ESSENCE - International Journal for Environmental Rehabilitation and Conservation

Volume VII: No. 2 2016 [81 – 85] [ISSN 0975 - 6272]

[www.essence-journal.com]

Role of certain Biochemicals In Maintanance of osmotic balance in *Philosamia Ricini* during starvation

Chandorkar, Shuchita; Shouche, Shobha[2] and Pathak, J.P.N.[2]

Received: August 08, 2016 | **Accepted**: October 10, 2016 | **Online**: December 31, 2016

Abstract

The impact of starvation was observed in fifth instar larvae of *Philosamia ricini*. Larvae were kept starved for three days under normal temperature and humidity. The haemolymph was taken for the analysis of carbohydrates, proteins and free amino acids. These biomolecules showed a significant decrease in concentration with respect to the control.

Keywords: Haemolymph | Starvation | Larvae | Biomolecules

For Correspondence:
[1]Future vision, college Ujjain (M.P.)
[2]Govt. MVM Ujjain, (M.P.)
Email: **shuchita.chandorkar@gmail.com; shobha.shouche@gmail.com**

Introduction

Insect haemolymph contains a number of solutes, which maintain osmotic balance. Among them organic molecules are very important because they are related to physiology of insect. It is evident that amino acids and protein play an important role to maintain the internal environment of haemolymph in different stages of insect life. They largely affect various metabolic pathways as well as physiological conditions of insect (Edwards,1982). Chen (1962) described the presence of various amino acids in seven different orders of insects. Wyatt *et al*.. (1955) studied the concentration of sugar, proteins and free amino acids in the silk worm *Bombyx mori* and other species of insect. Treherne (1958) investigated the absorption and metabolism of some sugars in the locust, *Schistocerca gregaria*. Cohen *et al.* (1982) determined the role of free amino acids during dehydration and rehydration of insect which loses water even in mildly stressful conditions. Various forms of stress such as dietary inadequacy (Collet, 1976) starvation (Lim and Lee, 1981) have been shown to influence haemolymph contents. Free amino acid concentration was measured in the haemolymph samples of third instar

larvae of blow fly, *Calliphora vicina*, at various stages prior to the pupation by Evans and Crossley, (1974). In 1991, Ali *et al.*. investigated the biochemical composition including the amino acids, carbohydrates and lipids in a lepidorteran insect *Schoenobius inotata*. They reported the presence of 17 amino acids along with their concentrations in the insect. In this study, we have attempted to determine impact of starvation on the haemolymph composition of *Philosamia ricini*.

Material and Methods

Control

The worms of *Philosamia ricini*, were reared under normal conditions *i. e.* at 29ºC ±2ºC, R.H. 90%±5% (Pant and Agarwal, 1965). The larvae of *Philosamia ricini* are voracious feeder, specially fifth instar larvae, and were provided full diet as per recommendation of Sericulture Department.

Thermal stresses

Fifth instar larvae were kept at 36ºC±2ºC, and relative humidity was 90%±5% for three days. Haemolymph was withdrawn on the fifth day for analysis (*i.e.* they were exposed to high temperature stress for three days). Worms for low temperature stress, were kept at 10ºC±2ºC, R.H. 90%±5%, for three days. Thus in both cases, haemolymph was withdrawn on the fifth day for analysis.

Estimation of total carbohydrates

The estimation of carbohydrates was performed by the method of Dubois *et al.* (1956).

Estimation of total Proteins

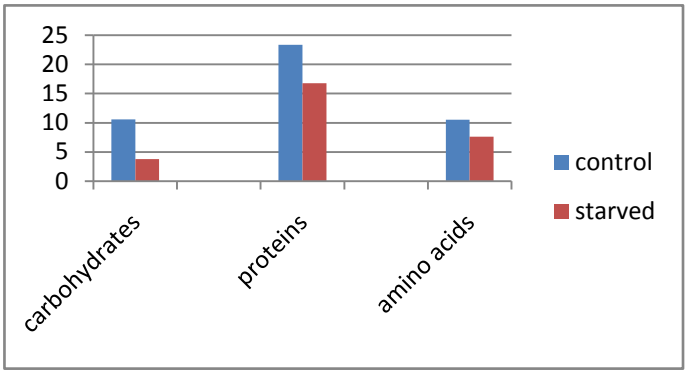
The total proteins were estimated by the method of Lowry *et al.* (1951).

Estimation of total free amino acids

The free amino acids were analyzed by colorimetric method *(Lee and Takahashi; 1966*).

Observation and Results

The worms which were kept without food for three days, showed a drastic decrease in the concentration of carbohydrates. They showed a decrease in the concentration of proteins as well as free amino acids significantly.



	Carbohydrates	Proteins	Amino acids
control	10.6±1.1	23.33±1.1	10.54±0.67
starved	3.8±0.06	16.8±0.83	6.9±0.79

Graph & Table 1: Concentration of carbohydrates, proteins and amino acids (mg/ml) in the fifth instar larvae of *Philosamia ricini* during starvation

Discussion

According to Satake *et al.* (2000) Trehalose concentration in the hemolymph increased slightly during the first 6 h of starvation and decreased thereafter, whereas glucose concentration decreased rapidly immediately after diet deprivation. Starvation-induced hypertrehalosemia was completely inhibited by neck ligation, suggesting that starvation stimulates the release of a hypertrehalosemic factor(s) from the head. The percentage of active glycogen phosphorylase in the fat body increased within 3 h of starvation and

its glycogen content decreased gradually. These observations suggest that production of trehalose from glycogen is enhanced in starved larvae. However, hypertrehalosemia during starvation cannot be explained by the increased supply of trehalose into hemolymph alone, as similar changes in phosphorylase activity and glycogen content in the fat body were observed in neck-ligated larvae, in which hemolymph trehalose concentration did not increase but decreased gradually.

Waytt *et al*.. (1955) studied the concentration of sugar, proteins and free amino acids in the silk worm *Bombyx mori* and other species of insects. They examined haemolymph from a series of developmental stages of silk worm. They concluded that there is gradual rise in protein level during development of *Bombyx mori* form about 1.2% in fourth instar to a maximum of over 5% at the time of spinning. The free amino acids composition of haemolymph of *Bombyx mori* and two other species of insets, *Galleria and Diprion* differ strikingly in proportions. Treherne (1958) investigated the absorption and metabolism of some sugars in the locust, *Schistocerca gregaria*. He reported that the absorption of labeled glucose from the mid gut of the insect has been related to the rate of its conversion to trehalose, which is accumulated in the haemolymph.

During the investigations of the composition of haemolymph of Australian black tipped locust, *Chortoicetes terminifera*, it was found that the concentration of free amino acids varied individually and collectively in locust fed on different diets (Djajakusumah and Milles (1966). They found that the dehydration of insects show a loss in the volume of haemolymph, but little change in osmotic pressure. When dehydrated insects imbibe distilled water in the absence of food, the volume of haemolymph also increases. The decrease in volume of haemolymph was with a loss of amino acids and gain of soluble proteins and the increase in volume is associated with the loss of soluble proteins and gain of amino acids. This result is consistent with the suggestions that free amino acids in the haemolymph of insects are involved in the osmoregulation (Beadle and Shaw 1950; Schoffeniels 1960).

In 1982, Edwards, showed that the organic molecules play an important role in osmoregulation. He reported that the changes in the inorganic ion composition of haemolymph from fourth instar larvae of *Aedes aegypti* was correlated with the changes in the concentration of organic ions. Among organic molecules, free amino acids have a significant role in regulating haemolymph osmotic pressure with respect to the osmotic pressure of water in which the insect lives. The effect of water stress and rehydration on the haemolymph volume and amino acid concentration in *Cysteodemus armatus* was studied by Cohen *et al.* (1982) determined the role of free amino acids during dehydration and rehydration of insect which loses water even in mildly stressful conditions. When insect was rehydrated, then the amino acid concentration decreased significantly but during dehydration the concentration increased from that of control value.

In 2007, Nakamura *et al.* reported the haemolymph patterns of amino acids after three days of starvation in different salinities inbrine shrimp *Artemia franciscana.* They

found that in the haemolymph eight amino acids such as taurine, alanine, threonine, lysine, glycine, arginine and leucine comprised 70% of total free amino acids, due to internal proteolysis during the starvation. Cohen and Patana (1982) reported that in the fourth instar larvae of *Spodoptera exigua*, during starvation for 10 hrs, amino acid concentration decreased significantly in the haemolymph but concentration of proteins increased.

In *Philosamia ricini*, the concentration of carbohydrates, proteins and amino acid decreased significantly which may be due to starvation cause dehydration so solutes were removed from haemolymph.

References

Ali, M.; Ahmad, Z.; Zaidi, Z. H.; Ali, S. S. (1991): Biochemical building blocks of a lepidopteran insect, Schoenobius inotata. *Pakistan J. Pharmaceut. Sci*. 4(1) 11-19.

Beadle, L. C. and Shaw, J. (1950): The retention of salt and the regulation of the non protein nitrogen fraction in the blood of aquatic larva, *Sialis lutaria. J. Exp. Biol.*, 27: 96-109.

Chen, P. S. (1962): Free amino acids in insects. In *Amino Acid Pools*. Edited by J. T. Holden, 115-138.

Chen, P. S. (1966): Amino acid and protein metabolism in insect development. *Adv. Insect. Physiol*. 3, 53-132.

Chen, P. S. (1971): Biochemical aspects of insects of insect`s development. S. Karger, Basel., pp. 55-56.

Cohen, A. C. and Patana, R. (1982): Ontogenetic and stress related changes in the haemolymph chemistry of beet armyworms. Comp. Biochem. Physiol. 71A, 193.

Cohen A. C.; *et al.* (1986): Effects of water stress and rehydration on haemolymph volume and amino acid content in the bltster beetle, *Cysteodemus armatus*. *Comp. Biochem. Physiol*. Vol. 85A No. 4 pp. 743-746.

Collet J. I. (1976): Some features of the regulation of the free amino acids in adult *Calliphora erythrocephala*. *J. Insect Physiol*. 22, 1395-1404.

Djajakusumah, T. and Milles, P.W. (1966): Changes in the relative amounts of soluble proteins and amino acids in the haemolymph of the locusts, *Chortoicetes terminifera*, in relation to dehydration and subsequent hydration. *Aust. J. Biol. Sci*. 19, 1081-1094.

Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P.A. and Smith, F. (1956): Colorimetric method of determination of sugars and related substances. Analytical chemistry, 28, pp. 350-356.

Edwards, H. A. (1982): Free amino acids as regulators of osmotic pressure in aquatic insect larvae. *J. exp. Biol.* 101, 153-160.

Evans P. D. and Crossley A. C.(1974). Free amino acids in the haemocytes and plasma of the larva of *Calliphora vicina. J. Exp. Biol*., 61, 463-472..

Lee, Y. and Takahashi, T. (1966): An improved colorimetric determination of amino acids with the use of ninhydrin. Analy. Boichem, vol. 14, pp 71-77.

Lim, S. J. and Lee, S. S. (1981): The effect of starvation on haemolymph metabolites, fat body and ovarian development in *Oxya japonica* (Acididae: Orthoptera) *J. Insect Physiol*. 27, 93-96.

Lowry, O. H.; Rosenberg, N.J.; Favor A.I. and Remdall, R. J. (1951): Protein measurement with the Folin phenol reagent. *J Biol. Chem*. 193: 265-275.

Nakamura, K.; Iwaizumi, K. and Yamadas, S. (2007): Haemolymph patterns of free amino acids in the brine shrimp *Atremia franciscana* after three days starvation at different salinities. *Comp. Biochem. Physiol*. A 147, 254-259.

Pant, R. and Agarwal, H. C. (1965): *Biochem. J.*, 96, 824.

Satake, S.; Kwabe, Y. and Misoguchi, A. (2000): Carbohydrate metabolism during starvation in the silkworm Bombyx mori. *Arch Insect Biochem Physiol* : jun;44(2) : 90-8

Schoffeniels, E. (1960): Role des acides amine´s dans la re´gulation de la pression osmotique du mileu inte´rieur des insectes aquatiques. *Archives Internationales de Physiologie et de Biochemie* 68, 507-508.

Treherne, J. E. (1958): The absorption and metabolism of some sugars in the locust, *Schistocreca gregaria. J. Exp. Biol*. 35, 611-625.

Wyatt, G. R. (1961): The biochemistry of insect haemolymph. *Ann. Rev*. 6, 75-102.

Wyatt, G. R.; Loughheed, T. C. and Wyatt, S. S. (1955): The chemistry of insect haemolymph. *J. Gen. Physiol*. 39, 853-68.