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A Study on Endocrine Cells in the Midgut of *Agrotis segetum* (Denn.and Schiff.) (Lepidoptera:Noctuidae)

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Abstract: The structure of midgut endocrine cells of *Agrotis segetum* larvae was examined by light and electron microscopy. It was observed that the endocrine cells were located on the basal lamina and among the columnar epithelial cells of the midgut. The cells were morphologically determined as columnar or coniform. The cytoplasm of endocrine cells was observed to contain rough endoplasmic reticulum, mitochondria, developed Golgi complex, vesicles and numerous small secretory granules. It was also observed that the secretory granules were often located in the basal region of the endocrine cells.

Key Words: *Agrotis segetum*, midgut endocrine cells, insect, electron and light microscopy

Agrotis segetum (Denn. ve Schiff.) (Lepidoptera: Noctuidae) Orta Bağırsak Endokrin Hücreleri Üzerinde Bir Çalışma

Özet: Bu çalışmada, *Agrotis segetum* larvalarının orta barsağındaki endokrin hücrelerinin yapısı ışık ve elektron mikroskobunda incelendi. Endokrin hücrelerin bazal lamina üzerinde ve silindirik epitel hücrelerinin arasında yer aldığı gözlendi. Morfolojik olarak hücrelerin silindirik veya konik şekilli olduğu belirlendi. Endokrin hücrelerin sitoplazmasında granüllü endoplazmik retikulum, mitokondriler, gelişmiş Golgi aygıtı, kesecikler ve çok sayıda küçük salgı granüllerinin bulunduğu gözlendi. Salgı granüllerinin, çoğunlukla hücrelerin bazal bölgesine yerleşmiş olduğu gözlendi.

Anahtar Sözcükler: *Agrotis segetum*, orta bağırsak endokrin hücreleri, böcek, elektron ve ışık mikroskobu

Introduction

Three types of cell have been recognized in insect midgut epithelium: the columnar epithelial cells, goblet cells and endocrine cells (1-6). These cells are believed to have originated from the regenerative crypt. The regenerative crypt is composed of stem and undifferentiating cells. The midgut endocrine cells are differentiated from stem cells and located among the columnar ones. The occurrence and immunoreactivity of endocrine cells have been described in many insect orders (1-3). In recent years, endocrine cells have been described in terms of the diffuse neuroendocrine system (DNES) cells of vertebrates. In some insect orders, pancreatic polypeptide (pp), somatostatin, and glucagon-like immunoreactive material has been shown to be present in the midgut endocrine cells of the insect (1,2,4,7). The midgut endocrine cells in some species of insect have been examined by electron microscopy. These insect species are *Ephestia kühniella* (6), *Agrotis ipsilon* (8), *Japyx* sp., *Nauphoeta cinerea*, *Gryllus bimaculatus* (9), *Periplaneta americana* (10), *Lepidocampa weberi* (11), *Anacridium aegyptium* (L)(12), *Apis mellifera* (13,14), and *Abracris flavolineata* (15). Some researchers have examined the midgut endocrine cells in *P. americana*. They have reported that the general structure of these cells in this insect shows structural differences from the others (10). Definition of these cells is done by immunohistochemical techniques for *P. americana* and *Blaberus craniifer* (1,7). In the present paper, the midgut endocrine cells of *Agrotis segetum* larvae were examined by light and electron microscopy to describe the general structure of these cells in relation to their endocrine activity.

Materials and Methods

The larvae of *Agrotis segetum* were reared on honeycreepers (*Beta vulgaris* var. rapa) and held under a

14 h light cycle at 26 ± 1°C and 60-65% relative humidity (16). In this study, fifth instar larvae of *A. segetum* were used in all experiments. The larvae were starved for a few days and then the midgut portions were dissected. For electron microscopic observation, the dissected midgut was fixed in 2.5% glutaraldehyde in sodium phosphate buffer at pH 7.2 for 2 h at +4°C, washed several times in sodium phosphate buffer for 2 h, postfixed in 1% OsO_4 for 2 h at +4°C, dehydrated in graded ethanol series and embedded in araldite. Thin sections were stained with uranyl acetate and lead citrate before examination (17). The samples were then examined by electron microscope (JEOL 100 CX II). Semithin sections were stained with toluidin blue and examined by light microscope (OLYMPUS).

Results

The midgut is the largest part of the digestive tract of Lepidopteran larvae. The midgut is lined with columnar cells. The endocrine cells of *A. segetum* are rarely located among the columnar epithelial cells and on the basal lamina (Figure 1 a, b). We observed that these cells do not come into contact with the luminal surface (Figures 1a, 2). In the semithin sections, they were observed in groups or individually (Figures 1, 2).

The midgut endocrine cells are columnar or coniform and, in addition, their axes are parallel to the axes of the columnar epithelial cells (Figures 1, 2). The nucleus of the coniform cell is suitable for the type of cell and they are completely filled with dense masses of euchromatin. The nucleolus is fairly clear in the nucleus (Figure 3).

The cytoplasm of these cells is poor in organelles. However, rough endoplasmic reticulum (RER) is clearly seen in the cytoplasm (Figure 3). The Golgi complex is well developed (Figure 4). Numerous secretory granules are located on the basal region of the endocrine cells (Figures 3, 4). As seen in the photographs, secretory granules release their content into the basal lamina and intercellular space (Figures 5, 6). Secretory granules range from 0.07 to 0.35 µm in diameter. These granules are surrounded by an unit membrane and contain dense material. Some of the endocrine cells contain a number of vesicles and mitochondria (Figure 7).

Discussion

In insects, the endocrine cells of the midgut show similarity in terms of their individual existence in the basal region of the epithelium (1-3,6,8,10-12,15). However, if the secretion granules are taken into consideration, some researchers separate the cells into various types (9,13,14). Four types of secretion granules of midgut endocrine cells in *Gryllus bimaculatus* have been identified: thick-stick, opaque heterogeneous, spherical and ovoid; three types in *Locusta migratoria*; two types in *Petrobius maritumus*; and only one type in *Folsomia candida* (9). Many granules of various types have been observed in the midgut endocrine cells of *A. segetum*. The

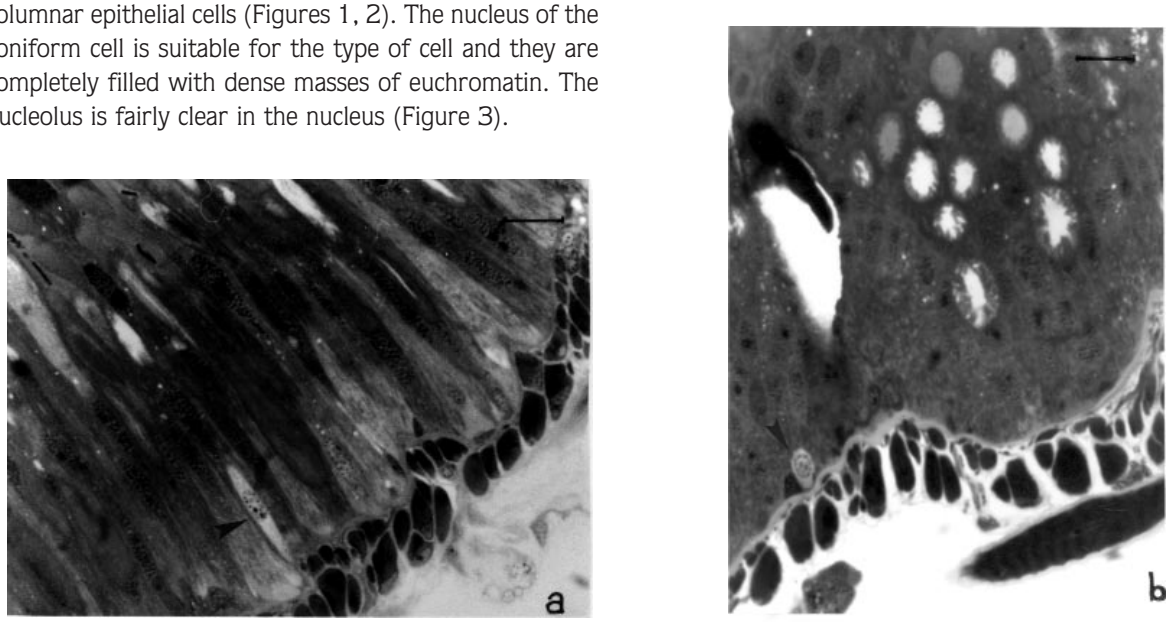

Figure 1. Endocrine cells a) The columnar endocrine cell among the columnar epithelial cells (➤). b) Endocrine cell located on the basal lamina (➤). Bar: 9.8 µm.

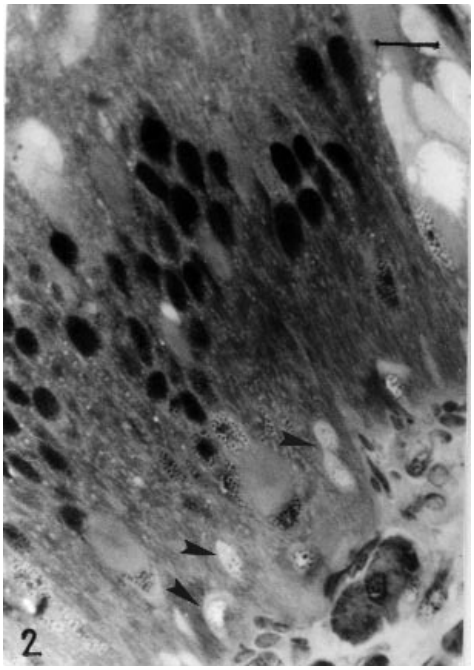

Figure 2. Endocrine cells solitary or in groups (➤) located among the columnar epithelial cells. Bar: 9.8 µm.

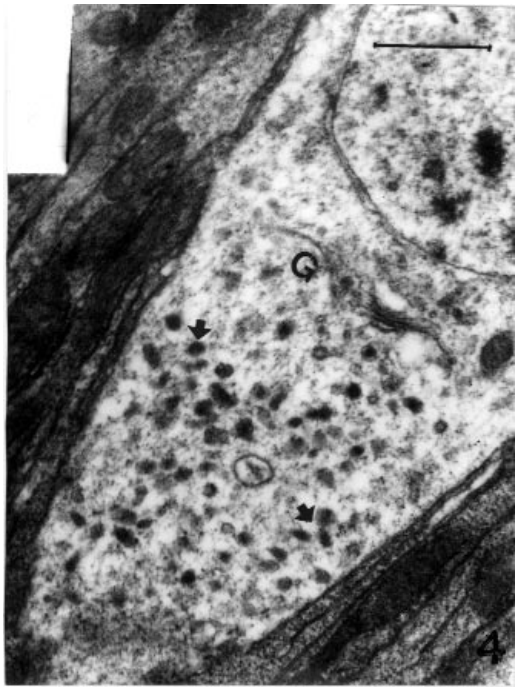

Figure 4. Golgi complex (G) and secretory granules (➨) in endocrine cell. Bar: 1 µm.

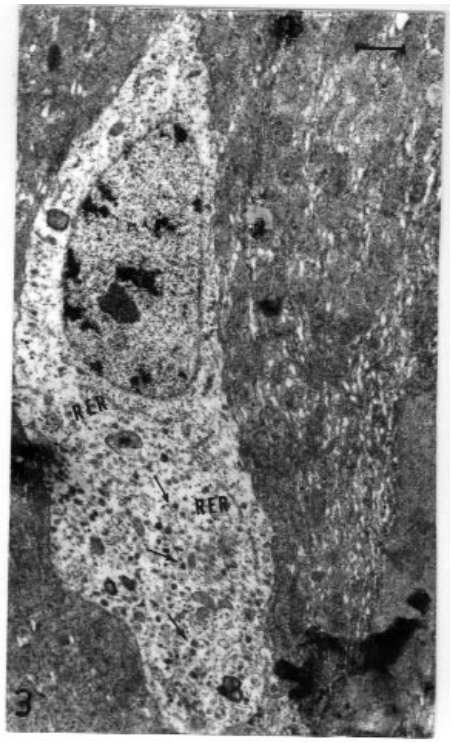

Figure 3. The coniform endocrine cell on the enlarged basal region. Secretory granules widespread on the basal half(→),RER (rough endoplasmic reticulum). Bar: 1 µm

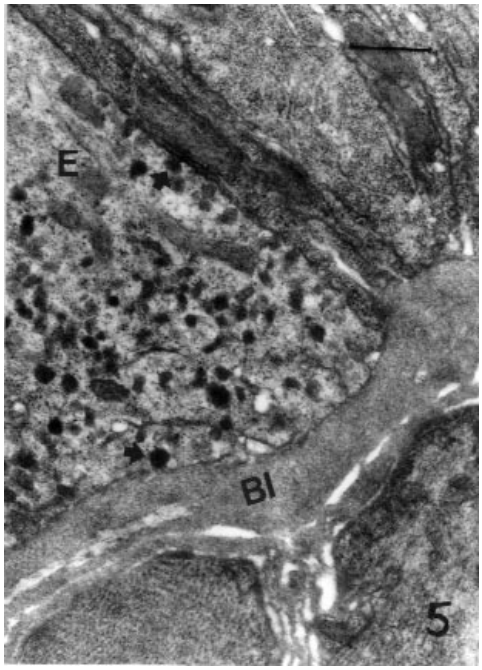

Figure 5. Endocrine cell (E) and basal lamina (Bl). Granule (➨) about to be released into the basal lamina. Bar: 1 µm.

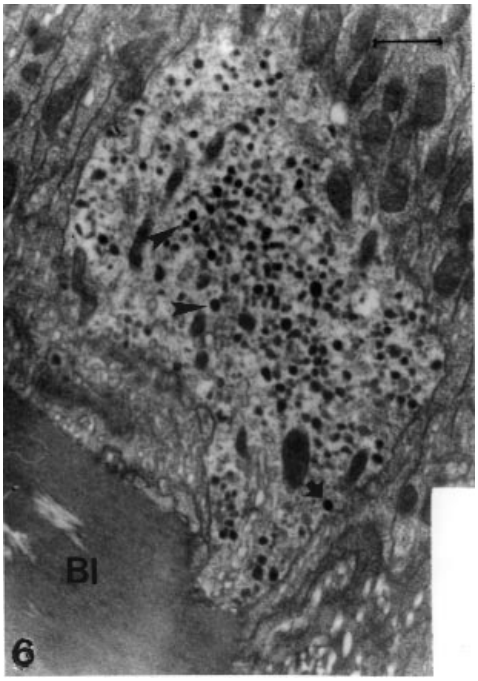

Figure 6. Endocrine cell located on the basal lamina (Bl). Secretory granules (➤) in the cytoplasm of the cell. Secretory granule (➨) discharged into intercellular space. Bar: 1 µm.

morphological structure of these granules show variations from spherical to thick-stick. According to our findings, the variations of secretion granules may be related either to the cytophysiological conditions or the direction of the cross sections. The secretory granules of *A. segetum* were released at the basal lamina and intercellular space (Figures 5, 6). Raes and co-workers (13,14), reported the same case in *A. mellifera*. Two types of endocrine cells have been identified in *A. mellifera* (13,14). One type of these endocrine cells is rich

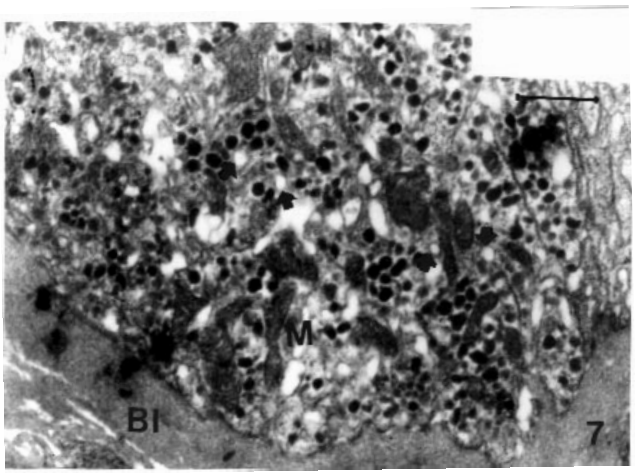

Figure 7. Vesicles (➨) and mitochondria (M) in the endocrine cell. Bar: 1 µm.

in terms of vesicles; the other one, however, is rich in granules. No classification of endocrine cells has been done in *A. aegyptium* (12), but the granules differ in terms of size and electron-density.

Endocrine cells which have been observed in *A. segetum* showed conformity in terms of cytomorphology with the endocrine cells of the insects which have been studied (2,8,10,13,14). As reported previously (10,13,14), the endocrine cells of *A. segetum* are generally columnar, bowl– or bottle (coniform) shaped and also located on the basal lamina. As seen in many cells, the existence of the vesicles and mitochondria within endocrine cells in *A. segetum* were similar. This similarity seemed to be due to the cell differentiation along with physiological activity of a cell. At a further stage, more detailed studies are planned on the structure granules of the endocrine cell of the midgut in *A. segetum*.

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