

PROTEOLYTIC ENZYME CONCENTRATION, WEIGHT CHANGE AND STARVATION IN LARVAL *Aedes aegypti* (L.) (DIPTERA: CULICIDAE)

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ABSTRACT. Changes in proteolytic enzyme concentration were measured between the 2nd-3rd and 3rd-4th larval ecdyses (i.e. during one complete molt) in fed and starved mosquitoes. Weight changes were determined during the same period.

Growth in enzyme-secreting tissue appears

Although proteolytic activity in adult mosquitoes has been studied, the larval stage has been relatively neglected. Gooding (1972) provided a recent review of digestive processes of hematophagous insects which included consideration of digestive enzymes.

Yang and Davies (1971a) found high concentrations of chymotrypsin and trypsin in 3rd and 4th instar *Aedes aegypti* larvae of unspecified ages within a given stadium. Yang and Davies (1971b) measured the amount of chymotrypsin egested during the larval period. Samples of larvae were taken at 2, 4, 24, 48 and 72 hr after hatching from eggs, but ages within a given stadium were not recorded. The amount of enzyme egested by starved larvae was very small compared to fed larvae. A particularly large amount of enzyme was egested at larval-pupal ecdysis. This would be expected since at this time the gut contents are voided.

to be isogonic with total growth. Enzyme secretion is not interrupted by molting and ecdysis. Since enzyme secretion is apparently continuous with feeding and terminates abruptly in the absence of food, it is regulated in close conjunction with feeding.

The purpose of this study was to determine change in proteolytic enzyme concentration relative to growth (weight change) in fed and starved larvae in the period beginning with the shedding of the 2nd larval exuvium and ending with the shedding of the 3rd larval exuvium. This time period insured that one complete molt, i.e. apolysis to ecdysis, would be covered.

MATERIALS AND METHODS

Larvae of *Ae. aegypti*, Rockefeller strain, were studied. They were fed a mixture of finely ground Purina® lab chow, lactalbumin hydrolysate and brewer's yeast, in equal volumes.

Following eclosion, larvae were placed singly in individual ice tray cubicles. They were supplied with an ample amount of food and incubated at $27 \pm 1^\circ\text{C}$. When ready to undergo 2nd-3rd ecdysis, larvae were checked every half-hour. The time

between the 2nd-3rd and 3rd-4th larval ecdyses was 23.5 ± 1.3 hr ($n = 14$; range, 21.5-26.0 hr; 95% confidence limits, 22.8-24.2 hr.). Thus the 24 hr samples mentioned below represent larvae approximately at the time of 3rd-4th ecdysis.

Larvae were collected 0, 6, 12, 18 and 24 hr following the 2nd-3rd larval ecdysis. Post-ecdysial age was accurate to within 30 min. Individual larvae, removed at appointed times, were placed in BEEM capsules (8 mm O.D. \times 13 mm long; Entomological Supplies, Inc.) and stored in a freezer. Three larvae were pooled in 1 capsule as 1 test sample.

After larvae of all post-ecdysial ages to be studied were collected, they were packed in ice. The weight of each sample was determined on a Mettler analytical balance by weighing each capsule before and after removal of the larvae.

Enzyme assays were performed with azure-blue hide powder (Calbiochem) (Rinderknecht et al. 1968). Four milligrams of azure-blue hide powder were used for each milliliter of 0.01 M sodium borate buffer solution (pH 8.5-9.0). All preparations were incubated at $27 \pm 1^\circ\text{C}$. The greatest resolution between different enzyme concentrations occurred after approximately 2 hr of incubation.

After weighing, each sample was placed

in 5 drops of buffer in a tissue grinder. The grinder was rinsed twice with 5.0 ml of buffer which was poured into a wide mouthed vial containing 40 mg of hide powder. The vial was capped and placed in ice.

Subsequently, all samples were incubated at $27 \pm 1^\circ\text{C}$ for 2 hr 15 min. A control of 40 mg of hide powder in 10.0 ml of buffer was incubated with the test samples. After incubation, the test samples and control were filtered and absorbance read at 595 nm on a Bausch & Lomb Spectronic 20 spectrophotometer which was zeroed with the control.

To study the effects of starvation, larvae were maintained as above, with the exception that upon the 2nd-3rd ecdysis, they were removed from the original ice cube tray and placed in another which was filled with tap water and contained no food. At 0, 12, 18 and 24 hr following ecdysis, samples were weighed and assayed for enzyme as above.

RESULTS

Weight and enzyme concentration (Table 1) increased at a constant rate in fed larvae. Doubling time for the enzyme and weight was about 5 hr. To compare rates (i.e. slopes of the regression lines), optical density units were multiplied by

Table 1. Weight and enzyme in *Aedes aegypti* larvae at intervals after 2nd-3rd larval ecdysis.

Age ^a	Number Weighed ^b	Weight (mg) ^c	Number Assayed ^b	Optical Density ^c
Fed				
0	8	0.8 ± 0.2	7	0.114 ± 0.028
6	3	1.7 ± 0.4	6	0.174 ± 0.033
12	10	3.2 ± 0.8	4	0.291 ± 0.052
18	12	4.0 ± 0.9	6	0.419 ± 0.064
24	14	5.0 ± 1.3	8	0.524 ± 0.072
Starved ^d				
12	7	1.4 ± 0.3	8	0.116 ± 0.052
18	9	1.6 ± 0.6	8	0.117 ± 0.046
24	8	1.4 ± 0.3	8	0.131 ± 0.055

^a Age in hours after ecdysis.

^b Number of test samples. Each sample consisted of 3 larvae.

^c \pm standard deviation.

^d 0h, same as fed.

10. Since variance was not equal among the weight and new optical density values, the data were transformed with logarithms in order that a single classification analysis of variance could be performed. The results of this test showed that the progressions of weight and optical density were the same ($F = 0.3714^{n.s.}$, d.f. 4 & 76).

Among starved larvae, the 0 hr samples (same as fed) weighed less than the 12, 18 and 24 hr samples ($F = 8.3851$, d.f. 3 & 28, $p \leq 0.001$), but there were no differences among the 12, 18 and 24 hr samples ($F = 0.4590^{n.s.}$, d.f. 2 & 21) (Table 1).

There was no significant change in proteolytic enzyme concentration in starved larvae during the test period ($F = 0.2241^{n.s.}$, d.f. 3 & 27) (Table 1).

DISCUSSION

Since rates of enzyme and weight increase are the same, it appears that growth of enzyme-secreting midgut cells and total growth are isogonic. It is possible that the quantity of enzyme secreted per cell increases with time or that enzyme accumulates within the midgut. However, as shown by Yang and Davies

(1971b) enzyme is egested in fed larvae. In any case, it appears that enzyme is secreted continuously with feeding and is not interrupted by molting or ecdysis.

Despite starvation, there was an increase in weight during the first 12 hr following 2nd-3rd larval ecdysis. Since there was no food available, this was probably due to uptake of water.

Since we found no change in enzyme concentration, and there is little egestion (Yang and Davies 1971b) of enzyme in starved larvae, both secretion and egestion must be regulated in close conjunction with feeding.

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