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HISTOLOGICAL AND CYTOCHEMICAL STUDIES ON THE EFFECT OF JHA ON BODY WALL, FAT BODY AND MIDGUT EPITHELIUM OF *CULEX PIPIENS QUINQUEFASCIATUS* SAY

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Fourth instar larvae (24 h old) were reared in 0.05 ppm and 0.10 ppm if JHA *viz.* 1- (3'-carbpropoxy phenoxy) -3, 7-dimethyl - 6 -octene, for 24 h alongwith controls. Histological and histochemical studies of cuticle, epidermal cells, fat body cells and midgut epithelium showed necrotic changes after 0.05 and 0.10 ppm. These studies revealed that incomplete moulting and juvenoids (intermoults) formation was due to the interference of JHA in the digestion and absorption of food in the midgut epithelium, biosynthesis and storage of reserves of glycogen, fats and proteins in fat body cells.

INTRODUCTION

Williams (1956) proposed the use of insect juvenile hormones in controlling insect pests. A very few workers have reported the effect of juvenoids on midgut, fat body and body wall (Gordon & Burford, 1984; Raja *et al.,* 1987; Sharma,1994). All the three organs are important in insects as reported by Chapman (1971). The midget plays role in digestion and absorption of digested food. The fat body is the reserve house of fats, proteins and glycogen which are needed for metabolic activity of insect. It also contributes metabolites needed for the synthesis of cuticle during metamorphosis. Epidermis of body wall plays an important role in metamorphosis. Keeping in view all these factors the present report deals with the effect of JHA, *viz.* 1-(3'-carbpropoxy phenoxy) - 3,7 - dimethyl -6-octene on these organs in *Culex pipiens quinquefasciatus* Say.

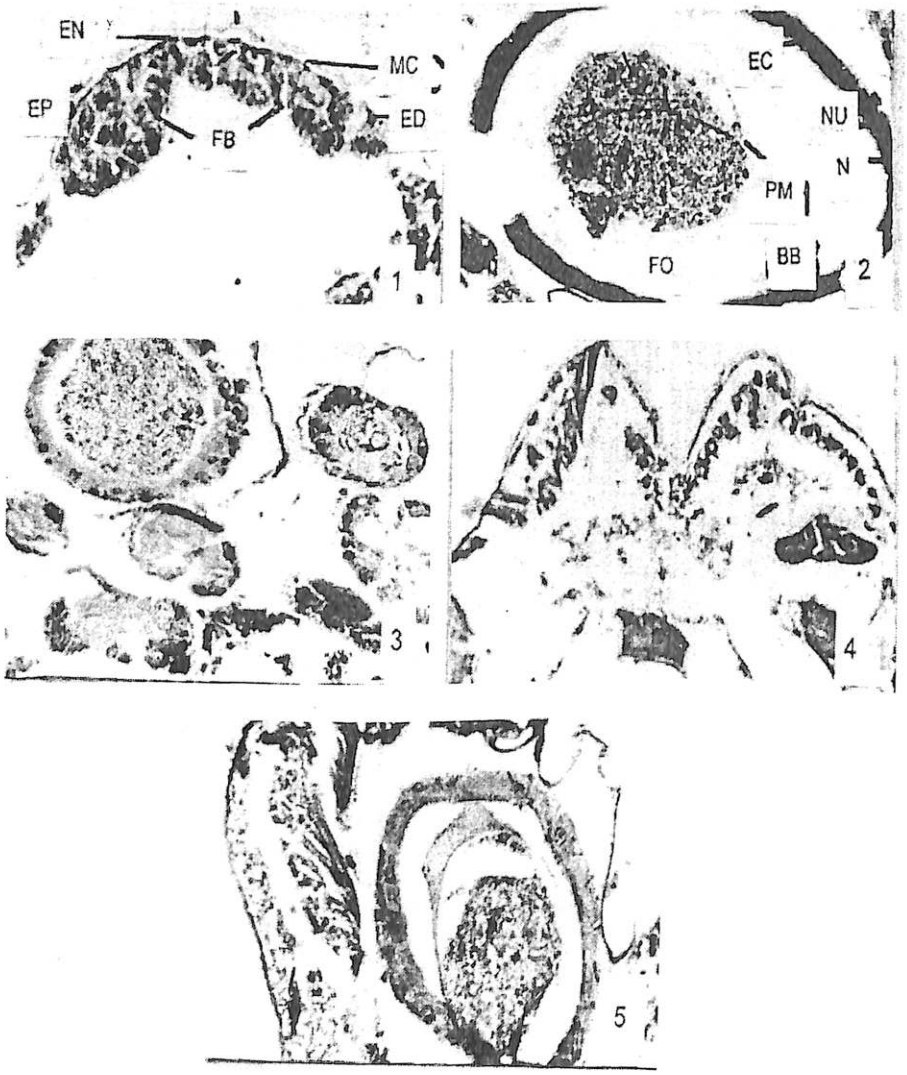
MATERIALS AND METHODS

Fourth instar larvae (approximately 24 h old) were reared in sublethal doses, *i.e.* 0.05 ppm and 0.10 ppm of JHA, *viz.* 1-(3'-carbpropoxy phenoxy) - 3,7-dimethyl-1,6 octene for 24 h. For control, acetone used in stock solution to dissolve JHA was diluted with water to obtain 0.05 ppm and 0.10 ppm in acetone. The whole larvae of treated and control were fixed in Bouin, Zenker and formaldehyde calcium. These were processed and embedded for block making according to the procedure of Pearse (1968). For histology, Bouin-fixed larvae were stained in 0.5% Heidenhein's haematoxylin/eosin. For cytochemical tests, periodic acid - Schiff (PAS) for 1:2 glycol groups of carbohydrates, Best's carmine for glycogen, mercuric - bromophenol blue (Hg - BPB) and ninhydrin - Schiff (NHS) for proteins, Feulgen for DNA, methyl green/pyronin G (MG/PG) for DNA and RNA, Sudan black B (SBB) for general lipids, oil red O (ORO) for triglycerides and Nile blue sulphate (NBS) for neutral and acidic lipids were employed on the sections of treated and control larvae according to the procedures prescribed by Pearse (1968).

OBSERVATIONS

Histological studies

Body wall : In control fourth instar larvae, after staining with Z/1H, three layers of cuticle *i.e.*epicuticle, mesocuticle and endocuticle overlying above the epidermal cells were observed



Figs. 1-5. 1. T.S. of control 4th instar larva showing body wall and fat body. Z/1H, x 500; **2.** T.S. of control 4th instar larva showing midgut epithelium. Z/H; x 250; **3.** T.S. of 4th instar larva treated with 0.05 ppm showing midgut. 2/1H. x 250; **4.** T.S. of 4th instar larva treated with 0.05 ppm of JHA showing body wall and fat body. Z/1H; x 250; **5.** T.S. of 4th instar larva treated with 0.1 ppm of JHA showing body wall, midgut and fat body. Z/1H, x 250. (BB = Brush border; EC = Epithelial cells; EPC = Epicuticle; EDC = Epidermal cells; ENC = Endocuticle; FB = Fat body; FO = Food; MC = Mesocuticle; N = Nucleus; NU = Nucleolus; PM = Peritrophic memberane)

(Fig. 1). After treatment with 0.05 ppm JHA, endocuticle was distrupted at certain places. Epicuticle and mesocuticle were damaged and were very lightly stained. After treatment with 0.1 ppm the cuticle was more badly damaged and very lightly stained. The epidermal cells were smaller than control and their cytoplasmic contents also showed a decrease (Figs. 4 & 5).

Fat body : In control, the fat body cells of the normal larvae having eccentric nuclei with darkly stained nucleoli were observed (Fig. 1). A few vacuoles were also present in the cytoplasm. In the cytoplasm darkly stained thick granules concentrated along the cellular membrane were also observed. With 0.05 ppm JHA a very slight change in cytoplasmic granules was observed. After 0.10 ppm, the fat body cells were badly damaged (Fig. 2).

Midgut : The midgut cells of control were observed to be flattened with central rounded nuclei having nucleoli and cytoplasmic granules. These have ciliated brush border towards the lumen. Peritrophic membrane was also present covering the food (Fig. 3). After 0.05 ppm cytoplasmic granules were lightly stained and with 0.10 ppm, the midgut cells were found to be severely damaged. Their cytoplasmic contents were not clear (Figs. 2 & 4).

Cytochemical studies

Body Wall : After 0.05 ppm all the layers of cuticle and epidermal cells showed depletion in Hg-BPB and NHS staining showing decrease in proteins. After 0.05 and 0.10 ppm nuclei of epidermal cells showed negligible stain in Feulgen, and MG/PG for DNA and RNA. After 0.10 ppm nuclei of epidermal cells showed negligible stain in Feulgen, and pyroninophilia in MG/PG in cytoplasm also decreased very much.

In Hg-BPB, the depletion in staning due to proteins was observed with both the doses. In 0.1 ppm the protein granules showed a decrease.

In PAS after both doses there was depletion in staining due to 1 : 2 glycol groups of carbohydrates. The PAS positive granules showed a decrease along the border of the cell.

The mitochondria in epithelial cells stained blue in AH, NBS showed some decrease in lipids after both doses. Phospholipids in their cytoplasm showed a decrease.

The depletion in metabolites in all the three organs was dose dependent.

DISCUSSION

According to Chapman (1971) and Wigglesworth (1972) midgut epithelium is very important part of the alimentary canal in insects as it acts as a secretory seat of digestive enzymes for the digestion of food in midgut lumen and absorbs the digested food with the help of microvilli on the epithelial cells. The decrease in DNA, RNA and proteins after JHA treatment reveals that there is decrease in the synthesis of digestive enzymes by these cells. The overall decrease in lipids, carbohydrates and proteins after treatment may be due to necrotic changes in epithelial cells of midgut.

Chapman (1971), Wigglesworth (1972), Mittal & Kanta (1987) have reported the presence of glycogen, lipids (triglycerides) and proteins as reserves in fat body cells. Locke & Collins (1967) and Dean *et al.* (1984) state that all insects take up haemolymph proteins, particularly at metamorphosis and are stored in fat body cells. During present study the depletion in proteins has been reproted after JHA treatment. This is in agreement with Raja *et al.* (1987) who reported that methoprene inhibited the sequesteration of haemolymph proteins in fat body of *Chilo partellus.* Gordon & Burford (1984) reported that methoprene caused alterations in the concentration of haemolymph metabolites and in the metabolism of proteins and carbohydrates by the fat bodies

of 4th instar larvae and pupae of *Aedes aegypti.* Sharma (1994) also reported similar observations after JHA treatment.

The decrease in glycogen after JHA treatment in fat body cells may be responsible for the decreased glycogenolysis resulting in reduced trehalose in haemolymph required for metabolic activity.

Beenakkers *et al.* (1985) stated that lipid contents and lipid composition of the fat body are the results of various processes including storage of dietary lipids, *de novo* synthesis, degradation and modification of fat body lipid and subsequently release of their transport to sites of utilization. The test chemical during present study seems to interfere in the storage of lipids in fat body cells and seems to affect all the functions of lipids described by Beenkkers *et al.*(1985). It also seems that JHA interferes with adipokinetic hormone of corpora cardiaca which is required for lipids mobilization. These observations are in conformity with Sharma (1994) who also reported interference of JHA's in lipid biosynthesis in fat body cells.

According to Chapman (1971) the onset of moulting is usually first indicated by changes in the epidermal cells which divide mitotically and so become close-packed and columnar in shape. Since the substance of the cuticulin layer of epicutical (*i.e. wax layer*) is probably produced in oenocytes and then transferred to the epidermal cells which secrete it to the outside. The necrotic effects of test JHA on epidermis seem to be responsible for incomplete moulting and juvenoid formation.

It can be concluded that the test chemical interferes in the digestion and absorption of food in the midgut epithelium; and storage of reservoirs of glycogen, fats and proteins in fat body cells. The necrotic changes in body wall changes seem to be responsible for intermoults (juvenoid) stages formed after test chemical treatment.

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