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Thermal Acclimative Changes in the Different Lipid Fractions Composition of Fat Body of Eri-Silkworm, *Philosamia Ricini* (Ward.)

G. B. Singh*, ** and M. K. Singh***

Silkworm Research Laboratory, Udai Pratap College, Varanasi, India.

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Present communication deals with quantitative determination of total lipid, triglycerides, total free fatty acids, phospholipids and total cholesterol in the fat body tissue of the silkworm adapted to low and high temperatures. At the end of spinning process is characterized by a marked cellular reorganization of the different lipid fraction of the fat body irrespective of thermal acclimation. Accordingly, the per cent composition of triglycerides of the total lipid is increased accompanied by a corresponding decrease in free fatty acids, phospholipids and cholesterol.

Key words : Cellular reorganization, Hexose Mono-Phosphate pathway, Larval-pupal transformation

Introduction

Although the thermal acclimation of biosynthetic processes and the concomitant compensatory changes in cellular concentrations of macromolucules, even of some substances of low molecular weight, which are of structural and functional significance in the organisms, seems to be fairly widespread phenomena in poikilotherms, information in insects is quite fragmentary and debatable (Singh and Das, 1980). Characterization of cold adaptation by an increase in the degree of unsaturation of lipids is well documented in a wide variety of poikilotherms. Frankel and Hoff (1940) noticed an augmented saturation of fat during warm adaptation of Phormia and Calliphora larvae. In adult of *Phormia terranovae* an increase in the degree of saturation of the depot fats with increasing temperature of acclimation was reported by Cherry (1959). Quantitative increase (14%) of total fat in *Ephestia* larvae was observed during cold adaptation (Somme, 1972). Investigation of Danks and Tribe (1977) indicated that alteration of fatty acids composition of phospholipids in the dipteran flight muscle mitochondrial membrane might be significant in the modulation of certain enzymetic activities (like glycerophosphate-cytochrome c-oxidoreductase) during thermal adaptation.

The present article envisages certain aspects of quantitative determination of total lipid, triglycerides (neutral fat), total free fatty acids, phospholipids and total cholesterol in the fat body tissue of the insects adapted to low and high temperature.

Materials and Methods

The eri-silkworm *Philosamia ricini* was reared according to Singh and Singh (1984). Laboratory reared larvae were transferred right on the first day of the 3rd instar in B.O.D. incubators maintained at 15±1C and 30±1C. The fat body of larvae were removed (Singh and Singh, 1992) from the first day of 5th instar onwards. Thus, the period of whole 3rd and 4th instar was available to the insects for thermal adaptation. Biochemical assays for different lipid components of fat body tissue of cold and warm adapted insects were carried out using the following methodologies: Total lipid (Folch *et al.*, 1957), phospholipids (Bieri and Prival, 1965), triglyceride (Natelson, 1971a), free fatty acids (Natelson, 1971b), and cholesterol (Natelson, 1971c).

The significance of all the data , was statistically tested by the standard '*t*'-test and its modifications for un-

*To whom correspondence should be addressed.
Central Sericultural Research and Training Institute, Srirampura , Mysore- 57008, Karnataka, India.
**Present address.
Central Sericultural Research and Training Institute, Srirampura , Mysore- 57008, Karnataka, India.
***Present address. Regional Sericultural Research Station, Ranchi, Bihar, India.

known variance in the two samples. The significance was accepted at P<0.05 only.

Results

The changes in the lipid composition of the fat body of eri-silkworm induced by thermal acclimation have been presented in Table 1. As it evident from Table 1 the total lipid content of fat body of the larvae from both thermal groups sharply increases during the 5th instar larval growth reaching the maximum level just prior to spinning process, this parameter in the insects of both thermal groups decreases gradually till the middle days of the period followed by only a detectable rise in the late days. However, the cold adapted insects always demonstrate higher level of total lipid content of their fat body in comparison to that of the warm adapted insects throughout the developmental stages studied. This difference is gradually widens from 12.3 per cent in the early days to 29.3% in the late days of the 5th instar. However, enhancement in this parameter due to cold adaptation during the spinning period remains almost unaltered (24 - 27%).

The variation in the level of triglycerides is not significantly influenced by thermal acclimation in the early and middle days of the 5th instar however, in late days an appreciable increase (15%) in the parameter is observed due to adaptation of larvae to a low temperature and it persist even during the spinning period (12.4, 22.6 and 12.8% in early, middle and late days).

The concentration of free fatty acids in the fat body of cold and warm adapted eri- silkworm (Table 1) is significantly altered during the progressive developmental stages and gradually increases and reaching to peak level in the late days of 5th instar, followed by a gradual decrees during the spinning period. Adaptation to low temperature brings an appreciable increase in the level of free fatty acids of fat body throughout the developmental stage. The enhancement in this parameter due to cold adaptation is more pronounced in the 5th instar (194.4, 266.3 and 113.3% in early middle and last days respectively) than that in the spinning period (92.1, 157.4 and 103.0% in early middle and late days respectively).

The phospholipids content of the fat body of insects from both thermal habitats sharply increases during the 5th instar larval growth reaching the maximal at the end of the instar. It is followed by an identical decrease in this parameter during spinning period (Table 1). Significant increase in phospholipid content is observed due to cold adaptation. The degree of difference between cold and warm adapted insects is 18.4, 50.0 and 37.1% in early middle and late days of 5th instar and it is 42.0 in early, 38.6 in middle and 30.1% in late days of spinning.

The level of total cholesterol in the fat body of both adapted groups (Table 1) gets abruptly increased in the late days of 5th instar from an initial level . It is followed

Table 1. Changes in the different lipid fractions composition (mg/g wet weight of tissue of the fat body of cold and warm adapted eri-silkworm, *Philosamia ricini* in relation to spinning process

Parameter	Nature of adaptation	Developmental stages					
		Instar V			Spinning period		
		Early	Middle	Late	Early	Middle	Late
Total lipid	Cold adapted	37.237±2.010	84.390±3.861	127.96±4.416	85.508±2.242	55.152±1.897	69.263±2.401
	Warm adapted	33.166±2.109	67.689±3.399	99.087±3.784	67.273±1.813	44.506±2.515	54.872±2.501
	Signif.	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Triglyceride	Cold adapted	24.104±1.340	62.501±3.213	86.627±3.191	66.958±2.632	43.090±1.583	53.578±2.383
	Warm adapted	24.491±1.464	62.566±2.102	75.474±2.390	50.688±1.641	35.135±1.125	47.482±1.951
	Signif.	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Free fatty acid	Cold adapted	09.779±0.347	16.050±0.856	18.373±0.783	10.565±0.560	08.212±0.201	04.549±0.422
	Warm adapted	03.316±0.226	4.382±0.229	08.614±0.381	05.500±0.227	03.190±0.168	02.241±0.135
	Signifi.	NS	NS	P<0.001	P<0.001	P<0.001	P<0.001
Phospho lipid	Cold adapted	01.293±0.077	03.329±0.111	04.605±0.230	02.603±0.239	02.714±0.104	01.840±0.180
	Warm adapted	01.092±0.039	02.219±0.145	03.360±0.180	02.539±0.137	01.959±0.110	01.414±0.113
	Signifi.	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Cholestrol	Cold adapted	1.283±0.081	04.186±0.126	08.505±0.080	07.184±0.151	03.024±0.158	02.528±0.137
	Warm adapted	01.144±0.136	03.576±0.070	06.652±0.184	05.128±0.110	01.776±0.085	02.141±0.060
	Signifi.	NS	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

NS = Non significance

by a gradual decrease since the commencement of spinning process. Thereafter, the level sharply decreases in the middle days of spinning period with almost little change in late days. An extremely low cholesterol level of fat body in early days of the 5th instar is particularly noteworthy. Cold adaptation of the insects results in an enhancement in the level of total cholesterol of the fat body in both 5th instar and spinning period. It is further noted that total cholesterol level of fat body is appreciably high in the late days of 5th instar and early days of spinning period in the insects of both thermal groups, but always with a higher value for the cold adapted worms than that for the warm adapted individuals.

Table 2 highlights the per cent composition of different lipid fractions against total lipids (taken as 100) of the fat body of eri-silkworm adapted to low and high temperatures. The percentage calculation is based on the data already given in Table 1. The findings of the present investigation demonstrate that the triglycerides constitutes the major portion 62 - 86%) of the total lipid of the fat body followed by the free fatty acids (4 to 26%) total cholesterol (3 to 8%) and the phospholipid (3 to 8%) respectively. Cold adaptation brings about significant change in the proportional composition of different lipid fractions of the fat body of eri-silkworm.

The triglyceride composition gets appreciably depleted (8 to 10%) due to low temperature in 5th instar and in early spinning period, no change in middle days of spinning and in the late spinning days again reduced due to colder environment.

Discussion

The lipid content of the fat body tissue varies with the size and age of the insects and that the changes in the lipid composition during development of an insects is very important for the various regulatory mechanisms responsible for the various regulatory mechanisms responsible for histogenasis and histolysis during larval pupal transformations (Gilbert, 1967; Pant *et al.*, 1973) The majority of retrospective investigations in insects are restricted to the studies on changes in the degree of unsaturation of lipids and the variable capacity of glycerol accumulation during the chances in the ambient temperature (Frankel and Hopf, 1940; Somme, 1972). The sharp increase in the total lipid content of 5th instar larvae is in response to the maximal feeding efficiency during this period and significant fall in the lipid level during spinning period indicating the possibility of utilisation of lipid as a source of energy for spinning process and possibly this is reason the lipid level enhanced in the late spinning period when spinning process is about to end. These observations are in conformity with the reports already made by Bade and Wyatt (1962) and Pant *et al.* (1973). However, an adaptation to low temperature results in enhance in lipid levels this may be because of lipogenasis favors the hexose mono-phosphate pathway which is more functional in cold adapted worms (Singh and Singh, 1992). The enhanced level of total lipids (29.2%) of cold adapted tissue is consistently maintained (24 - 27%) throughout the spinning period suggest that the fat body of cold adapted insects is comparatively better equipped than warm ones with respect to reserved potential energy in form of total lipid for its utilisation during larval-pupal transformation. Almost similar conclusion were drawn by Somme (1972).

According to Wigglesworth (1972) triglyceride constitutes 70 to 80% of total lipids present report results also in same tune. Enhancement in triglyceride level in 5th instar larval period and spinning period due to cold adaptation is indicated that although the triglyceride accumulate in the fat body in cold, the rate of its increases lags behind that of the total lipid fractions in the fat body of cold adapted insects.

Cold adaptation brings about an appreciable increase in the level of free fatty acids throughout the developmental stages however, it is more pronounced in the 5th instar than in the spinning period. It may be be because of decreased inorganic phosphorus in the 5th instar and increase in spinning period due to cold adaptation leading to the more functional hexose mono phosphate level in the former than in the latter period (Singh and Singh, 1992; Singh, 1994). The per cent composition of free fatty acids is increased by 2-3 fold in cold than in warm (Table 2) accompanied by a marginal decrease in the per cent composition of triglyceride throughout the developmental stage this may be due to partly increased lipolytic activity and rest from the increased fatty acids biosynthesis through HMP shunt induced by cold stress. The free fatty acids involves in bringing about changes in lipoprotein structure of enzymes resulting in modulation of their activities during low temperature adaptation as also suggested by Hochachka and Somero (1973).

The phospholipids play an important role in cellular transport mechanisms, maintaining the structural and functional integrity of the membrane system of a cell. The phospholipids in the insects reveal hardly 2 to 4 per cent of the total lipid composition (Albrecht, 1961; Beament *et al.*, 1967). The results obtained in present investigation also confirm the earlier findings. Cold acclimation brings about a significant enhancement in phospholipids content and this is more pronounced during the period from mid 5th instar to mid spinning period (38 - 50%). This developmental stage coincides with maximal growth

Table 2. Per cent composition of different lipid fractions against total lipid of fat body of cold and warm adapted eri-silkworm *Philosamia ricini* in relation to spinning process

Parameter	Nature of adaptation	Developmental stages					
		Instar V			Spinning period		
		Early	Middle	Late	Early	Middle	Late
Total lipid	Cold adapted	64.9	62.5	67.6	67.0	78.3	77.6
	Warm adapted	75.7	77.3	75.2	75.6	79.8	86.3
Triglyceride	Cold adapted	26.4	19.1	14.3	12.4	14.9	6.5
	Warm adapted	10.03	6.4	8.7	8.2	7.2	4.1
Free fatty acid	Cold adapted	3.4	3.9	3.6	4.2	4.9	2.6
	Warm adapted	3.3	3.2	3.4	3.8	4.4	2.5
Phospho lipid	Cold adapted	3.4	5.7	6.5	8.4	5.5	3.6
	Warm adapted	3.4	5.2	6.7	7.6	4.10	3.8
Cholestrol	Cold adapted	3.4	5.7	6.5	8.4	5.5	3.6
	Warm adapted	3.4	5.7	6.7	7.6	4.0	3.8

of the larvae in preparation for the spinning process and the maximal level of physiological activities due to commencement of cocoon formation. Moreover, this is the period when the inorganic phosphorus level of the fat body tissue starts exhibiting a higher value in cold adapted insects (Singh, 1994). The enhancement of phospholipids level due to cold adaptation has been demonstrated in other poikilotherms by Nayeemunnisa and Rao (1972). As evident from Table 2, the per cent composite of phospholipids in total lipid is not appreciably altered due to acclimation of the insects either to low or high temperature. It is probably because of total lipid content of the fat body and its phospholipids fractions both demonstrate an enhancement to almost to same extent due to cold acclimation and hence, the per cent composition of phospholipids remains static.

The cholesterol constitutes an important sterol in the insects and helps the vital activities like development, moulting, oogenasis and hatching (Robbins and Shortino, 1962) as well as precursor for moulting hormone or ecdysone (Wiggelsworth, 1972).

In present investigation cholesterol level maximally increased just prior to spinning , followed by an abrupt fall in after the commencement of spinning. It indicates accumulation of cholesterol prior to spinning is in response to utilisation for the synthesis of moulting hormone during the process of spinning needed for the larval-pupal transformation. The efficiency of this process is more elaborated in cold than in warm and more pronounced in later days of 5th instar upto middle days of spinning (30 to 70%). Results indicates that cold adapted wormsrequire higher amount of moulting hormone than the warm adapted and it may be due to cold exposure act as a stimulus for cholesterol biosynthesis from the dietary phytosterol precursor (Gilbert and Goodfellow, 1965).

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