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Carbohydrate Levels in the Normal and Aposymbiotic *Chironomus barbatitarsis* (Insecta; Diptera; Chironomidae) during Development

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The pattern of fluctuation of sugars in the integument, gut, fat body and haemolymph during larval periods of *Chironomus barbatitarsis*, was organ-specific and instar-specific. Total sugars content depicted gradual increasing levels up to late instar periods in the intgument, a more or less uniform level in the gut, and a high level during mid-instars in the fat body. Haemolymph sugar level was low during mid-instar periods and high towards late-instar periods. In pharate pupa, the sugar content was high in the fat body and low in the haemolymph. Aposymbiotic larvae showed marked mobilization of glycogen and elevation of trehalose and glucose levels. Of the total sugars, trehalose accounted highest content followed by glycogen and glucose.

Key Words: Sugars, Larva, Moulting cycles, *Chironomus*

Introduction

Studies on the ontogenetic variations of different nutrients in insects have received much attention of the insect physiologists. Variations of these biochemical constituents in different insects have been interpreted as reflecting the balance of syntheses, storage and degradation in response to different developmental needs (Gilbert & Schneiderman 1961, Bade & Wyatt 1962, Agrell 1964, Pant & Pandey 1980 and Islam & Roy 1981). Ontognetic variations of different biochemical parameters during the post-embryonic development of dipteran insects have made significant contributions in understanding the developmental patterns in holometabolous insects (Chen & Levenbook 1966, Bjarnov 1972 and Firling 1977). A study of quantitative and qualitative variations of sugars during metabolic processes is important in understanding the energy needs during ontogenetic development (Shighematsu 1956, Wang & Patton 1969 and Woodring et al. 1977). Most of the previous investigations, considered the ontogenetic profiles of

different nutrients in either the whole insect or in the haemolymph and fat body, which failed to provide any idea of organ-specific ontogenetic variations of these nutrients.

We have studied here the quantitative variations of total sugars, glycogen, trehalose and glucose during the larval periods of *Chironomus barbatitarsis* in the integument, gut, the fat body and haemolymph.

Symbiotic micro-organisms and their roles in providing essential nutrients to the host are well-documented in literature (Brooks & Kringen 1972 and Brown & Chippendale 1975). Among various plant products having anti-microbial activity, garlic extract has proved to be a potent antibiotic compound (Sharma et al. 1977). The possible role of intracellular bacteria in maintaining the normal sugar-balance during larval development of *C. barbatitarsis*, is also discussed.

Materials and Methods

Inbred stocks of *C. barbatitarsis* reared in the laboratory, following the method of Nandi (1981), were classified into four instars. Intermoult stages were identified as early, mid and late periods on the basis of age and external characters. Duration of the four larval instars was 4, 4, 5 and 12 days respectively. The first day after the preceding moult was considered as the early, the intermediate days as mid, and the last day as the late instar periods. In case of fourth instar larvae, 11th day was considered as late and 12th day as pharate pupa stage.

The larvae were rendered aposymbiotic through application of aqueous extract* of garlic (*Allium sativum*) and terramycin (Dey's Med. Stores) incorporated in the culture medium. Three sets of culture media were maintained: (i) normal, (ii) with 5%, 10%, and 20% aqueous extract of garlic (v/v), and (iii) with same concentrations of terramycin.

Detection and identification of intracellular bacteria was done as described by Islam and Roy (1982).

Biochemical estimations of the whole larvae were done from the second-instar onwards, and organ-specific estimations from the third-instar onwards. After dissection in the ringer solution, the integument, the gut and the fat body from 5-8 larvae (depending on the developmental stages) were pooled for a single estimation. Haemolymph was collected with a graduated capillary tube earlier rinsed with 1% phenyl-thiourea. Haemolymph collected from 7–10 larvae was used for a single estimation.

The haemolymph samples were dissolved in 0.2 ml of methanol which was latter evaporated at room temperature. The resultant residue was re-suspended in 0.1 ml of distilled water and centrifuged at 450g for 5 min. The supernatant so obtained was used for various estimations (Hill & Goldsworthy 1968). The tissue samples were homogenized with double distilled water and processed for specific estimations. Total carbohydrate was estimated following the method of Roe (1955) using anthrone reagent. For the determination of glycogen, protein was precipitated by adding 5% TCA with the homogenate and estimated following the method of Carrol et al. (1956). Trehalose and glucose were estimated enzymatically using trehalase and glucose oxidase (Dahlman 1973). Dry weights of tissues were determined by the conventional method.

Results

Total body sugars in the second instar larva were 6.78% of the wet wt. In the third instar, 7.79% decrease was observed while in the fourth instar it depicted 1.27% increase

*Garlic extract was prepared following the method of Augusti and Mathew (1973)

(table 1). Within the moulting cycle periods, total body sugars exhibited relatively uniform levels with insignificantly higher levels in the mid-instar periods (table 1). Total sugar level presented: *Integument*—gradual increase upto late periods; *Gut*–a more or less uniform level with insignificantly elevated levels in the mid instar periods; *Fat body*–a high level during mid stages of the third instar followed by a decline, while in the fourth instar a gradual increase was noted with significant increase in the pharate pupa *Haemolymph*—depicted a gradual decrease up to mid-periods followed by a sharp rise in the late-periods of third instar and a more or less uniform level up to the mid periods followed by a peak which declined in the pharate pupa stage of the fourth larval instar.

Glycogen : It was highest in the fat body (52–65%) followed by the gut (30–34%) and least (6.5–9%) in the integument. Intra-instar level of glycogen in the integument and the fat body showed increase during the mid-instar periods though in the fat body of pharate pupa it depicted a steady increase (figure 1 A, C). In the gut, a gradual increase from early to late periods was observed (figure 1B).

Trehalose: Fatbody and haemolymph showed more or less equal contents of trehalose (30–38%), followed by gut (18–20%), and only 8-9% in the integument. Trehalose levels in the gut and the fatbody presented higher concentrations during mid-instars in respect of early and late periods (figure 1, B, C). In the integument, the level maintained more or less uniform contents

Table 1 *Different sugars in the whole body (mg/100 mg wet wt) during the larval periods of* Chironomus barbatitarsis*

Instars (age in days)		Total sugars	Glycogen	Trehalose	Glucose
2nd instar	1	6.16±0.5	1.98±0.1	2.82±0.2	0.47± 0.03
	2	6.12±0.1	2.06±0.2	2.91± 0.5	0.51 ±0.08
	3	6.78±0.6	2.09± 0.7	2.99 ±0.3	0.57 ±0.02
	4	6.59±0.1	2.01± 0.9	3.02±0.4	0.58±0.09
3rd instar	1	5.91±0.5	1.94± 0.3	2.94±0.6	0.44±0.07
	2	6.13±0.6	2.05±0.3	3.01 ±0.8	0.49± 0.05
	3	6.29± 0.5	2.19± 0.3	3.05 ±0.9	0.48 ±0.07
	4	6.21 ±0.5	2.19±0.8	3.06 ±0.9	0.48 ±0.03
	5	6.03 ±0.5	2.05 ±0.7	3.02± 0.5	0.48 ±0.03
4th instar	1	5.58±0.6	1.93 ±0.3	2.77±0.7	0.42± 0.05
	2	5.69± 0.8	2.08±0.6	2.91 ±0.7	0.42± 0.06
	4	5.95 ±0.9	2.13 ±0.8	2.95± 0.7	0.41 ±0.05
	6	6.37 ±0.4	2.24 ±0.6	2.86± 0.9	0,49 ±0.08
	8	6,36 ±0.6	2.31 ±0.8	2.91 ±0.5	0.46 ±0.07
	10	6.38 ±0.5	2.34 ±0.7	3.03 ±0.9	0.48± 0.07
	12	6.28± 0.8	2.91 ±0.9	2.73± 0.6	0.45 ±0.06

*Data are mean ±S.E. of 7 replications

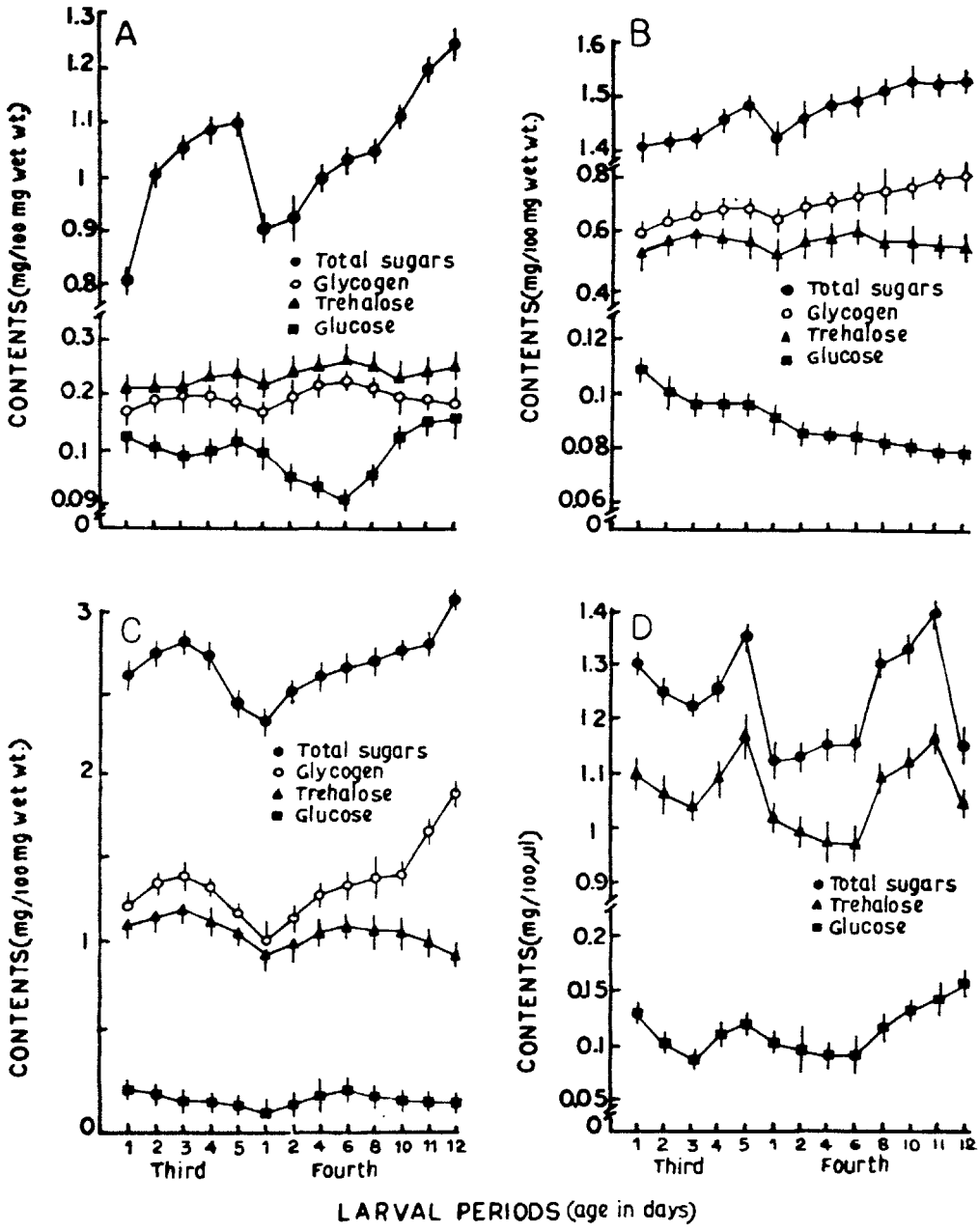

Figure 1 A-D Variation of sugars in the integument; *A*, the gut; *B*, the fat-body; *C*; the haemolymph; *D*, during third and fourth larval instars of *C. barbatitarsis* (Data are mean of 7 replications with S E at respective points)

up to mid instars while in the late instars the contents appeared high (figure 1, A). In the haemolymph, it depicted a gradual decrease up to the mid periods followed by a peak in the late periods; however the level showed a decreasing content in the pharate pupa (figure 1,D).

Glucose: In the integument, the gut-and the fat-body-glucose presented gradual decreasing level from early to late periods; in the haemolymph it depicted declining trend in the mid instar followed by gradual increase in late periods (figure 1, D).

Table 2 gives an idea of the growth rates in the third and fourth instars. Both instars showed rapid growth up to mid-instars followed by a plateau and a slight decrease on the last day.

In garlic-treated and terramycin-treated larvae, the number of bacteria/larva was notably lower. Among the three doses used, 20% garlic extract and terramycin were the most effective in reducing the number of bacteria/larva. The harbouring bacteria appeared to be gram-positive. Both the antibiotics caused marked mobilization of glycogen, slight increase of total carbohydrates and trehalose, and significant elevation of glucose levels (table 3). The treated larvae failed to pupate and the duration of respective instars was prolonged.

Discussion

The pattern of fluctuations of various sugars was instar-specific and did not follow the general trend in relation with age as reported by previous workers (Shighematsu 1956, Wang & Patton 1969, Woodring et al. 1977 and Islam & Roy 1981). Organ-specific fluctuations of various sugars often reflected the developmental dynamics of the system concerned. In the integument, active growth mainly occurred during the early (to reconstruct the shredded cuticle) and the late periods for the formation of new cuticle beneath the old cuticle (Condoulis & Locke 1966 and Hill & Goldsworthy 1968). The level of various sugars, however, did not reflect this rhythm; rather it demonstrated that the sugar-requirement for integumentary cells during these periods was largely met from other sources, mainly the fat body. The gut tissues are least affected by the rhythm of

Table 2 *Relation of total body wet wt and dry wt in the third and the fourth larval instar of* C. barbatitarsis. (*Weights are expressed as mg ± S.E. of 7 replications*

Age in days	Third Instar*		Fourth Instar**	
	Wet wt mg ± SE	Dry wt mg ± SE	Wet wt mg ± SE	Dry wt mg ± SE
1	5.1 ± 0.1 (25)†	1.1 ± 0.02 (25)	6.5 ± 0.2 (25)	1.26 ± 0.03 (25
3	6.8 ± 0.2 (26)	1.8 ± 0.03 (26)	6.8 ± 0.6 (22)	1.79 ± 0.04 (22)
5	6.9 ± 0.3 (25)	1.5 ± 0.04 (25)	9.7 ± 0.7 (21)	2.91 ± 0.03 (21)
7	——	——	10.3 ± 0.3 (23)	3.4 ± 0.02 (23)
9	——	——	10.7 ± 0.7 (26)	3.7 ± 0.05 (26)
11	——	——	10.9 ± 0.4 (22)	3.6 ± 0.06 (22)
12	——	——	10.8 ± 0.2 (21)	3.5 ± 0.08 (21)

*Larval duration is 5 days; **Larval duration is 12 days; †Data within the parentheses represent the number of insects used in each replication

Table 3 *Total carbohydrates (CHO), glycogen (GLY), trehalose (TRE) and glucose (GLU) contents (mg/100 mg mg wt) in relation to the number of bacteria (BN) in normal, garlic- and terramycin-treated* C. barbatitarsis *during third and fourth larval instars (mid) (Data are mean ± S.E. of 7 replications)*

		Third Instar					Fourth Instar				
		BN	CHO	GLY	TRE	GLU	BN	CHO	GLY	TRE	GLU
Control		65 ±1.8	6.29 ±0.2	2.19 ±0.1	3.05 ±0.6	0.48 ±0.3	71 ±1.6	6.37 ±0.7	2.24 ±0.4	2.86 ±0.1	0.49 ±0.05
Garlic-treated	5%	7.5 ±0.1	7.10 ±0.3	1.51 ±0.2	3.59 ±0.6	0.95 ±0.08	8.3 ±0.08	6.48 ±0.4	1.78 ±0.9	3.13 ±0.8	0.88 ±0.09
	10%	6.1 ±0.5	7.19 ±0.7	1.38 ±0.8	3.98 ±0.4	1.21 ±0.1	7.4 ±0.04	6.52 ±0.6	1.39 ±0.9	3.51 ±0.5	1.31 ±0.08
	20%	4.1* ±0.06	7.25** ±0.8	1.05** ±0.2	4.16** ±0.8	1.85** ±0.1	5.4* ±0 6	6.99** ±0.4	1.11** ±0.7	3.89** ±0.6	1.81* ±0.05
Terramycin treated	5%	7.2 ±0.06	7.11 ±0.8	1.51 ±0.7	3.61 ±0.3	0.89 ±0.6	8.1 ±0.06	6.45 ±0.9	1.66 ±0.1	3.02 ±0.5	0.81 ±0.03
	10%	6.8 ±0.02	7.18 ±0.7	1.36 ±0.3	3.89 ±0.4	1.15 ±0.4	7.6 ±0.03	6.54 ±0.9	1.32 ±0.2	3.54 ±0.7	1.33 ±0.04
	20%	4.3* ±0.03	7.26** ±0.8	1.12** ±0.3	4.08* ±0.8	1.79* ±0.04	5.5* ±0.08	6.98** ±0.5	1.12* ±0.1	3.82** ±0.5	1.79* ±0.07

* denotes $P<0.05$; ** denotes $P<0.1$ in comparison to control
The number of bacteria/larva is exprcssed as data X 10^7

ontogenetic events. The variation patterns of glycogen clearly reveal that the gut serves as an important site of glycogen storage. Relatively high trehalose level in the gut appears enigmatic, since it is neither a chief component of the diet nor has any functional significance in the gut. Probably, trehalose accumulation in the gut is due to its diffusion from the haemolymph (Laufer et al. 1964). The fat body, the main site of storage, syntheses and degradation and the haemolymph representing the transporting titre of various components showed positive fluctuation of different sugars in relation to developmental requirements. In the first half of the moulting cycle, high food consumption rate results in corresponding high rate of glycogen synthesis which mainly remains stored in the fat body whose content depends upon the food consumption rate and the period of development (Shighematsu 1956). In the late moulting periods, an inverse relation was noted between the fat body glycogen and haemolymph trehalose level. The high trehalose content in the haemolymph may be due to greater mobilization of glycogen in the fat body. However, trehalose content in the haemolymph is said to be greatly transformed to glucose to meet energy requirement during moulting (Chippendale 1978). Relatively lower trehalose level during early moulting

periods often corroborated the role of trehalose in the synthesis of chitin (Chippendale 1978).

In the pharate pupa, a steady accumulation of glycogen in the fat body signified the storage of food reserves to be used as an immediate source of energy in the early pupal periods (Islam & Roy 1981). Moderate decline of haemolymph trehalose level and increase of haemolymph glucose level might be due to the fact that glucose is needed both as an energy source and as a substrate for the synthesis of pupal cuticle (Chippendale 1978).

The fluctuation of total body sugars in relation to age (table 1) shows that the synthesis, storage and degradation of sugars correspond with the food consumption rate. Growth rate data reveal that active food consumption actually occurred in the first half of respective moulting cycle after which the feeding rate declined and became zero on the last day of respective instars as has also been observed by Woodring et al. (1977).

The reduction in the number of bacteria/larva in the antibiotic-treated larvae and the quantitative variation of sugars suggest that the bacteria play a role in the metabolism of these compounds (Brooks & Kringen 1972 and Brown & Chippendale 1975). The symbiotic bacteria provide most of the important B-vitamins to the host (Brooks & Kringen 1972). The absence of thiamine could have prevented the aposymbiotic larvae to utilize glucose completely since the vitamin acts as a co-factor in shuttling the glycolytic end-products into TCA cycle. Biotin defficiency would also limit the rate at which glycolytic end-products entered the TCA cycle. All these factors impaired the ability to metabolize glucose which results in premature utilization of glycogen (Brown & Chippendale 1975). Slight increase in trehalose concentration in the treated larvae may be accounted for by the fact that intracellular bacteria utilize a large part of host trehalose as an assimilable source of carbon (Bismanis 1976).

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