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Thermal Stress Induced Ionic Changes in *Philosamia Ricini* with Special Reference to Fifth Instar Larvae

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Abstract: *Haemolymph is only the extracellular fluid present in insects, which has ionic balance under the ideal conditions. The major cations in the haemolymph of insects are Na^+, K^+,Ca^{++} and Mg^{++}. The fifth instar laevae of Philosamia ricini were reared under thermal stress of high and low temperature, which showed an increase in monovalent cation Na^+ at high as well as low temperatures significantly while there was no significant change in K^+ ions at both temperatures. The divalent cations Ca^{++} and Mg^{++}showed a significant decrease at both high and low temperatures.*

Keywords*: temperature, stress, monovalent, divalent, cations, haemolymph.*

I. Introduction

The haemolymph of insects represents open circulation because it flows through body cavity. It resembles the blood of vertebrates in having various types of haemocytes and ions. It is involved in immune reactions (Mullins, 1985). It is different from vertebrate blood in the absence of respiratory pigment, so it plays no role in respiration. The major role of haemolymph is to maintain ionic equilibrium and distribution of nutrients.

Considerable amount of information has been available concerning ionic composition of blood. Probably earliest study on certain chemical constituents of blood and their osmotic effects was done by Bishop, Briggs and Rozony (1925) on the larvae of honey bee. Bone (1944) was probably the first to estimate the concentration of monovalent cations Na^+ and K^+ in approximately 27 species of insects representing different orders.

The early studies of Bone (1944, 47) Dechataeu *et al*. (1953), Clarck and Craig (1953), Clark (1958) tried to explain the presence of sodium and potassium ions in the haemolymph of insects and correlated them with food material of insects (diet). Some later studies by Harvey and Zerehn (1971) on *Hyalophora cercopia* Tucker(1977) in *Periplanata americana* have reported the presence of various ions in the haemolymph and related them with the osmotic pressure The stress related study on the changes in the ionic composition of haemolymph was reported by Cohen and Patana (1982) in beet army worm *Spodoptera exigua*.The ionic composition of haemolymph is modified when insects are exposed to any adverse ecological condition or stresses such as temperature fluctuations *i.e*. high temperature (dehydration) or low temperature.

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In the present study we have undertaken analysis of inorganic ions (Na^+, K^+, Ca^{++}, Mg^{++}) in *Philosamia ricini* during ideal and different stressed conditions. The insect is commonly known as Eri silk worm and produces poor quality of silk. The food material of insect is castor leaves (*Ricinus communis).*

II. Material and methods

2.1 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.

2.2 Thermal stresses

Fifth instar larvae were kept at 36ºC±2ºC, and relative humidity was 80±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. 80%±5, for three days. Thus in both cases, haemolymph was withdrawn on the fifth day for analysis.

2.3 Estimation of ions

The quantitative estimation of sodium, potassium and calcium was done with the help of automatic analyzer. The automatic analyzer, model number- 9180, a product of *COBAS* and is a trade mark of Roche

Diagnostic GmbH D-68298 Mannheim, Germany The Mg^{++} ions were measured by Xylidyl blue method (*Bouhen. Clin. Chem. Acta.16, 155:1957).*

III. Results

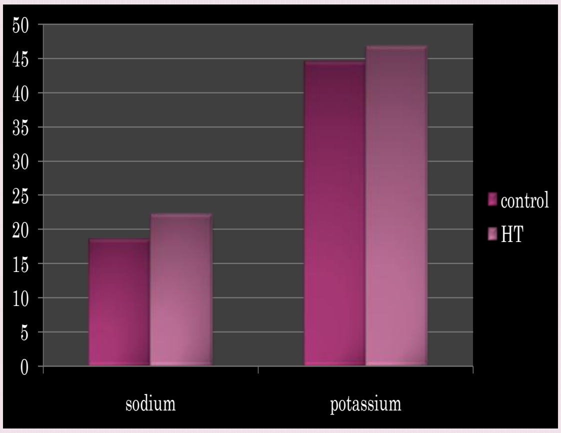
The concentration of sodium ions was 18.6±0.17mg/dl in fifth instar larvae under normal conditions. Whereas at higher temperature, it was 22.3±1.12 mg/dl as shown in graph and table (1). The rise in the concentration of sodium ions was found to be significant. It increased to 24.5±0.38mg/dl at low temperature stress significantly.

In the fifth instar larvae the value of K^+ ion was 44.6±0.30mg/dl. When the worms were reared at high temperature *i.e.* 36°C±2 the concentration of potassium ions was insignificantly higher as compared to the control worms (graph and table 1) As shown in graph and table (2), when the fifth instar larvae were reared at low temperature (10°C±2°C) the potassium ion concentration increased slightly (46.1±1.75 mg/dl) which was also insignificant statistically.

The concentration of Mg^{++} ions was 28±0.30mg/dl in the fifth instar larvae. It was significantly low when the worms were exposed to high and low temperatures. It was concluded that ions decreased significantly at high temperature (graph and table 3 and 4).

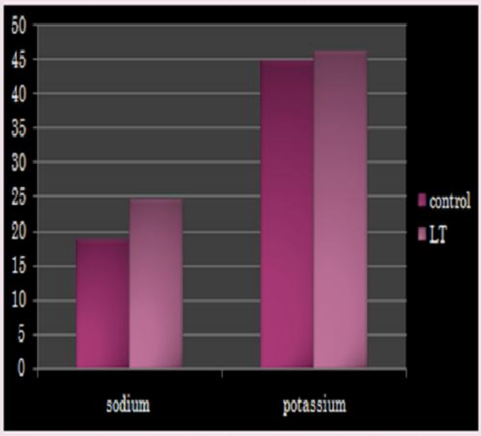
Calcium ion concentration in fifth instar larvae (graph and table 3 and 4) was17.7±0.15 mg/dl under ideal conditions. At higher temperature (36°C±2°C) the concentration of calcium ions decreased significantly to 13.8±2.34mg/dl. Similarly, low temperature stress caused a significant decrease in the calcium ion concentration in the larvae of *Philosamia ricini*.

Graph and table 1: Concentration of sodium and potassium ions (mg/dl) in the fifth instar larvae of *Philosamia ricini* during high temperature stress (36°C±2°C)



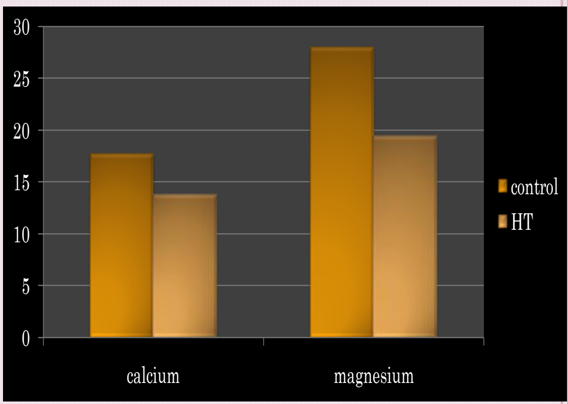
	SODIUM	POTASSIUM
Control	18.6±0.17	44.6±0.30
HT	22.3±1.12	46.8±0.89

Graph and table 2: Concentration of sodium and potassium ions (mg/dl) in the fifth instar larvae of *Philosamia ricini* during low temperature stress (10°C±2°C)



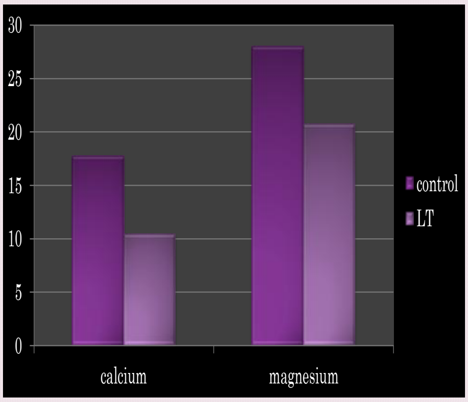
	SODIUM	POTASSIUM
control	18.6±0.17	44.6±0.30
LT	24.5±0.38	46.1±1.75

Graph and table 3: Concentration of calcium and magnesium ions (mg/dl) in the fifth instar larvae of *Philosamia ricini* during high temperature (HT) stress (36°C±2°C)



	Calcium	Magnesium
Control	17.7±0.15	28±0.30
HT	13.8±2.34	19.4±0.84

Graph and table 4: Concentration of calcium and magnesium ions (mg/dl) in the fifth instar larvae of *Philosamia ricini* during low temperature (LT) stress (10°C±2°C)



	Calcium	Magnesium
control	17.7±0.15	28±0.30
LT	10.4±2.64	20.7±1.75

- HT – High Temperature.
- LT – Low Temperature.

IV. Discussion

A number of studies have been performed by various workers, such as Wigglesworth (1939), Buck (1945) concluded that ionic composition vary according to physiological conditions of insects. Florkins *et al*. (1945) and Florkins (1950) found that Na^+/K^+ ratio of different insects may vary in different orders of insects. Treharne (1965) interpreted that in *Carosius morosus*, concentration of sodium ion was low in relation to potassium ions. Malik and Reddy (2009) found the same results as Treherne (1965).

Edney (1968) investigated the impact of high temperature on the water loss from haemolymph in *Arenivaga* sp. and *Periplanata americana*. On the basis of his observations and other reports, he considered the haemolymph as a water reservoir which is influenced by numerous factors like age, ontogenic development, diet, hydration etc. The same author in 1977 published a review on water balance in land arthropods. In the article he concluded that insects are able to regulate osmotic potential of haemolymph under different stressed conditions and ions play very important role in regulating osmotic potential. The high temperature may affect the K^+ transport by hind gut (Nicolson, 1974).

Cohen and Patana (1982) studied the impact of heat and cold in the beet army worms. In heat stressed larvae, they concluded that the decrease in osmotic potential which was partially accounted for the decreased removal of K^+ ions from the haemolymph. On the other hand the cold stressed larvae showed a significant increase in both haemolymph volume and osmotic potential. These workers mentioned that their findings agreed with the results of Somme (1966). Somme studied the impact of cold stress on the flour moth *Anagasta kuehniella* and concluded that cold stress induce the increase in haemolymph solutes and cause water movements in the haemocoel. Therefore, the haemolymph volume and potassium ions both increased.

Cohen and Patana (1982) reported the low concentration of sodium ion, high concentration of potassium ion, low concentration of calcium ion but the concentration of magnesium was more or less same.

The levels of Mg^{++} and Ca^{++} dropped significantly at higher as well as low temperatures (Malik and Malik, 2009) in *Bombyx mori*. They found increased monovalent cations at high temperature but significant decreased concentration of divalent cations.

Our study indicates a significant increase in Na^+ ions whereas insignificant change in K^+ ions. This variation may be due to change in membrane permeability. The excess of K^+ ions might be excreted by active transport through hind gut to maintain Na^+/K^+ ratio. There was a significant decrease in the concentration of Ca^{++} and Mg^{++} ions during stress, which may be due to less feeding, enzyme inactivation and weak nerve impulse conduction.

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References

[1]. Bishop., G. H. Briggs A. P. and Ronzoni E. (1925). Body fluids of the honey bee larva II. Chemical constituents of the blood and their osmotic effects. *J. Biol. Chem.* 66, 77.

[2]. Wigglesworth, V. B. (1939). The regulation of osmotic pressure and chloride concentration in the haemolymph of mosquito larvae. *J. Exp. Biol.* 15, 235-47.

[3]. Bone, G.H. (1944). La raport sodium / potassium dana le liquide coelomique des insects. 1. Ses relations avec le regime alimentaire, *Ann. Soc. Zool. Belg.* 75, 123-32.

[4]. Buck J.B. (1945). In insect Physiology (ed. K.D.Reoder). Wiley. Pages 135-154. Plenum press New York.

[5]. Bone, G.H. (1947). Regulation of sodium and potassium ratio in insects. *Nature, Lond.*, 160, 679.

[6]. Florkin, M. (1949, 50) Biochemical evolution. trans.S. Morgulis, Academic press, New York.

[7]. Clark, E.W and Craig, R. (1953). The calcium and magnesium content in the haemolymph of certain insects. *Physiol. Zool.* 26, 101-7.

[8]. Duchateau G. Florkin, M. and Leclerq J. (1953).Concentration des bases fixes, et types de composition de la base totale de l'hemolymphe des insects. *Arch. Int. Physiol*. 61, 518-49.

[9]. Clark, E.W. (1958). A review of literature *Ent. Soc. Amer.* 51, 142-54. Treherne J. E. (1961). Sodium and potassium fluxes in the abdominal nerve cord of the cockroach *Periplaneta americana. J. exp. biol.* 38, 315-22.

[10]. Somme, L. (1966). The effect of cation temperature anoxia or injection of various substances on haemolymph composition and supercooling in larvae of *Anagasta kuehniella. J. Insect.Physiol. 12*, 1069.

[11]. Edney E.B. (1968). The effect of water loss on the haemolymph of *Arenivaga* sp. and *Periplaneta americana. Comp. Biochem. Physiol*. 25, 149-158.

[12]. Harvey,W.R. and Zerehn, K. (1971). Active transport of sodium by the isolated mid gut of *Hyalophora cercopia. J. Exp. Biol.*, 54, 269-274.

[13]. Nicolson, *et al*. (1974). Effects of starvation and dehydration on osmotic and ionic balance in *Carasius morosus*. *J. Insect. Physiol*. 20, 2061-2069.

[14]. Treherne J. E. and Willmer, P.G. (1975). Hormonal control of integumentary water loss: evidence for a novel neuroendocrine system in an insect (*Periplaneta americana*). *J.exp.Biol.*63, 143-159.

[15]. Tucker, L. E. (1977). Effects of dehydration and rehydration on the water content and Na^+ and K^+ balance in adult male *Periplaneta americana. J. Exp. Biol.* 71, 49-66.

[16]. Tucker, L. E. (1977). Regulation of ions in the haemolymph of the cockroach *Periplaneta americana* during dehydration and rehydration. *J. Exp. Biol* 71, 95-110.

[17]. Edney E.B. (1977). *Water balance in Land Arthropods*. New York: Springer-Verlag.

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

[19]. Mullins D. E. (1985). Chemistry and Physiology of haemolymph. In; Comprehensive insect physiology, Biochemistry and Pharmacology (ed. Kerkut, G. A. and Gilbert, L.I.). Pegamonpress, Oxford 3, pp 355-400.

[20]. Malik F. A. and Reddy Y. S.(2009). Changes in the haemolymph cationic concentration in larvae of the silkworm *Bombyx mori* exposed to selected high temperature regimes. *Acad. J. Ent*. 2(1): 1-9.