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HISTOLOGICAL AND HISTOCHEMICAL STUDIES ON THE FAT BODY IN *CULEX* AFTER TREATMENT WITH A NEWLY SYNTHESIZED JHA

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The fourth instar larvae of *Culex pippins quinquefasciatus* (24 h old) were reared in sublethal doses (1, 2 and 3 ppm) of a newly synthesized JHA : 1-(3'-methyl-6'-isopropylcyclohexyloxy-3, 7-dimethyl-2(E), 6-octadiene for 24 h. Their histological and histochemical studies revealed that JHA treatment produced decrease in number of fat body cells, vacuoles in them and change to their cellular membranes. It was dose dependent. DNA, RNA, proteins, 1:2 glycol groups, lipids (acidic and neutral) decreased qualitatively whereas glycogen showed an increase. These changes may be responsible for larval mortality and incomplete moulting after JHA treatment.

INTRODUCTION

According to Chapman (1971) the fat body plays an important role in metabolism and serves as a depot for storage of fat, carbohydrate and protein reserves. Fatty acid synthesis takes place in the fat body. Blood proteins are synthesized from amino acids produced in the fat body. King & Akai (1984), Locke & Collins (1968), Collins & Downe (1970), Dean *et al.* (1984) reported the role of fat body in insects. Lea & van Handel (1970), van Handel & Lea (1970), Wright & Rushing (1973), Downer *et al.* (1976), Gordon & Burford (1984), Mittal & Kanta (1987), Chattoraj & Sharma (1988), Garcia *et al.* (1988), Sawby *et al.* (1992) and Sharma (1994) have reported the effect of JHAs on fat body of insects. Keeping in view of the importance of fat body, the present work has been undertaken to see the histopathological and histochemical effect of test compound on various metabolites in fat body of *Culex*.

MATERIALS AND METHODS

Fouth instar larvae (24 h old) were collected from the colony of *Culex pipiens quinquefasciatus* maintianed at 26 ± 2°C and relative humidity of 60-80% in B.O.D. incubator. 1mg of newly synthesized JHA : 1-(3'methyl-6'isopropylcyclohexyloxy-3, 7-dimethyl-2(E), 6-octadiene - was dissolved in 10 ml of acetone and was diluted with distilled water to prepare doses of 1, 2 and 3 ppm (Mittal & Navpree, 2000). These controls were also prepared by dissolving 10 ml acetone in distilled water to prepare 1 ppm, 2 ppm and 3 ppm doses. Fourth instar larvae were reared in these sublethal doses along with their controls for 24 h. After that these were narcotized in refrigerator. These were fixed in various fixatives, *viz.* Bouin, Zenker, formaldehyde calcium and weak Bouin. These were processed and embedded for section cutting. The sections were stained with iron haematoxylin (Baker, 1945). For histochemical tests, *viz.* mercuric bromophenol blue (Hg-BPB) and ninhydrin-Schiff (NHS) for general proteins, periodic acid-Schiff (PAS) along with controls for 1:2 glycol groups, Best's carmine (BC) along with control for glucogen, Feulgen along with controls for DNA, methyl green/pyronin G (MG/PG) along with control for DNA and RNA, Sudan black B (SBB) for general lipids, acid haematein (AH) along with control for phospholipids, and Nile blue sulphate (NBS) for acidic and neutral lipids were performed according to the procedures given in Pearse (1968).

RESULTS AND DISCUSSION

The results obtained from histological and histochemical studies are as under :

Zenker/Iron Haematoxylin : In the control fat body cells, the circular nuclei are generally eccentric having darkly stained nucleoli. A few vacuoles were also observed in the cytoplasm. In the cytoplasm, darkly stained thick granules are present along the cellular membrane (Fig. 1). After treatment with 1 ppm, the size of fat body cells decreased. The nuclei and nucleoli were lightly stained (Fig. 2). After 2 ppm and 3 ppm doses, the cells were observed tc be badly damaged and these took very light colour as compared to control.

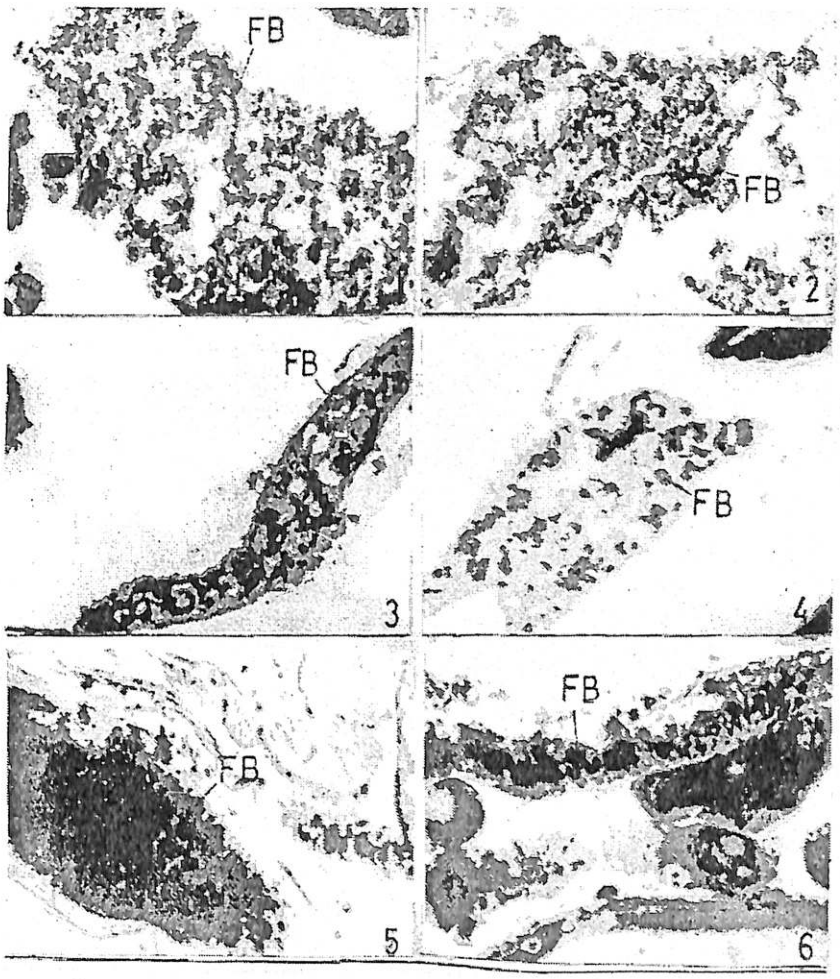
MG/PG and Feulgen's test for nucleic acids : In the control fat body cells, the nuclei stained blue (-ive after control) for DNA and the cytoplasm stained pink (-ive after control) in MG/PG for RNA. After 1 ppm and 2 ppm there was depletion in staining in nuclei and cytoplasm granules for DNA and RNA respectively. After 3 ppm the nuclei of some fat body cells became pycnotic and others showed depletion in staining for DNA. The pyroninophilia due to RNA ed abundant depletion in MG/PG.

Hg - BPB and NHS tests in proteins : The nuclei and nucleoli of the fat body of control larvae took blue and pink colour after Hg-BPB and NHS respectively. The protein containing bodies were concentrated near the nuclei and cellular periphery. After treatment with 1 ppm and 2 ppm, the protein sites showed a decrease in colour in Hg-BPB (Fig. 3) and NHS. With 3 ppm dose the fat body cells took very light colour (Fig. 4).

The present observations are in conformity with Mittal (1991) and Mittal *et al.* (1991) who after treatment with newly synthesized JHAs : 1-(3-carbpropoxy phenoxy)-3, 7-dimethyl 1-6, octene and 2-methyl-5-isopropyl phenyl ether, respectively reported necrosis of fat body cells in *Culex*. They also reported a decrease in DNA, RNA and proteins. Patel & Madhwan (1968) and Grezlak & Krishnakumaran (1985) studied the effect of exogenous JH and reported that it inhibited ecdysteroid stimulation of general transcription as well as synthesis of specific mRNAs. The fat body becomes the main protein store of an insect when the haemolymph storage proteins are transferred to it (Locke & Collins, 1968; Tojo *et al.*, 1981; Locke *et al.*, 1982; Dean *et al.*, 1984). It seems that transfer of haemolymph storage proteins in the fat body is interfered due to necrosis caused by JHA during the present observations. Gordon & Burford (1984) and Raja *et al.* (1987) also reported that methoprene inhibited the sequestration of haemolymph proteins by the fat body. So it can be concluded that depletion in proteins in fat body cells is on account of depletion in DNA and RNA; and due to inhibition of sequestration of proteins by fat body.

BC and PAS tests for carbohydrates : In PAS uniform distribution of PAS positive material in cytoplasm of fat body cells in addition to a few dense granules (-ive after PE and restoration of colour after KOH treatment) was observed. After treatment with 1 ppm and 2 ppm there was .a lot of decrease in PAS staining. With 3 ppm treatment staining was almost negative. In BC, cytoplasm granules along with some deposits of glycogen along cortical cytoplasm were observed. With 1 ppm and 2 ppm, the glycogen sites took dark colour. After 3 ppm the glycogen sites were observed to be darkly stained. Mittal (1991) also reported an increase in glycogen in fat of *Culex* after JHA : 1-(3-carbpropoxyphenoxy)-3, 7-methyl 1-6 octene. The increase in glycogen in fat body cells may be due to inhibition of glycogenolysis by JHA during presnt stdies. This may be responsible for the decrease in trehalose in the haemolymph required for metabolic activity.

SBB, AH and NBS for lipids : In SBB (Fig. 5) and AH (-ive after control) and NBS the cytoplasm of fat body cells took deep blue colour for phospholipids and some vacuoles were also observed. In NBS the pink globules containing triglycerides were also observed. After 1 ppm, 2 ppm and 3 ppm the phcspholipids and triglycerides showed decrease in various tests for lipids (Fig. 6) as compared to control. According to Beenakkers *et al.* (1985) lipid contents and lipid



Figs. 1 - 6 : T.S. fouth instar larva of *Culex*. **1.** Fat body cells in control, B/IH, x400; **2.** Necrotic fat body cells after 1 ppm treatment, B/IH, x400; **3.** Necrosis in fat body cells after 2 ppm treatment, Z/Hg. BPB, x400; **4.** Necrosis and depletion in staining in fat body cells after 3 ppm treatment, Z/Hg.BPB, x400; **5.** General lipids in fat body cells in control, FCa/SBB, x400; **6.** Depletion in general lipids in fat body cells after 2 ppm treatment, Fca/SBB x400.

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 subsequent release for modification of fat body lipid and release for transport to sites of utilization. Mittal (1991) and Mittal *et al.* (1991) reported decrease in phospholipids and triglycerides in fat body like those of present observations. Mittal & Navpreet (1998), and Mittal & Ruchita (1999) reported decrease in phospholipids, triglycerides and cholesterol in whole larvae after JHAs. It seems that JHA inhibits fat body cells to sequester fatty acids and triglycerides from haemolymph to synthesize lipids from non-lipid precursors and may also inhibit secretion of adipokinetic hormone from corpora cardiaca.

The necrosis of fat body cells caused by JHA and changes in various metabolites after various doses may be responsible for larval moulting and incomplete moulting as observed by Mittal &

Navpreet (2000) after treatment with the same JHA.

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