CHANGES IN ESTERASE ACTIVITY IN THE SPINNING GLAND CELLS OF *DIACRISIA OBLIQUA* (LEPIDOPTERA) DURING SEQUENTIAL STAGES OF DEVELOPMENT

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Esterase activity has been studied for the first time during different phases of cellular activity i.e. growth, prefunctional, functional and post functional (degenerating) ones in the spinning gland cells of the Indian moth *D. obliqua*. Esterase activity is in keeping with the needs of energy of the growing spinning gland cells which is associated with increased metabolism of the cell rather than the secretion of silk. The enzyme also seems to play a significant role in the termination of larval period.

The investigators have expressed conflicting ideas regarding the localization and activity of esterases in different tissue cells of insects. While some have confined their study to single instar only (2, 5), others have reported the presence of lipolytic enzymes in different cell types during growth and metamorphosis (8, 9). Present work is an attempt to study the pattern of localization and activity of esterases in only one cell type at various phases of activity in sequential developmental instars and metamorphosis.

MATERIALS AND METHODS

The insects *D. obliqua* were collected from the castor gardens around Allahabad and reared in the laboratory at a temp. 25–27°C. Spinning glands were dissected out at different stages of development (larvae, prepupae and pupae) and sectioned 6–8 μ m thick, with cryostat. α -naphthyl acetate method (6) was employed to detect esterase activity in the cells. Control experiment were run side by side in which: (a) the substrate α -naphthyl acetate was omitted from the incubating mixture. (b) 0.1 M solution of arsanilic acid and 1 gm potassium fluoride were used as inhibitors.

OBSERVATIONS

The enzyme esterase detected by α -naphthyl acetate method, shows positive activity and specific localization in the cytoplasm of the spinning gland cells. In the first, second and third instars the cells show moderate enzymatic activity in the basal and apical cytoplasm as well as around the nuclei (Fig. 1).

FIG. 1. Spinning gland cells of third instar larva of *Diacrisia obliqua* showing esterase activity.

During fourth instar stage the cells of the gland grow in size. The activity of enzyme also increases considerably. Fig. 2 shows reaction product mainly in the basal and apical cytoplasm of the gland cells. In the middle region esterase activity is comparatively less.

In the cells of the fifth instar larva of *D. obliqua* there is remarkable increase in

FIG. 2. Cells of the spinning gland of fourth instar larva of *D. obliqua*. Enzyme activity is mainly confined to the apical and basal cytoplasm.

FIG. 3. Spinning gland cells of fifth instar larva showing dense deposits of enzyme reaction product in the middle cytoplasm.

FIG. 4. Esterase activity in the spinning gland cells of prepupa of *D. obliqua* mainly confined to the basal cytoplasm.

FIG. 5. Degenerating spinning gland cells of 2-day old pupa of *D. obliqua* showing reduced enzymatic activity.

the enzymatic activity. Dense deposits of enzyme reaction product are formed mainly in the middle region of cells. Basal as well as apical cytoplasm is devoid of reaction product. Thus there is alteration in the enzyme active sites in the cytoplasm of the gland cells (Fig. 3).

In prepupa a notable change in the sites of enzyme activity in the cells of the spinning gland is observed. It is now mainly confined to the basal cytoplasm. The activity of the enzyme in the apical and middle region gets reduced. What appeared as an expansion of enzymatic sites during mid developmental period is now qualified with shrinkage of enzyme active area in high secretory phase of the gland cells (Fig. 4).

In post-secretory phase (pupation) the activity of the spinning gland cells is precipitously reduced. With the onset of degeneration esterase activity does not remain confined to the periphery as it was in the preceding stage. The entire cytoplasm shows diffuse enzyme activity as shown in Fig. 5. Later in advanced stage of degeneration, the activity of the enzyme appears extinguished.

In controls the observations regarding esterase distribution and activity in the spinning gland cells were compared with those in the usual test cases. Negative results were obtained in the former.

DISCUSSION

On the basis of the biochemical estimation of esterases Eguchi and Sogimoto (1) found changes in esterase zymograms in *Bombyx mori* during development. Schiff and Sonnenschein (7) applied esterase enzymology to understand the variations in enzymatic activity in normal and diseased tissue cells. According to them, esterases are present during growth phases accompanied *inter alia* with quantitative changes.

Pant and Srivastava (5) found esterase to be highly active during larval development and it declined sharply with the commencement of spinning. They could not, however, pinpoint intracellular changes in the patterns and sites of enzyme activity in the cells. In the silk gland cells of *D. obliqua* there is not only variation in the enzymatic activity but also obvious expansion in the area of enzymatic activity in early, mid- and peak secretory phases characterised by first, second and third; fourth and fifth instars and prepupal stage respectively. The decline in esterase activity prior to spinning in the mid-gut cells of *Phylosamia ricini* is considered to be associated with the transport of enzymes from mid-gut to lumen in the course of histolysis. In the degenerating cells of the spinning gland of *Diacrisia obliqua* during pupation, although showing enfeebled enzymatic activity; no transport of enzyme into the lumen of the gland could be observed. In fact, the entire gland is in a state of collapse, and the lumen itself loses its identity. Moreover, the passage of the secretion across the lumen of the gland or its duct is no longer there at that time. There is reason to believe that pupal decline of esterases in the silk gland cells occurs as a result of depletion of the substrate and transport of the remaining enzyme into the haemolymph of the insect. It is corroborated by the fact that the apical region of the gland cells becomes free of esterase activity. Increased esterase activity, prior to commencement of silk-spinning, has been suggested by Pant and Srivastava (5) to be involved either in silk synthesis or in the termination of the larval period. In their opinion the significant decrease in enzyme activity on the onset of metamorphosis could be attributed to its role in spinning. Enzyme activity is, no doubt, associated with substrate degradation to fuel the metabolic process in the cells. In the silk gland cells of *Diacrisia obliqua* esterase activity in the fifth instar stage is highest, and there is notable decline of enzyme activity in the prepupa. Moreover, the secretion of silk already starts in the first instar larva. In prepupa, when silk secretion is maximum, there is sharp decline in esterase activity. These facts do not agree with the participation of esterase in silk synthesis as far as the silk gland cells are concerned. Had it been so, then the peak (Maximum) esterase activity could be seen in the cells of prepupa, but it is not so. It is concluded that the esterase activity is in keeping with the needs of energy of the growing silk gland cells, which is associated with increased metabolism of the cell in general rather than the secretion of fibroin. Silk spinning may be taken as cocoon formation, but silk secretion is a process entirely different. Pant and Srivastava (5) consider that silk is synthesized only during spinning, which is, however, not the case. The silk synthesis is an intracellular process. The secretion is released into the lumen of the gland. It is spun into threads and woven around the animal in the prepupal stage enclosing it by an envelope (cocoon). The investigators, referred to above, have considered that silk is synthesized and immediately released and spun into cocoon only in the prepupal stage. To this extent their study is incomplete and unsatisfactory.

Esterases have been assigned a major role in the termination of insect embryonic diapause and in the resumption of embryogenesis (3, 4). It has also been suggested that they participate in the termination of the larval period in non-diapausing ones. Pant and Srivastava (5) emphasised that esterase activity could be associated with spinning. Present findings on the cytochemistry of silk glands of the moth studied point to the role of esterases in the termination of the larval period rather than in

silk synthesis.

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Abbreviation L; Lumen, BM; Basement membrane, SG; Silk globules, SC; Silk core, DGC; Degenerating gland cells.

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