LOW TEMPERATURE ACCLIMATIVE STUDIES ON *PHILOSAMIA RICINI* LARVAE

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ABSTRACT

Low temperature studies on insects have shown that cooling profoundly affects carbohydrate level as well as the enzyme and respiration rates. Fifth instar larvae of *Philosamia ricini*, having a life span of 5 days, could be acclimated for 10 days at 4° C without any significant disturbance in their development. Extending the acclimation period for further 10 days did not visibly affect the larvae except for their becoming flaccid and somewhat inactive.

Cooling to 4° C results in the accumulation of glucose and acetylglucosamine. Trehalose is the major carbohydrate in the acclimated *P. ricini* larvae. Triacylglycerols appear to have exerted 'sparing' action on the carbohydrates during later stage of acclimation.

INTRODUCTION

MANY reviews have appeared on the studies of short-term acclimation and long-term adaptation to temperature[1]. Insects exhibit acclimative changes in respiration and the activity of some enzymes[2] either by extensive supercooling or by production of cryoprotectants such as glucose, trehalose, sorbitol and glycerol[3].

Low temperature studies on insects have shown that cooling profoundly affects carbohydrate level[4]. Further, direct relationships between the degree of cold-hardiness and level of polyols[3], carbohydrates[5] and unsaturated fatty acids[6] have been shown for several overwintering insect species. In *Protophormia terranovae*, accumulation of glycerol to which several protective roles have been ascribed[7] was observed in cold-stressed adults[8] although some species accumulate it even during normal diapause[9]. Interestingly the non-diapausing fifth instar larvae of *Philosamia ricini* was observed to accumulate glycerol[10]. Therefore, with a view to providing an insight into the physiological strategy of survival adopted by this insect at winter temperature, studies were carried out at regular intervals of acclimation.

MATERIALS AND METHODS

Larvae of *P. ricini* were reared in the laboratory at 29 ± 2° C as described earlier[11]. Ninety-five freshly ecdysed fifth instar larvae, picked at random from the colony and reared in wooden trays, were refrigerated at 4° C. Insects were picked at regular intervals randomly and the change in their weight noted. Rate of their mortality was also recorded.

From the colony, three sets of larvae, each consisting of three insects were subjected to homogenization (20%, w/v) as described earlier[12] and various assays were conducted on them as stated below.

For the analysis of total soluble carbohydrates, glycogen, fructose, pyruvic and lactic acids, trichloroacetic acid extract was employed[12], while glucosamine, acetylglucosamine, trehalose and total amino acids were assayed in the alcoholic extract[12] from the homogenate.

Total soluble carbohydrates were determined by the method of Trevelyan and Harrison[13], fructose by resorcinol method[14], aminosugars by the method of Elson and Morgan[15] and trehalose, by that of Wyatt and Kalf[16]. Glycogen was extracted from the lipid-free residue, with trichloroacetic acid and determined as described previously[12]. Pyruvic acid was assayed by the method of Friedman[17] and lactic acid by that of Barker and Summerson[18].

Total amino acids were determined by the method of Rosen[19]. Lipids were extracted from the entire tissue homogenate according to Folch *et al.*,[20] and gravimetrically determined[21]. Triacylglycerols were quantitated according to Fletcher[22].

Trehalase activity was assayed according to Derr and Randall[23] by estimating the rate of trehalose hydrolysis, the glucose released being estimated by the method of Somogyi[24]. Protein was assayed according to Lowry *et al*[25].

RESULTS

Exposing *P. ricini* larvae to 4° C renders them inert and significantly affects their rate of food consumption. This reflects in their normal metabolism resulting in retarded growth and development. Eighty per cent of the experimental insects survived for twelve days and lost 30% of their initial weight. Maintaining them for a further period, another 5% died while the rest regained their initial weight on days 14 and 21. When returned to the ambient temperature after ten days' exposure to 4° C, 80% of the larvae survived and pupated, the process being delayed by five days. It is noteworthy that the reverted larvae behaved exactly as the control in that they also took the same time period for moth emergence.

Larvae acclimated for a further period grew very weak and flaccid but continued to nibble at the food. When transferred to room temperature after twenty

TABLE I

The effect of low temperature (4° C) on various carbohydrate profile, protein, lipids and body weight

	Amount** (mg/g fresh weight)			
	Control	After 4 days at 4° C	After 14 days at 4° C	After 21 days at 4° C
Total soluble carbohydrates (calculated as glucose)	3·0 ± 0·3 (6)	6·1 ± 0·4 (6)	3·0 ± 0·5 (6)	3·1 ± 0·6 (5)
Trehalose	1·8 ± 0·2 (5)	1·7 ± 0·1 (5)	0·2 ± 0·1 (6)	1·0 ± 0·2 (6)
Glycogen*	78·0 ± 15·8 (4)	91·6 ± 17·2 (6)	111·2 ± 11·0 (6)	21·4 ± 8·9 (6)
Acetylglucosamine	0·1 (6)	0·3 (6)	1·1 ± 0·1 (6)	0·2 ± 0·02 (6)
Glucosamine	0·5 (6)	0·5 (6)	0·4 ± 0·1 (6)	0·1 (5)
Fructose	0·7 (6)	1·0 ± 0·1 (6)	0·6 ± 0·1 (4)	0·4 (6)
Glucose	0·7 ± 0·3 (5)	1·4 ± 0·3 (5)	0·7 ± 0·1 (6)	1·3 ± 0·3 (5)
Lactate	0·2 (6)	0·2 (6)	0·3 (6)	0·3 (6)
Pyruvate	0·9 ± 0·1 (6)	1·3 ± 0·1 (6)	0·1 (6)	0·1 (5)
Total amino acids	0·8 ± 0·3 (5)	2·0 ± 0·3 (6)	0·6 ± 0·2 (5)	0·9 ± 0·2 (5)
Trehalase activity (μg glucose liberated per mg protein at 37° C)	121·2 ± 24·3 (3)	60·6 ± 6·2 (3)	123·4 ± 20·5 (4)	42·5 ± 7·2 (5)
Protein	18·9 ± 1·0 (6)	24·4 ± 1·5 (6)	13·0 ± 1·0 (6)	17·3 ± 0·2 (6)
Total lipids	7·5 ± 0·9 (6)	9·3 ± 0·4 (6)	10·8 ± 2·9 (6)	4·0 (3)
Triacylglycerols	0·5 ± 0·1 (5)	0·6 ± 0·1 (6)	1·3 ± 0·4 (6)	0·3 (3)
Body weight per insect (g)	5·0 ± 1·0 (48)	2·6 ± 0·2 (48)	4·9 ± 0·5 (36)	5·0 ± 0·4 (8)

* Expressed in micrograms per gm wet weight.

** Expressed as the mean ± S.E.M. of the number of determinations stated in parentheses.

days' cooling, they spun cocoons within 24 hr. However, no moth emergence took place. Exceptionally, two larvae were seen to spin silk on day 20 even at 4° C.

Influence of Low Temperature on Various Carbohydrates

Mature Eri silkworm larvae contain about 0·3% of total soluble carbohydrates, mainly consisting of trehalose, glucose, fructose and aminosugars (Table I). During the first four days of acclimation total soluble carbohydrates increased by 202% while trehalose decreased only slightly. The 50% loss of trehalose activity observed on day 4 did not significantly reflect on the trehalose concentration. Total carbohydrates reattained the initial level on days 14 and 21.

Whilst the control insects contain equal quantities of both fructose and glucose, the experimental ones exposed to 4° C reveal a significant increase in both the hexoses, the latter exceeding the former. Prolonged exposure (21 days) further enhances glucose accumulation when fructose appears to have been significantly utilized. Presence of high concentration of acetylglucasamine was noteworthy during this period.

While lactate level remained more or less unchanged initially (first four days) during acclimation, pyruvate accumulated significantly (Table I). Total amino acids varied in a similar manner to pyruvate.

Protein, Total Lipids and Triacylglycerols

Although acclimation at 4° C apparently produced little change in protein content on day 4, it caused a significant depletion on day 14. The protein content returned to the initial level on day 21 (Table I).

Total lipids recorded significant decrease by 47% on day 21 suggesting their mobilization for energy purposes.

Triacylglycerols which are ordinarily utilized to regulate the energy needs for larval growth showed a 158% increase on day 14 (Table I).

DISCUSSION

In *P. ricini*, the majority of the fifth instar larvae, acclimated for 10 days at 4° C, did not lose the capacity to spin cocoons when transferred to the ambient temperature (29 ± 2° C). In those reverted after twenty days' cooling, though spinning occurred as in the controls, further development ceased and the insects died within the cocoons. Likewise, Cymborowski and Bogus[26] noticed serious disturbance following cooling in *Galleria mellonella* during late fifth instar larval development.

Glycogen has been reported to be the main carbohydrate source of energy in a majority of non-migratory insect species[4]. Development of *P. ricini* larvae at normal temperature (27–31° C) proceeds through instars 2–4 with significant increase in both total soluble carbohydrates and glycogen[27]. However, in the present investigation glycogen was present in microquantities leading one to speculate that during first four days of acclimation, carbohydrates, trehalose excepted, are not utilized as a source of energy in the colder environment as there is an appreciable increase in the content of total soluble carbohydrates and monosaccharides including acetylglucosamine. Perhaps, trehalose alone meets the energy requirement during this period. Since the amount of glycogen in *P. ricini* was very small and there appeared to be a fall in the trehalose content after fourteen and twenty-one days at 4° C, it is possible that trehalose functions as the major carbohydrate source of energy in the acclimated fifth instar larvae.

In *P. ricini* larvae, there has been a net increase in glucose and amino acid content. In another investigation on the larvae chilled at 2° C for a week, glyceride-glycerol decreased not very significantly while triacylglycerols increased substantially. In addition, haemolymph carbohydrates, trehalose, aminosugars, sorbitol and sorbitol-6-phosphate underwent decrease while total periodate-oxidizable substances, chiefly glycerol, increased considerably[28]. It is, therefore, presumed that in *P. ricini* larvae glycerol affords cryoprotection from freezing damage during long exposure to low temperature. This speculation follows from the established fact that increase in glucose and glycerol contributes to the lowering of the freezing point in the tissues and thus proves advantageous for their survival at low temperature[7]. Recently, a cryoprotectant system comprising of glycerol, sorbitol and trehalose has been demonstrated in the third instar larvae of the gallfly, *Eurosta solidagensis*[29].

Total lipid and neutral lipid contents in *P. ricini* increase all through larval development and attain maximum concentration at the fifth instar stage[27]. Immediately after spinning, however, they decrease suggesting the use of lipid as a source of energy for spinning purpose[21].

Triacylglycerols have been considered as a secondary source of energy for insects[30]. In the present investigation it is possible that total lipids and triacylglycerols are mobilized for derivation of energy on day 21 in preference to the carbohydrates. It is noteworthy that carbohydrates initially protected 'lipid stores' during cold exposure on day 14.

The important role of cholesterol as a precursor of ecdysone(s) is well recognized and documented. The observed retarded growth, disturbance in development and moth emergence could be traced to the diminished intake of phytosterols *via Ricinus* leaves.

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