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The Effect of a Juvenoid on the Acid Phosphatase Activity in the Midgut Epithelium of *Spodoptera litura* Fabr.

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Using modified lead nitrate method of Takeuchi and Tanoue, acid phosphatase (AP) activity was studied in the midgut cells of the normal larval and pupal stages of *Spodoptera litura* Fabr. and changes in AP activity in abnormal insects, produced as a result of feeding larvae with synthetic juvenile hormone (SJH), were also studied. In the normal feeding larvae, AP was rich in secretory and absorptive cells and was released in the lumen. In the prepupae and pupae, it was noted in the discarded cells.

Irrespective of the difference in external effects, SJH treatment inhibited degeneration and regeneration of midgut epithelial cells and the AP activity in the secretory and absorptive cells. The accumulation of the enzyme at the free surface of the cells and its release were not seen. Changes in AP activity, however, occurred after considerable delay.

Key Words: Acid phosphatase, Juvenoid effect, Midgut epithelium, *Spodoptera litura*

Introduction

The regulatory effect of juvenile hormone (JH) on insect metamorphosis is well established. Plantevin (1977) has shown by *in vivo* and *in vitro* studies in *Galleria mellonella* that the intestinal epithelium and epidermal cells are sensitive to JH. Srivastava and Singh (1978, 1980) have demonstrated that in *Sarcophaga ruficornis* the cells of the fore- and midgut are highly sensitive to JH and that the various processes of cellular degeneration, regeneration, secretion and absorption are influenced by it. These changes are likely to be associated with changes in the enzymatic system, particularly in the activity of acid phosphatase. It was, therefore, considered appropriate to study changes in the AP activity in the midgut cells of *Spodoptera litura* Fabr. during normal metamorphosis as well as differences in its activity as a result of treatment of last instar larva with synthetic juvenile hormone (SJH) (Roller's compound).

Materials and Methods

A culture of *S. litura* was maintained in the laboratory at 25 ± 2°C. Larvae were fed on castor leaves. The last larval instar had a total life-span of 6 ± 1 days. It fed actively for 4 days. During the last 2 days, feeding stopped and the larva was transformed into the non-feeding prepupal stage with characteristic external morphological changes.

Forty μg of the hormone, dissolved in acetone, were administered on a small piece of castor leaf (about 1 × 1 cm) with a microapplicator. Acetone was allowed to evaporate completely, following which, one-day-old last instar larva, which had not been given food for about 4 hr, was brought to feed on the leaf. When it had completely consumed the treated piece of leaf, it was given untreated leaves to feed upon as usual.

Normal and SJH-induced abnormal larvae and larval-pupal mosaics of the desired stages (as indicated in the

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observation), were chilled for 10 min and then quickly dissected in cold distilled water. Their midguts were removed, fixed in chilled neutral formalin for 24 hr, embedded in gelatin at 37°C and frozen sections, 10μ thick, were cut. Modified lead nitrate method of Takeuchi and Tanoue (Pearse 1982) was adopted to stain acid phosphatase. Parallel checks for ensuring correct identification of acid phosphatase were run by incubating sections in: (i) the medium devoid of substrate, and (ii) the medium supplemented with 0.1M sodium fluoride, a specific inhibiter of AP. In both these checks, AP was not detected and hence it was not considered worthwhile to include their photographs.

Results

Normal Insect

The midgut epithelium of the following stages of the normal, untreated insects was examined for AP: *24 hr old, feeding, last instar larva.* The midgut is histologically differentiated into three regions—the anterior, purely secretory region; the middle secretory and absorptive region; and the posterior, mostly absorptive region. AP was noted in abundance in the secretory (goblet) and absorptive (columnar) epithelial cells of the three regions of the midgut. The distribution of the enzyme, however, varied in the different regions. The enzyme which occurred richly at and near the apical border of all the cells in the anterior region, was also found throughout the entire cytoplasm at different sites (figure 1). In the middle region of the midgut, AP activity appeared to be distinctly located in the central cytoplasmic region of both the cell types (figure 2). Cells of the posterior region, as in the case of the anterior region, also exhibited strong AP activity near their free borders (figure 3). Release of this enzyme into the lumen of the midgut by rupture of the free borders of the cells was observed in the anterior and posterior regions of the midgut, but not in the middle region. Thus, although, the enzyme appeared to be synthesised in the epithelial cells in all the three regions of the midgut, its release into the lumen occurred only in the anterior and posterior regions.

Non-feeding prepupae: The original epithelial layer of the prepupa was shed off into the lumen and replaced by a new layer arising from the new regenerative cells. The larval epithelial cells in the early prepupal stage showed conspicuously rich AP activity throughout the cytoplasm. This intense AP activity was also noted in the cells which had already been shed off into the lumen. The regenerated cells, on the other hand, showed the enzyme in relatively small concentrations (figure 4). This condition of the enzyme in cells of the old and new epithelia was observed in all the three regions of the midgut.

24-hr-old-pupae: The distribution and intensity of the enzyme in different regions of the midgut in the 24-hr-old pupa were comparable to what was observed in the prepupae

Treated Insects

(i) Larvae showing prolonged life-span after treatment

Feeding stage (24 hr after SJH treatment): the nature and distribution of AP activity in the three regions of the midgut resembled those in the respective regions of the midgut of the normal 24-hr-old feeding larvae.

Non-feeding stage (8 days after SJH treatment): Histologically, degeneration and regeneration of the midgut epithelium was not observed. Discarded cells were not seen in the lumen. All along the midgut, epithelial cells showed the enzyme only in the basal half and there was no sign of its impending release (figure 5). Consequently, the difference, if any, between secretory and absorptive cells, was not clear. This was also true for the normal feeding larva, thus, the difference between conditions in the treated and non-treated larvae was confined to the anterior and posterior regions of the midgut.

(ii) *Larvae which suffered ecdysial failure*

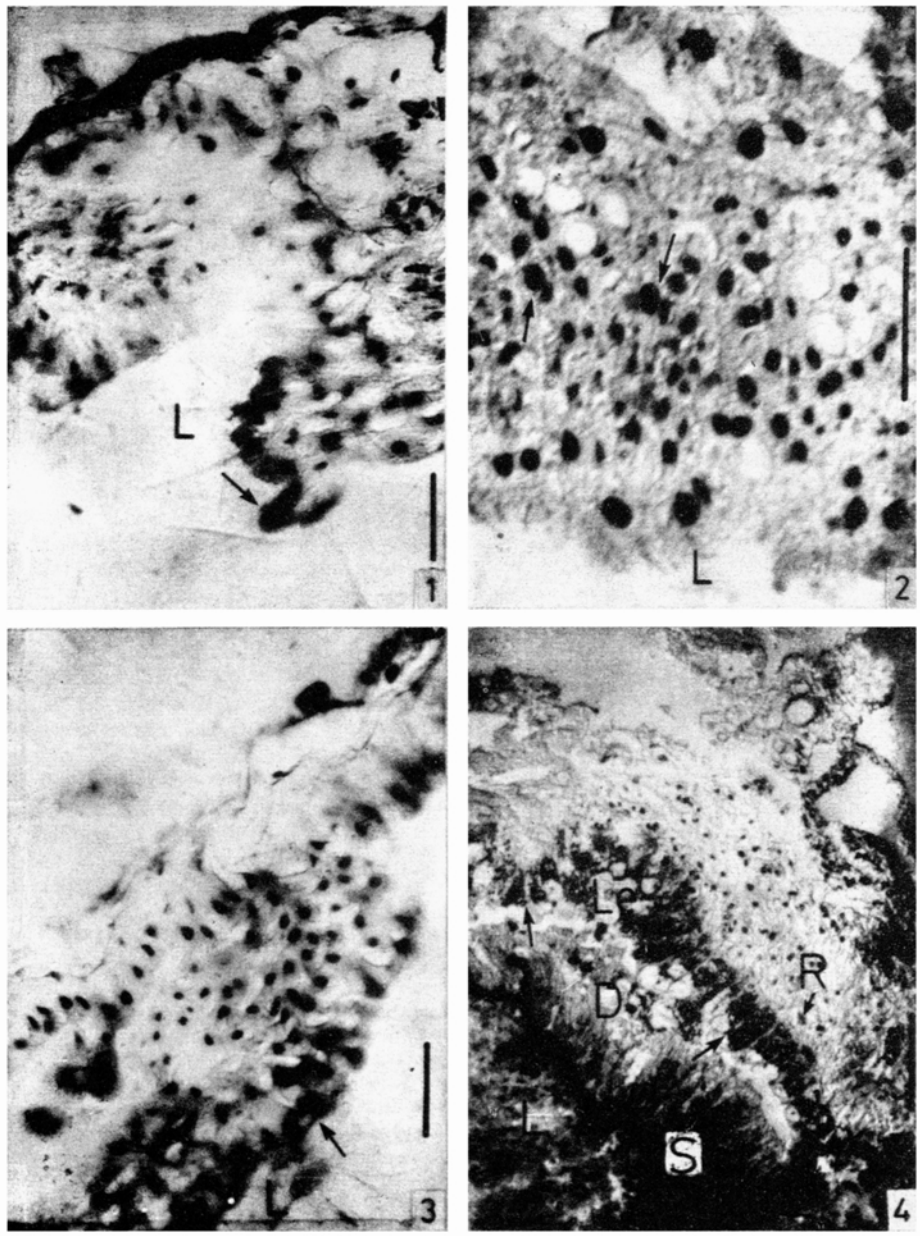
In all the three stages, viz. (i) at the onset of moulting; (ii) 24 hr after (i), when the posterior half had moulted; and (iii) 48 hr after (ii),—the pattern of enzyme distribution in the midgut was more or less similar. In all the three regions, while the apical half of the epithelial cells was devoid of AP, their basal half was particularly rich in this enzyme. The condition is somewhat similar to the one in the non-feeding stage of the SJH-treated larvae (figure 6).

The larval-pupal mosaic produced by SJH-treatment was examined on the 2nd day after the last moult.

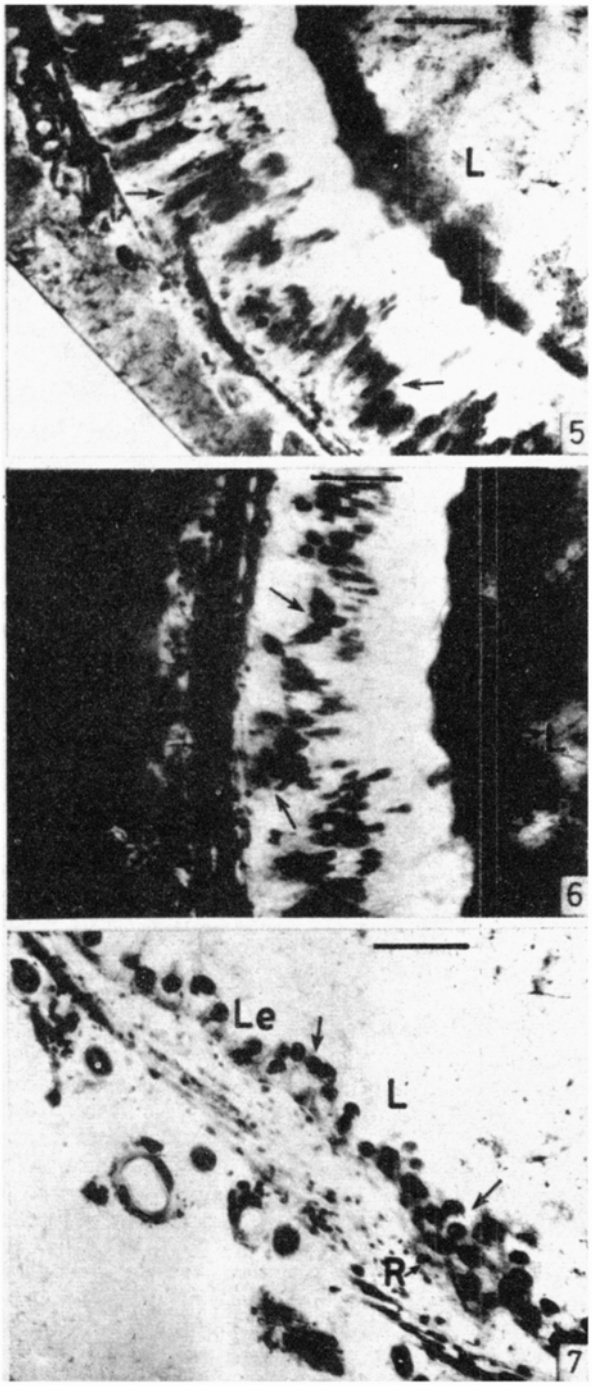
The cells lining the midgut in stage (iii), as far as we could see, were not shed off into the lumen. The epithelium was relatively much thinner, and there was no distinction between secretory and absorptive cells. The enzyme gave intense reaction in a small area far from the free border and near the basal region. As in the case of the prepupae, the apical half of the cells was distinctly free of the enzyme and the regenerative cells in the larval pupal mosaic multiplied to form a layer of regenerative cells. Some of these cells showed a small enzyme-rich area (figure 7).

Discussion

The present study on the midgut epithelium of *S. litura* brings to light certain interesting facts. For instance, in the normal last instar larvae, AP is released by secretory cells of the anterior and posterior regions of the



Figures 1–4 **1,** Acid phosphatase in the epithelial cells of the anterior region of midgut of the larva of *S. litura*. AP is seen at and near the apical border of cells and at different sites in the cells (L, Lumen). Arrow shows AP activity; **2,** Acid phosphatase in the epithelial cells of the middle region of the midgut of larva. AP is located in the central cytoplasmic region of the cells; **3,** Acid phosphatase in the epithelial cells of the posterior region of the midgut of larva. The distribution of AP is similar to that of figure 1; **4,** Acid phosphatase in the cells of the midgut of pre-pupa. Rich AP activity is noted in larval cells, including those which have been shed off. Regenerative cells show poor AP activity (Le, Larval epithelial cells; R, Regenerative cell; D, Degenerative cells; S, Shed off cells). Arrows show AP activity



Figures 5–7 **5,** Acid phosphatase in the midgut epithelium of larva after 8 days of SJH treatment (Non-feeding stage). AP is located in the basal half; there is no sign of its release; **6,** Acid phosphatase in the midgut epithelium of larva which suffered ecdysial failure following SJH treatment. The apical half of the epithelial cells is devoid of AP while the basal half is rich in it; **7,** Acid phosphatase in the midgut epithelium of the larval-pupal mosaic produced by SJH treatment. Larval cells show rich AP activity in a small area near the basal region. Some regenerated cells also show small enzyme rich area; Bars on all the figures represent 50μm

midgut but when SJH treatment is given on 6th or 7th day, when the larva stops feeding, the enzyme is seen confined to the basal region and no sign of its release is noted in any part of the midgut. This is also the condition in the case of ecdysial failure. On the other hand, in the feeding condition, 24 hr after SJH treatment, no difference from the normal midgut was found in the distribution of the enzyme. Beel and Feir (1977) have noted changes in AP, attributable to JH treatment, occur after considerable delay which we have also observed. However, we do find that the changes are distinct, and, in general, it can be stated that SJH treatment exhibits AP activity.

The larval midgut epithelium during normal metamorphosis has been seen to be discarded into the lumen, digested and reabsorbed (Kathuria 1971, Whitten 1976, Singh 1977) and the new imaginal epithelium arises from the interstitial cells. Evidently, AP released in the normal early metamorphosing midgut epithelium helps in the disintegration and digestion of the discarded cells. As a result of SJH treatment, the process of degeneration of larval epithelial cells is wholly or partly stopped, and AP activity and secretion are severely inhibited. This is the condition in insects with prolonged larval life or those suffering ecdysial failure. Couch and Mills (1968) had earlier shown an association of AP in the secretory cells with the intracellular autolytic process. They observed that the escape of the enzyme may be brought about by the disintegration of discharged cells or by the release at the borders of cells. In either case, the discharged enzyme is regarded by them as a constituent of the digestive fluid. Radford and Misch (1971) have also noted an increase in AP in the midgut during metamorphosis in *Sarcophaga bullata* and regarded it as a general feature of histolysis, occurring during such transformation.

On the basis of the generally accepted histolytic (including degenerative) function of AP, and the present observations that SJH treatment inhibits its synthesis, it may be assumed that among other factors, SJH titre controls the entire series of processes involved in the metamorphosis of midgut epithelium like the cells being discarded, their disintegration and digestion and finally their absorption, by regulating the synthesis of AP.

The absence of the processes which follow, e.g. the origin and differentiation of imaginal cells, may be the result of the inhibition of the earlier process of degeneration. Radford and Misch (1971) claim to have demonstrated the effect of ecdysterone in increased AP activity in the midgut cells. If so, the action of ecdysterone and JH seem to be antagonistic.

Postponement of larval-pupal moult and ecdysial failure resulting from JH administration (Riddiford 1972, Slama et al. 1974) also indicate the interference by excess of JH in the action of the moulting hormone. In the mid gut cells one of the ways in which this interference is manifested consists of reduced AP activity. It would imply that excess of JH somehow causes suppression of the genes which regulate AP synthesis either at the level of transcription and/or translation. It would be interesting to work the exact molecular mechanism.

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