NUTRIENT ACCUMULATION IN THREE MOSQUITOES DURING LARVAL DEVELOPMENT AND ITS EFFECT ON YOUNG ADULTS[1]

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Information on the accumulation of nutritional reserves during larval development of mosquitoes is practically non-existent. Wigglesworth (1942) had shown with elegant histological evidence that fourth instar larvae of *Aedes aegypti* (Linn.) synthesize protein, fat and glycogen, but no quantitative measurements are available for any instar. In this note, I will present the accumulation of larval protein, lipids, glycogen and trehalose of 3 mosquitoes maintained under identical conditions of diet, light and temperature. The results are a measure for larval growth (protein) and nutritional reserves (lipids and carbohydrates) that influences the survival and flight range of the newly eclosed adult before it finds a sugar or blood meal. The advantage of using protein as a criterium for larval growth instead of weight has been applied and discussed in a previous article (Van Handel 1986). In the present work, where larvae, pupae and adults are compared, the advantage of using protein instead of weight is even clearer.

Aedes aegypti, Culex nigripalpus Theobald and *Culex quinquefasciatus* Say, all from established colonies, were raised under 12 hr light- 12 hr dark at 26°C in 22 × 32 cm enamel pans containing 500 ml water and 150–160 larvae. Each treatment consisted of 2 pans. On the day of hatching, and 3, 4, 5 and 6 days later 100 mg food (3 parts dried brewer's yeast and 1 part porcine liver powder, purchased from ICN Biochemicals) was added. Larvae were collected at 0900 hr and food added thereafter. Larvae were analyzed in duplicate in pools of 25 on day 3, older larvae and adults in pools of 10. All chemical assays were repeated until the difference among replicates was less than 5 percent. Early pupae are predominantly male and late pupae predominantly female. Pupae were therefore analyzed when most larvae had pupated, but before adult emergence had begun, to minimize sex bias. The larval experiment was repeated 10 times over a 6 month period.

Adults for chemical analysis and starvation were collected within a few hours after eclosion. Starvation was determined at 26°C with 50 mosquitoes per cup (400 ml) in duplicate. The adults had access to water only and were predominantly at rest. These experiments were carried out a year later and repeated 6 times. The assays for protein, lipids, glycogen, trehalose and other sugars have been recently described (Van Handel 1985a, 1985b, 1986). The average result of each test was treated as a single data point and the averages of the experiments were used in the calculation of standard errors.

Aedes aegypti reached the fourth instar after 4 days, as judged by size, and pupated on the sixth day. *Culex nigripalpus* and *Cx. quinquefasciatus* reached the fourth instar after 5 days and pupated on the seventh day.

Protein. As expected by the difference in growth rate, *Aedes* larvae accumulated more protein during the first 3 days than the two *Culex* species, but between days 3 and 5 the rates of protein accumulation were similar in the 3 species. The maximum rate was between day 3 and 4 for *Aedes* (140 μg/day) and between day 4 and 5 for *Culex* (185 μg/day). However, the 3 species reached maximal protein content (approximately 400 μg each) at the same time (on the 5th day) with little or no further addition (Fig. 1). These results are in agreement with earlier experiments in which the effect of different diets was investigated (Van Handel 1986).

Lipids. The accumulation of lipids closely followed that of protein for all 3 species. However, the maximum lipid content of *Cx. nigripalpus* (40 μg) was only half that of *Ae. aegypti* and *Cx. quinquefasciatus* (80 μg), as shown in Fig. 2.

Carbohydrates. Glycogen reached its highest level after 6 days in all 3 species, but at pupation that for *Ae. aegypti* (55 μg) exceeded that for *Cx. quinquefasciatus* (40 μg) and *Cx. nigripalpus* (30 μg) as shown in Fig. 3 (closed circles). Trehalose, the circulating sugar of hemolymph, is limited to a few μg per larva. In *Ae. aegypti* it reached 7 μg in 4 days and then varied only a little. In *Culex* it rose to its maximum level (4.5 μg) in 4 to 5 days (Fig. 3, open circles). In addition to trehalose the larvae contain sugars that are anthrone-positive but destroyed by treatment with alkali. Only a small portion (20–25%) of these labile sugars was identified as glucose; fructose was absent. In all 3 species the sugar reached a maximum in 4 to 5 days and declined to a much lower level in the pupae (Fig. 3, open triangles).

[1] Institute of Food and Agricultural Sciences, University of Florida Experiment Station Journal Series 7564.

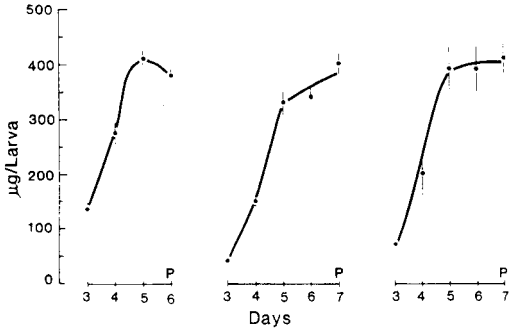

Fig. 1. Protein content of larvae of *Aedes aegypti* (left), *Culex nigripalpus* (center) and *Cx. quinquefasciatus* (right) until pupation (P). All values are expressed as µg per insect ± SE.

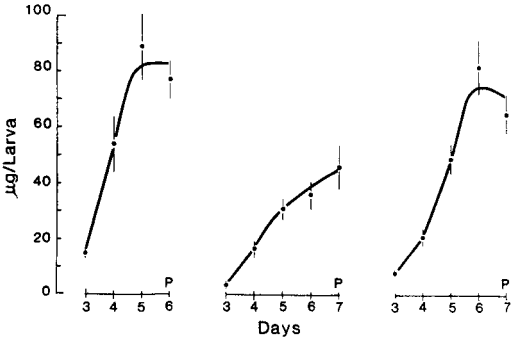

Fig. 2. Lipid content of larvae of *Aedes aegypti* (left), *Culex nigripalpus* (center) and *Cx. quinquefasciatus* (right) until pupation (P).

Since the protein levels at pupation were about the same in all 3 species, the fuel reserves (lipids and carbohydrates) per pupa or per mg protein would yield the same information and can therefore be directly compared (Fig. 1). The data suggest that adults from pupae with the lowest fuel reserves (*Cx. nigripalpus*) would have to find a food source sooner than the other 2 species. To test this hypothesis, pupae were allowed to emerge and males and females compared with larvae and pupae. Lipid reserves were close to those of pupae, with *Ae. aegypti* having the highest and *Cx. nigripalpus* the lowest levels. Glycogen reserves were almost the same in all 3 species and considerably lower than in pupae. Glycogen may have been used as a respiratory substrate by pupae. Protein levels were somewhat lower in adults, especially in the (smaller) males (Table 1a). This difference between pupae and adults may be due to excretion of some proteinaceous material (meconium) during the first few hours after emergence. When lipid levels are expressed per unit of protein, *Aedes aegypti* seems to have an even greater nutritional

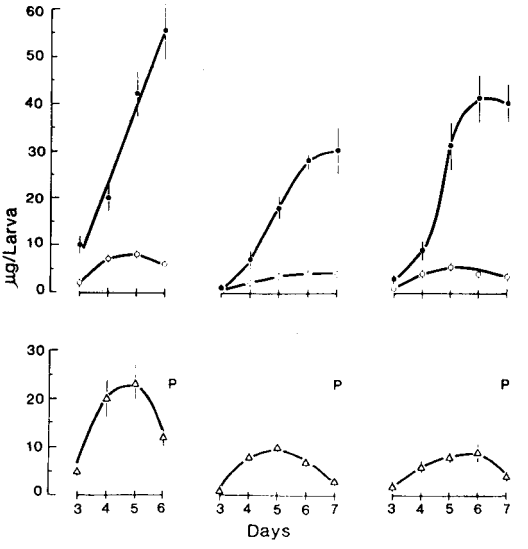

Fig. 3. Carbohydrate content of larvae of *Aedes aegypti* (left), *Culex nigripalpus* (center) and *Cx. quinquefasciatus* (right) until pupation (P). Closed circles: glycogen. Open circles: trehalose. Open triangles: glucose and other alkali-labile sugars.

Table 1a. Protein, lipid and glycogen content (µg/insect ± SE) of recently emerged adults.*

	Proteins	Lipids	Glycogen
	Aedes aegypti		
Males	180 ± 15	80 ± 5	20 ± 3
Females	290 ± 30	100 ± 15	27 ± 2
	Culex nigripalpus		
Males	220 ± 10	37 ± 3	24 ± 2
Females	360 ± 20	48 ± 8	24 ± 3
	Culex quinquefasciatus		
Males	230 ± 5	55 ± 10	26 ± 4
Females	350 ± 25	62 ± 7	26 ± 3

* In addition, all adults contained 4–6 µg trehalose and less than 2 µg of other sugars. The 3 species were raised simultaneously, and each experiment was repeated 6 times.

Table 1b. Mortality (days ± SE) in above mosquitoes starved from emergence.*

Species	Males	Females
Aedes aegypti	4.0 ± 0.40	4.3 ± 0.30
Culex nigripalpus	2.1 ± 0.15	2.6 ± 0.18
Culex quinquefasciatus	2.4 ± 0.25	2.6 ± 0.25

* Means of 6 experiments, each started with 100 mosquitoes, until 50% had died.

advantage. This was confirmed by mortality experiments in (resting) unfed adults. Male and female *Ae. aegypti* could postpone adult nutrition longer than either *Culex* species (Table 1b).

The technical assistance of Mrs. Hilda Lynn is appreciated.

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