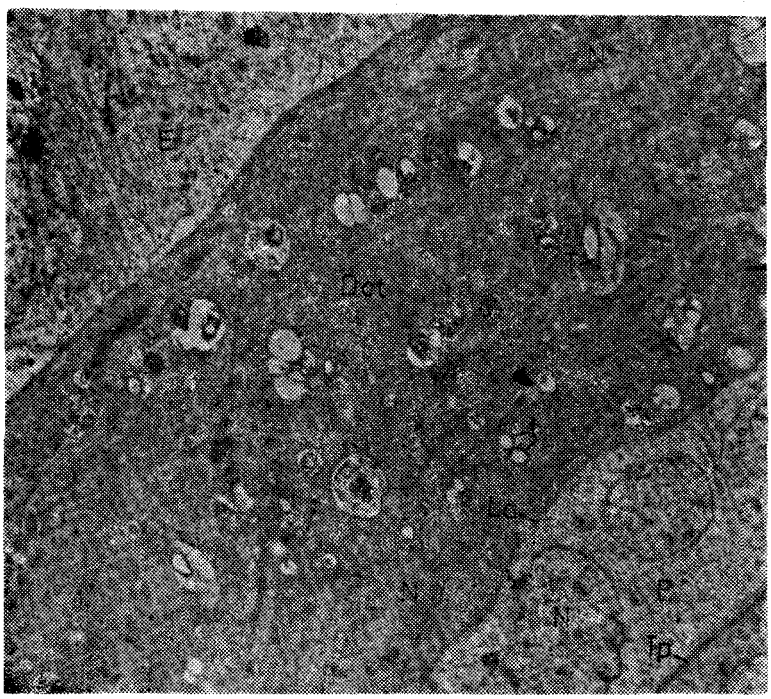

Figure 4. Dense connective tissue between two ovarioles are seen in lognitudinal section. 3000X

nuclear envelope. Lipid granules are smaller and fewer than those of the loose connective tissue. The sections of tracheoles can be seen frequently. Small vesicles and myelinated bodies are also seen almost every part of this connective tissue.

The ovarioles are separated from the dense and the loose connective tissues with a lamina. This lamina rests upon a single epithelial coat (Fig. 5). The thickness of the epithelial coat decreases at different

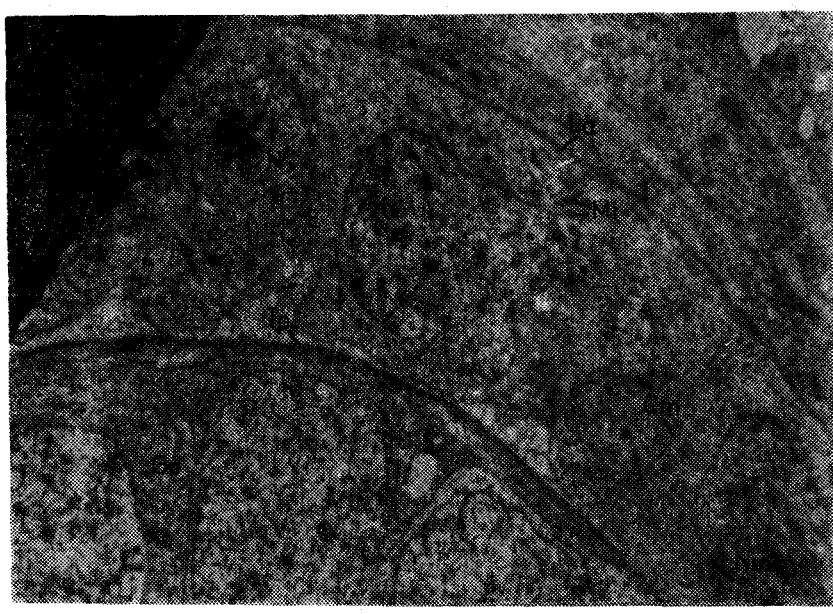

Figure 5. Epithelial layer, lamina and tunica propria are seen in the cross section of ovariole. 5000X

regions of the ovariole. Epithelial cells are closely packed and are cuboidal or polygonal in shape. The nuclei are round, oval or polygonal. Cytoplasm is rich in mitochondria and in free ribosomes. Granular endoplasmic reticulum is scarce. A nucleolus-like body can be seen in the cytoplams in some microphotograph (Fig. 6). The epithelial coat runs up to the beginning of the pedicel (See Fig. 13). Tracheae or tracheoles are found neither in this epithelial coat nor in the lamina.

On the other hand, the epithelial coat rests on a tunica propria which completely surrounds each ovariole (See Fig. 1,5). Tunica

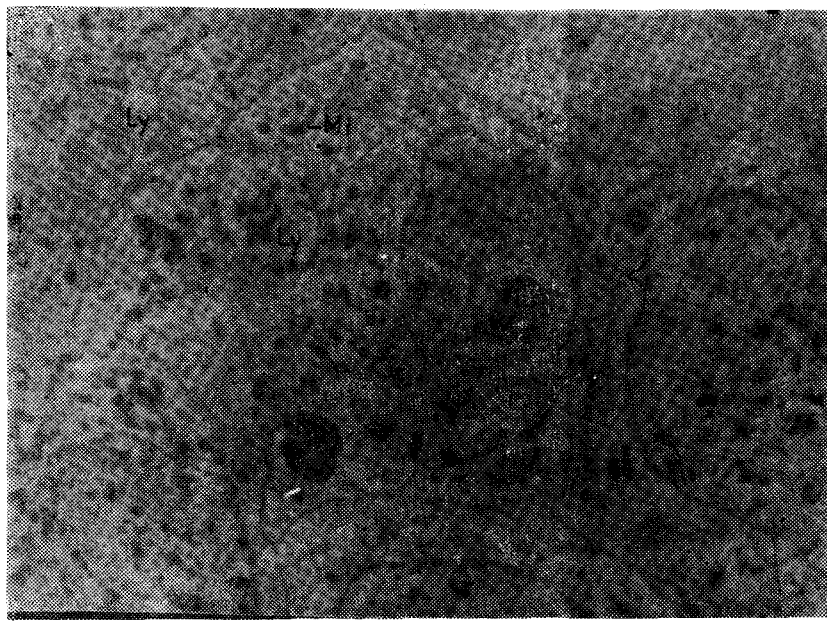

Figure 6. Epithelial cells in a tangent section. 5000X

propria is thinner than ovarian coat and is not also penetrated by tracheae or tracheoles.

The ovarioles are full of two types of cells. One type cell is almost round and is called germ cell. The second type cell is small and irregular in shape. The second one is called profollicular cell (Fig. 7).

At this stage, it is difficult to distinguish the cysts with eight cells that would develop from germ cells after three successive mitotic divisions. The germ cells were not differentiated yet and there is not any distinguishable structure that separates the cysts (Fig. 7). During the cyst formation, the cystocytes seemed to be at the same stage in one cyst. The cysts which formed in previous stage were found close to the pedicel. Cysts do not display any difference except their volumes. It is not also possible to distinguish the oocyte or the nurse cell in the cyst. Synaptinemal complexes which are characteristics of meiosis are not seen, either.

Some differences in electron density can be seen among the neighboring cysts. In the nuclei of cystocytes, which were divided early, dark chromatin patches and a nucleolus are observed. Their cytoplasm are dense and contain numerous free ribosomes. In the nuclei

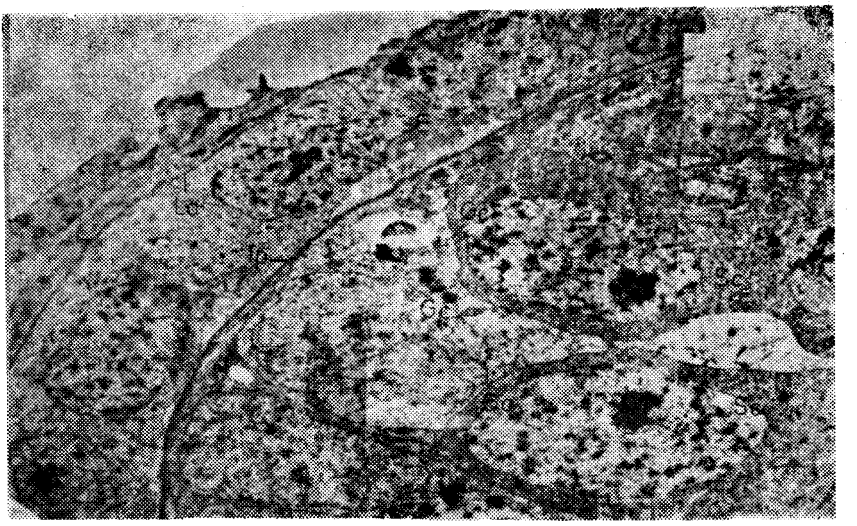

Figure 7. Some part of ovariole in cross section. 3000X

of the less dense cells, chromatin is distributed quite homogenously (Fig. 8). Both the nuclei of denser cells and those of less dense ones

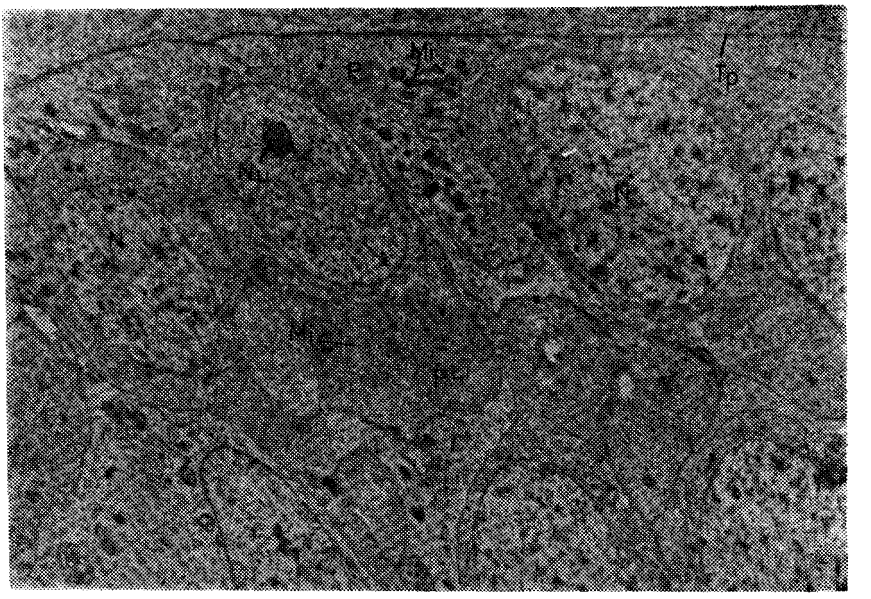

Figure 8. Epithelial layer and some part of ovariole in cross section (Photomontage). 5000X

are large enough to fill the three-fourths of the cells. Some Golgi complexes are visible in each cell (Fig. 9). Granular endoplasmic reticulum is scarce, mitochondria are generally accumulated on one side of the cell (See Fig. 7).

Intercellular bridges which are formed after incomplete cytokinesis between the sister cells of a cyst are clearly seen in Figure 9

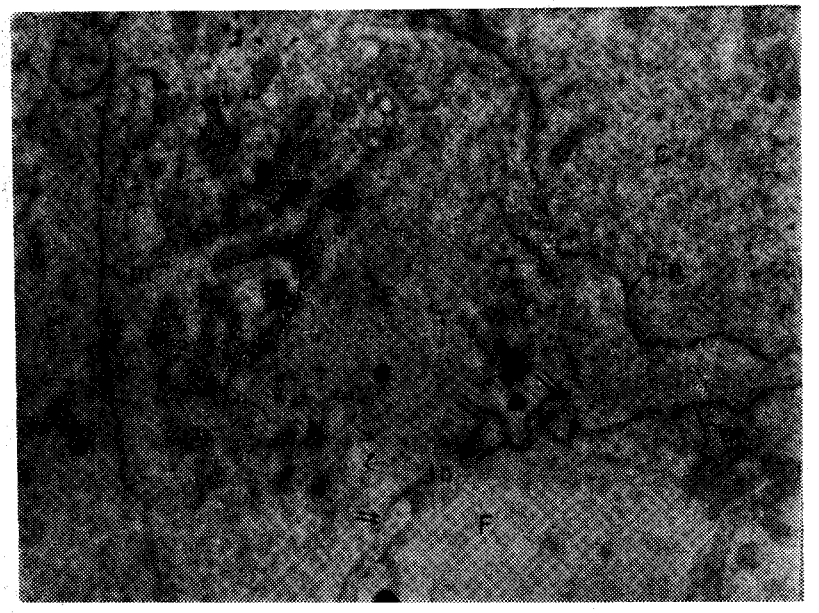

Figure 9. Intercellular bridge and fusome material between two germ cells. Small vesicles (→) in cytoplasm and small outpocketings of the cytoplasmic membrane (⇒) are seen. 10000X

and 10. The cytoplasmic membrane around the intercellular bridges shows some small outpocketings. An amorphous fibrous material of fusome fills the bridge field. In the same cyst, the fusome material exists between all the cells that are divided successively by mitosis (Fig. 10). The fusome material is easily distinguished from the cytoplasm. It seems granulated and does not contain mitochondria. The mitochondria are situated in the cytoplasm just next to the fusome material. An electron dense region called mid-body can be seen right in the middle of the intercellular bridge and in the fusome material.

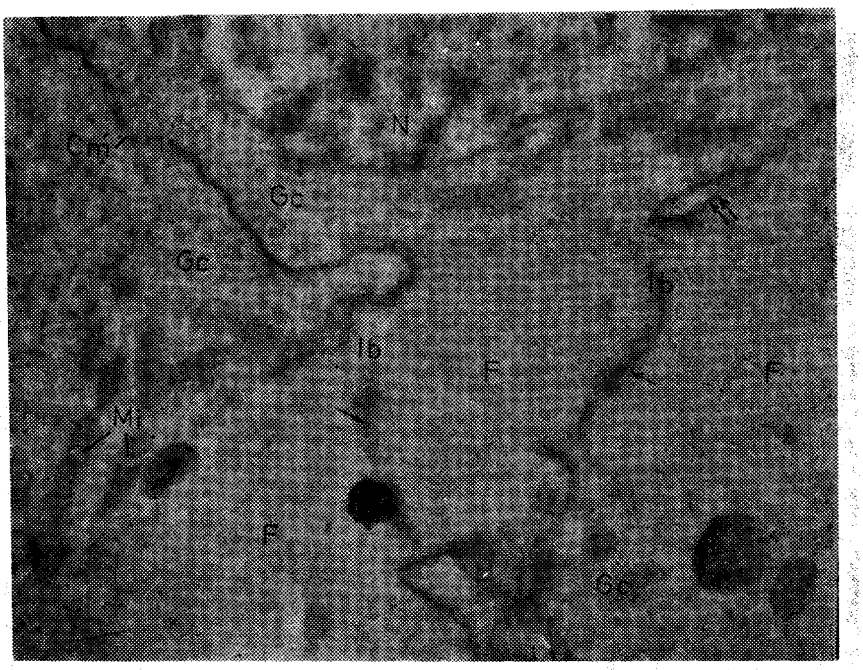

Figure 10. Two intercellular bridges among three germ cells, the fusome material, midbody (→) and outpocketings of cytoplasmic membrane (⇉) are seen. 10000X

Profollicular cells found among the germ cells, have some mitochondria and granular endoplasmic reticulum (See Fig. 7). Some small masses of cytoplasm can be seen at sections of ovariole. These masses are full of mitochondria and small vesicles (Fig. 11).

The pedicels of ovarioles are filled with the closely packed cells. Each cell contains a very big nucleus. Their cytoplasms are like narrow bands around their nuclei. These nuclei have nucleoli and homogenoously distributed chromatin material (Fig. 12). Pedicel is covered by a tunica propria.

DISCUSSION

The ovaries of *A. ipsilon* in the last instar larvae are fusiform in shape. Each ovary contains four ovarioles which have not yet developed into their characteristic polytrophic type. There is a sheet all over the ovary. Bonhag and Arnold (1961) stated that this sheet

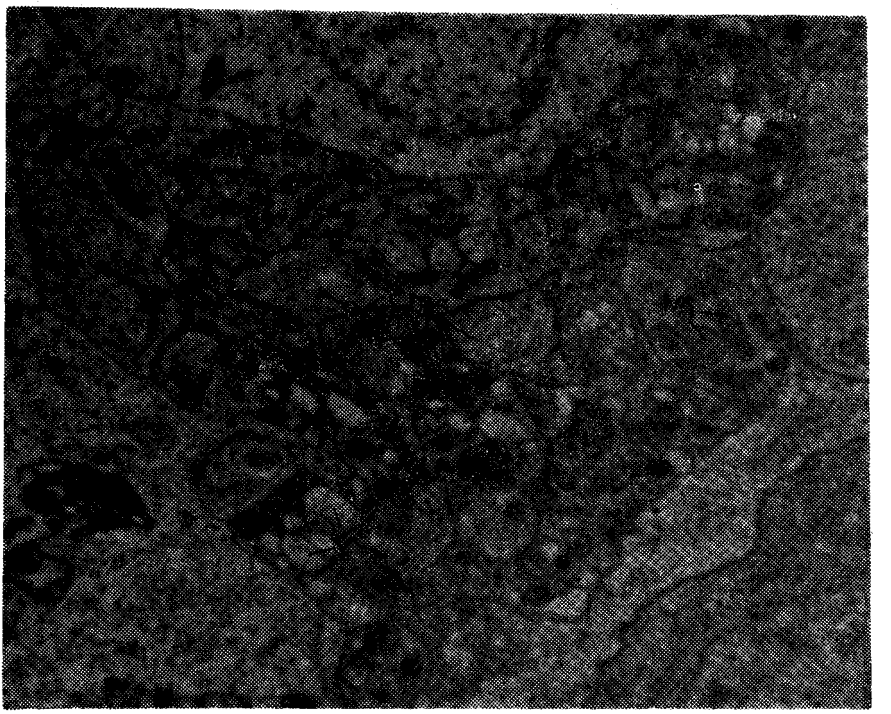

Figure 11. Some cytoplasmic masses among the germ cells. 5000X

did not exist in most of mature insects. They showed that there was not such a sheet in *Periplaneta americana*. Mandelbaum (1980) described this sheet as a capsular membrane in the larvae of *Hyalophora cecropia* (Lep.).

In the ovary of *A. ipsilon* larvae, there are a loose connective tissue just under the ovarian sheet, which surrounds the ovarioles, and a dense connective tissue among the ovarioles. These layers were reported with different terms such as "outer and inner envelope", "epithelial sheaths", "peritoneal sheath" in *Oncopeltus fasciatus* (Bonhag and Wick, 1953), in *Anisolabis maritima* (Bonhag, 1956), in *P. americana* (Bonhag and Arnold, 1961, in *Tomocerus minutus* (Matsuzaki, 1973) respectively. In *A. ipsilon*, these loose and dense connective tissue layers possess lipid spherules and glycogen. Matsuzaki (1973) saw lipid granules and glycogen in great amount in *T. minutus* and also described tracheae and tracheoles among the cells of this layer as it is seen in

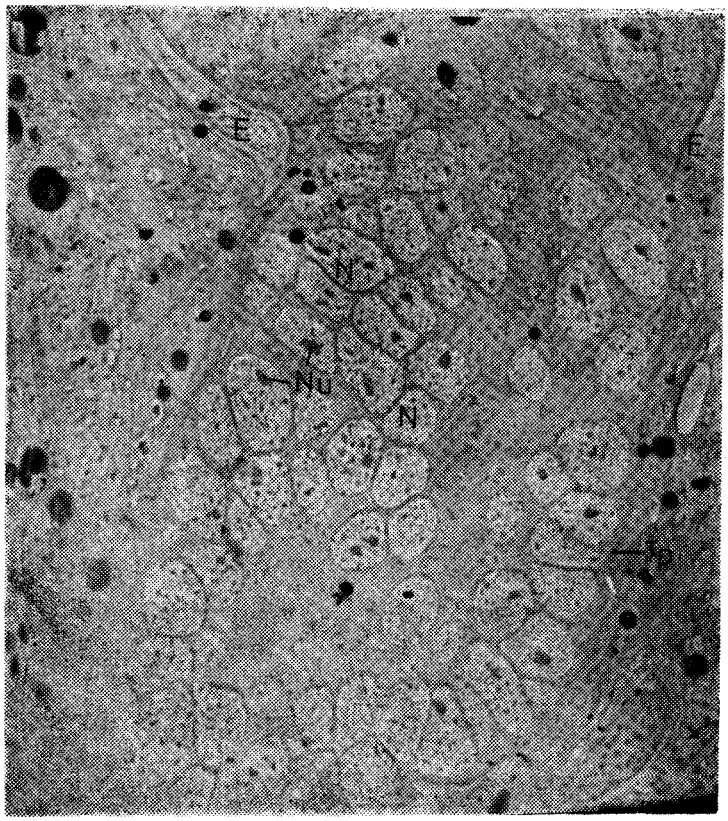

Figure 12. The longitudinal section of pedicel. Epithelial layer extends up to the beginning of the pedicel. 1000X

A. ipsilon. Bonhag and Arnold (1961) stated that tracheae and tracheoles did not penetrate into *tunica propria* and ovarioles. This is also true for *A. ipsilon.* Besides, in *A. ipsilon,* tracheae and tracheoles do not penetrate into the lamina and the epithelial layer, either.

Mahowald (1972) explained that there was a thin connective tissue, in which the muscle bands extend obliquely, around the *tunica propria* in *Drosophila melanogaster.* There is no muscle cell in the connective tissue of *A. ipsilon.* Bonhag and Arnold (1961) also stated that the muscle cell did not exist in *P. americana.*

An epithelial layer that exists in the ovarian coat of *A. ipsilon* larvae, was also shown by Bonhag and Wick (1953) in *O. fasciatus.*

In the ovary of *A. maritima*, Bonhag (1956) described ameoboid cells at the same region of the epithelial layer.

The epithelial layer in the larval ovary of *A. ipsilon* is covered with a lamina from outside and with a tunica propria from inside. Bonhag and Wick (1953) showed these two layers in *O. fasciatus*. Bonhag and Arnold (1961) considered *tunica propria* as a connective tissue and stated that these layers did not exist at the stalk of ovariole. In the ovary of *A. ipsilon*, *tunica propria* surrounds all the ovariole, including the pedicel, but the epithelial layer does not pass over the pedicel. Anderson (1964) suggested that *tunica propria* was a product of follicle cells or interfollicular tissue. This view is also acceptable for *A. ipsilon*.

The nucleolus-like body which is also called "nematosome" is seen in the cytoplasm of the epithelial cells surrounding ovarioles in *A. ipsilon*. It was also shown by Halkka and Halkka (1975) in the previtellogenic oocyte of *Cordulia aenea* (Odonata). They reported that these bodies possessed RNA.

At the beginning of the last instar larvae, the ovarioles of *A. ipsilon* are not differentiated into terminal filament, germarium, vitellarium and pedicel. At this stage, it is difficult to decide which cell is oocyte or nurse cell. Since the profollicular cells are still small and irregular in shape, it is fairly easy to distinguish them from the germ cells. The fact that profollicular cells change into follicular cels after the formation of egg chambers was described by Mahowald (1972) and by Brunt (1971). During the vitellogenesis, the follicular cells play important roles. Ramamurty (1964) and Stay (1965) showed by autoradiography that the haemolymph proteins reached the oocyte as vitelline precursor by passing into ovarian coat and follicular cells. It is explained that the function of follicular cells was to synthesize the yolk bodies and to form the vitelline-membrane and the chorion, as well as to from the design over the chorion in *Lygaeus kalmii* (Kessel and Beam, 1963), in *Bombus terrestris* (Hopkins and King, 1966), in *Drosophila melanogaster* (Cummings and King, 1966), in *Tenebrio molitor* (Ullmann, 1973), in *P. americana* (Bell and Sams, 1974), in *Simulium vittatum* (Liu et al., 1975).

The small masses of cytoplasm which are seen among the germ cells are the elongations of the profollicular cells. They will form the interfollicular cells between the egg chambers.

During the development of ovarioles of *A. ipsilon*, the intercellular bridges between the germ cells also exist. In *P. communis* (Ramamurty, 1964), in *B. terrestris* (Hopkins and King, 1966), in *D. melanogaster* (Cummings and King, 1969; Mahowald, 1972), in *H. cecropia* (Pollack and Telfer, 1969; Mandelbaum, 1980), there were intercellular bridges between the germ cells, through which lipid, RNA, ribosomes, mitochondria were transferred to the oocyte.

In *H. cecropia*, Mandelbaum (1980) showed that there were some fusome materials in these interecellular bridges. Still, she was unable to explain precisely whether this material was a remnant of spindle or some other material. Matsuzaki (1973) showed that there were ribosomes, mitochondria and some endoplasmic reticulum in the intercellular bridges and electron dense, RNA positive, fine granulated bodies in the cytoplasm next to the bridges in the nurse cells in *T. minutus*. His desctriptions and electronmicrographs clearly show that these RNA positive, electron dense bodies are nothing but fusome materials. As a matter of fact, Mandelbaum explained that, in the late phase of bridge formation, fusome material moved into the cytoplasm and this area replaced by mitochondria, ribosomes, microtubules and agranulated endoplasmic reticulum. Although Mandelbaum said that a microtubule-midbody was found in the bridge and this was previous stage of the fusome formation, such succeeding stages might not exist in *A. ipsilon* for fusome and midbody were seen at the same bridge together.

Further investigations are necessary to describe the development of oocytes into eggs showing all the intracellular differentiation. All the other structures of ovaries should also be studied in detalis.

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ABBREVATIONS

C	: Cytoplasm	Mi	: Mitochondria
Ch	: Chromatin	N	: Nucleus
Cm	: Cell membrane	Nu	: Nucleolus
Cms	: Cytoplasmic masses	Nb	: Nucleolus-like body
Dct	: Dense connective tissue	Os	: Ovarian sheet
E	: Epithelial layer	Ov	: Ovariole
F	: Fusome material	P	: Pedicel
G	: Golgi Complexes	Pc	: Profollicular cell
Gc	: Germ cell	R	: Ribosome
Gl	: Glycogen	Sc	: Sister cell
Ib	: Intercellular bridge	T	: Trachea
L	: Lipid spherules	t	: Tracheole
La	: Lamina	Tp	: Tunica propria
Lct	: Loose connective tissue	V	: Vacuole
Ly	: Lysosomal structure		

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