The Effect of Ecdysone on the Fat Body Cells of the Penultimate Larvae of *Mamestra brassicae*

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**Summary.** The capability of β-ecdysterone to induce autophagocytosis in the fat body cells of penultimate larvae of *Mamestra brassicae* was investigated both in vivo and in vitro. The hormone proved ineffective when applied for 3 h on the first 3 days of the instar, but it induced the formation of autophagic vacuoles on the 4th day (24 h before ecdysis). This effect became more pronounced when the hormone was administered 10 h before ecdysis. Cells incubated in vitro reacted to ecdysterone more sensitively than cells of similar age treated in vivo. It was concluded that the responsiveness of the fat body cells to ecdysterone as evaluated on the basis of autophagy depends on the age of the larvae. The increased sensitivity of the cells to ecdysterone at the end of the penultimate stage may be related to the decrease of juvenile hormone titre during this period.

**Key words:** Ecdysterone – Autophagy – Fat body – *Mamestra* – Insect.

**Introduction**

The degradation of larval tissues during the last larval instars of holometabolous insects has been extensively studied (Butterworth, 1972; Lafont et al., 1975; Locke and Collins, 1965; Locke, 1970; Wyatt, 1972). In previous experiments we have shown that in the fat body cells of the last larval instar of *Mamestra brassicae* the development of autophagic vacuoles which are the sites of intracellular degradation of cytoplasmic fragments, begins at the end of the 3rd day of the stage (Sass and Kovács, 1974). JHa applied externally inhibited or delayed this process, while ecdysone greatly enhanced it and was capable of inducing the formation of autophagic vacuoles as early as during the 1st day of the instar both in vivo and in vitro (Sass and Kovács, 1975).

The present study was undertaken to determine whether this sensitivity of fat body cells to ecdysone exists in earlier instars. For this reason the hormone was
applied to the penultimate larval instars both in vivo and in vitro. During this stage neither autophagy nor heterophagic protein granule accumulation can be found in the fat body cells of normally developing larvae (Dutkowski, 1974; Ishizaki, 1965; Locke and Collins, 1965; Walker, 1966).

Material and Methods

Larvae of *Mamestra brassicae* kept at 28°C, in 70% humidity, under a light-dark regime of 12:12 h were used in the experiments. Under such circumstances the penultimate larval stage lasted 4 days. About 24 h prior to ecdysis, the larvae stopped eating, 8 to 10 h before ecdysis they ceased moving and finally clung motionless to the top of the vessel, while the colour of their bodies gradually darkened.

The lobes of fat bodies taken from 8 animals on each day of the penultimate larval stage and 10 h prior to ecdysis served as controls. In experiments performed in vivo, 10 µg/g body weight of β-ecdysterone (SIGMA Chem. Comp. St. Louis, USA) were given in the form of microinjections (20 µl) to animals on each day, 6 larvae being processed from each age group.

Experiments in vitro were carried out on fat bodies taken from animals on each day. The lobes of the organ of each animal were divided into two nearly equal parts which were then incubated separately in 1 ml of Grace medium at 28°C for 3 h. The medium in one of the incubation tubes contained 10 µg β-ecdysterone, while the other sample served as control. The tissues were fixed in 4% glutaraldehyde dissolved in 0.1 M phosphate buffer, postfixed in 2% osmium tetroxide and embedded in araldite. The sections were contrasted with uranyl acetate and lead citrate and examined in a UEMV-100B electron microscope.

Results

Untreated Animals. The fat body of the penultimate larval instar is composed of thin lamellae consisting of one or two cell layers. In the cytoplasm large lipid vacuoles are present which are, however, smaller than those characteristic of the last larval stage. Areas containing stored glycogen can also be found in the peripheral part of the cells. The rough-surfaced endoplasmic reticulum (RER) is well developed; free ribosomes can also be discerned. The amount of fat and glycogen increases with age, but otherwise no appreciable ultrastructural changes are noted in the cells during the stage.

In untreated fat bodies incubated in vitro the cells showed the same features as those in unincubated controls (Fig. 1). In certain cases the sacs of the RER appeared to be more dilated than usual.

Ecdysone Treatment in vivo. The hormone proved ineffective when applied on the 1st, 2nd or 3rd days of the instar. Given, however, on the 4th day (24 h before ecdysis) it induced the formation of autophagic vacuoles in the cells. The effect of treatment was slight as shown by the small size and sparse number of the vacuoles.

Ecdysone applied 10 h before ecdysis induced marked ultrastructural changes. In the vicinity of the cell nuclei a vast number of autophagic vacuoles of various sizes appeared containing sequestered parts of the cytoplasm. In addition, many multivesicular bodies and numerous small protein granules were discernible in the cytoplasm (Fig. 4). In this way, all the features characteristic of the fat body