Commun. Fac. Sci. Univ. Ank. Series C
V. 5, pp 19–25 (1987)

AMOEBOID CELLS IN THE OVARIOLAR SHEATH OF THE LATE LAST INSTAR LARVAE OF AGROTIS IPSILON (HUFNAGEL) (LEPIDOPTERA: NOCTUIDAE)

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(Received 10 April 1987 Accepted 21 April 1987)

ABSTRACT

The amoeboid cells are observed between the tunica propria and the lamina of the ovariolar sheath in the late last instar larvae of *A. ipsilon*. These cells derive from the epithelial cells in the same place in the early last instar. In their cytoplasm, numerous ribosomes, rough endoplasmic reticulum and granules exist. This structure indicates a synthetic activity. The cytoplasmic extensions, lysosomal bodies and pinocytic vesicles show the properties of phagocytosis of these cells.

INTRODUCTION

In the ovaries, oocyte differentiation and vitellogenesis have been studied extensively in a wide range of insects (Anderson, 1964; Hopkins and King, 1966; Cummings and King, 1969; Huebner and Anderson, 1972; Mahowald, 1972; Matsuzaki, 1973; Ullmann, 1973; Wightman, 1973; Junquera, 1984; Buning, 1985; etc.). In addition, the follicular epithelium, the nurse cells or the trophocytes have been the subject of many investigations, especially concerning the roles on the acquisition and the synthesis of vitellogenic substance (Bier, 1962; Ramamurty, 1964; Stay, 1965; Cruicshank, 1972; Bell and Sams, 1974; Liu et al., 1975; Mandelbaum, 1980; Dey and Walker, 1982; Huebner and Sigurdson, 1984; Mazzini and Giorgi, 1985, 1986; Gutzeit and Huebner, 1986; Huebner and Gutzeit, 1986). But yet, all these completed studies are not sufficient if the whole insect groups are taken into consideration.

The structure and the function of the ovariolar sheath have been studied generally in the mature insects such as *Oncopeltus fasciatus* (Heteroptera) (Bonhag and Wick, 1953), *Anisolabis maritima* (Dermaptera) (Bonhag, 1956), *Periplaneta americana* (Bonhag and Arnold, 1961), *Dysdercus fasciatus* (Heteroptera) (Brunt, 1971), *Drosophila melanogaster* (Cummings, 1974). The differentiation and the function of the ovariolar sheath have received much less attention during the development of the caterpillars.

From above mentioned information, It may be concluded that a detailed knowledge on the comparative morphology of the ovariolar sheath, especially that of the larval stage is still incomplete. The aim of this work is to clarify the structure and the origin of the amoeboid cells in the ovariolar sheath of the late last instar larvae of *Agrotis ipsilon*.

MATERIALS AND METHODS

The larvae of *A. ipsilon* were collected and reared as described previously (Suludere, 1986). The larvae reached to the last instar in 15 th days and to the pupae in 19 th days after hatching at 26 $\pm$ 1°C in laboratory. The larvae, 16–17 days old, were dissected in buffer solution and ovaries were removed. They were immersed in ice-cold gluteraldehyde (5 % in phosphate buffer, pH 7.2) for one hour. After the rinsing with the buffer solution (with 7.5 % sucrose) and postfixation with 1 % $Os0_1$ for one hour, the tissue was washed again with the same buffer and then transferred to the saturated uranyl acetate (in 50 % ethanol). Following the dehydration in a graded series of acetone, materials were embedded in araldite.

Ultrathin sections were mounted on formvar coated -copper grids and stained in uranyl acetate and lead citrate. The sections were examined in a Jeol 100CX II Electron microscope.

OBSERVATIONS

In the late last instar of *A. ipsilon* larva, there is a pair of ovaries which are located on the both side of the alimentary canal. Each ovary is spindle shaped and comprises four ovarioles, embedded in connective tissue. These ovarioles have no the characteristics of polytrophic

type yet as it is seen in Lepidoptera. In the ovarioles the germarium, the vitellarium and their appendages are not distinguishable at this stage.

Each ovariole is covered by an amorphous sheath, tunica propria (Fig. 1). In the sections of ovaries, some amoeboid shaped cells are seen in the space between the tunica propria and the lamina of the connective tissue. These cells are easily distinguishable from the connective tissue by their electron density. The width of the space is almost equal to the diameter of an amoeboid cell and there is a flocculent material among the cells. These cells do generally not contact with each other and placed irregularly in this space. These amoeboid cells have some cytoplasmic extentions and a number of pinocytic vesicles (Fig. 2). The cytoplasm of the cell has many free ribosomes, rough endoplasmic reticulum, some lysosomal bodies, small vesicles, and granules which are in groups. The nucleus is lobated, irregularly shaped and has some chromatin patches. The mitochondria are small and few in number.

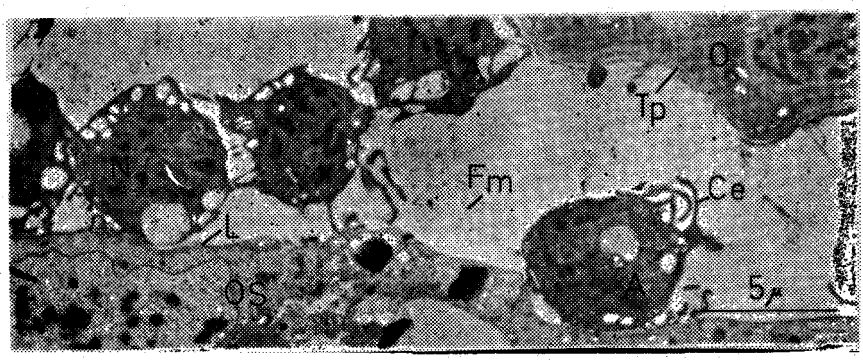

Figure 1. A longitudinal section of the ovariole (O) and the ovariolar sheath (OS) of *A. ipsilon* larva. The structure and the distribution of the amoeboid cells (A) between the tunica propria (Tp) and the lamina (L). Ce. Cytoplasmic extention, N. Nucleus, Fm. Flocculent material. 2000x

DISCUSSION

As it was described in the previous paper (Suludere, 1986), a single layer of epithelial cells exists between the lamina and the tunica propria. The closely packed epithelial cells are in cuboidal or polygonal shape during the early last instar of *A. ipsilon* larvae.

In this paper, the observations made on the late last instar larvae indicate that the epithelial cells do not appear in a single layer as it was

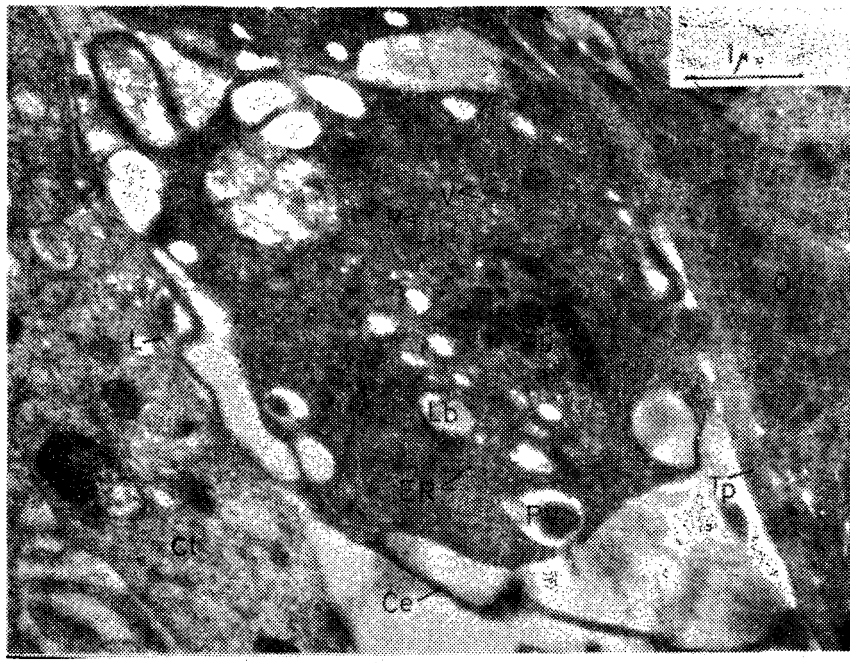

Figure 2. An amoeboid cell. Ce. Cytoplasmic extention, Ct. Connective tissue of the ovariolar sheat, ER. Rough Endoplasmic Reticulum, Lb. Lysosomal body, PV. Pinocytic vesicle, R. Ribosomes, L. Lamina, g. granules, Tp. Tunica propria, V. Small vesicles. 10 000x

seen in the early last instar larvae. In the late last instar, the lamina and the tunica propria are morphologically similar to those of the early last instar larvae. In this stage, the cuboidal epithelial cells change into the amoeboid cells more or less separated from each other. The lateral contact between the cells remains close at some points, while at other places, the contact maintained by thin cytoplasmic extentions or absent. These cells may be considered to be phagocytic for they have some pinocytic vesicles, lysosomal bodies and cytoplasmic extentions.

Some authors have studied the ovariolar sheaths of other insects and described the cells between these two layers as amoeboid cells, muscle cells, or haemocytes. In the simple type of polytrophic ovariole of *Anisolabis maritima*, Bonhag (1956) reports that the inner envelope of the sheath tissue consists of a layer of loose, amoeboid cells enclosed within two membranous laminae. In the polytrophic ovariole of *Drosophila melanogaster*, Koch et al. (1967) illustrate the lumen cells between the

tunica propria and the epithelial sheath. Mahowald (1972) reports the muscle cells, existing over the basal lamina in the same species. Cummings (1974) also reports in *Drosophila*, a discontinous layer of the muscle fibers existing in the median epithelial sheath and a group of haemocytes existing between the tunica propria and the inner epithelial sheath. In the polytrophic ovariole of *Calpodes ethlius* (Lepidoptera), Griffith and Lai-Fook (1986) demonstrate that the ovariolar sheath is a tightly knit network of longitudinal and circular muscle fibers, the latter being adjacent to the basal lamina. During the observations in the electron microscope on the ovariolar sheath, no evidence on the presence of the muscle cells and haemocytes were found between the lamina and the tunica propria.

It is generally said that the precursor of the yolk bodies pass through the ovariolar sheath, the tunica propria and the follicular epithelium before reaching the oocytes. The cells in the ovariolar sheath participate the transport of the precursors of the yolk bodies from the haemolymph and the fat body to the oocytes (Ramamurty, 1964; Hopkins and King, 1966; Ullmann, 1973; Raikhel, 1986). As we did not use any autoradiographic or immunocytochemical technics, we could not observe the transport of the precursors of the yolk bodies directly. There is, however, not any reason that the amoeboid cells should not participate to this transport. Whether the cells of the ovariolar sheath is responsible for any step of synthesis from the precursors to the yolk body is not clear.

The difference of the electron density between the amoeboid cells and other ovariolar sheath cells is due to the abundant ribosomes. The abundance of ribosomes, rough endoplasmic reticulum, and granules in the cytoplasm of these amoeboid cells might be considered as an evidence of a synthetic activity.

This work, with the above mentioned points, should be regarded as an initial one, not only *A. ipsilon* but also other insects. Confirmations among the closely allied groups will be contributed to this subject.

ACKNOWLEDGMENT

I am grateful to Prof. Dr. Sevinç Karol for her advice and help on the accomplishment of this research.

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