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URIC ACID TITRE IN THE HAEMOLYMPH OF THE DEVELOPING SIXTH INSTAR LARVA OF THE MOTH *ORTHAGA EXVINACEA* HAMPSON

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Abstract: **High level of uric acid was found in the haemolymph of the sixth instar larva of the moth, Orthaga exvinacea Hampson. The uric acid level was found to be varying from 3.8 µmole/ml to 5.59 µmole/ml in the unit volume of haemolymph. In the total volume of haemolymph, the uric acid concentration was varying from 0.08 µmole/total tissue to 0.20 µmole/total tissue during sixth instar development. It is suggested that uric acid is playing an important role in the maintenance of the homeostatic equilibrium of the internal environment of the insect larva.**

Keywords: **Uric acid, haemolymph, Orthaga exvinacea, insect larva.**

1. INTRODUCTION

Terrestrial insects are generally uricotelic. One of the characteristics of insect haemolymph is the high concentration of uric acid and this character distinguishes insects from all other invertebrates [1]. The occurrence of uric acid in haemolymph has been demonstrated in a number of insects [2], [3]. This is in tune with the uricotelic nature of excretion of terrestrial insects. The relative changes in the uric acid concentration of haemolymph during the development of insect larvae were rarely been studied [4]. In the present study, an attempt was made to study the changes in the concentration of uric acid in the haemolymph of the sixth instar larva of the moth, *Orthaga exvinacea* Hampson.

2. MATERIALS AND METHODS

The larvae of *Orthaga exvinacea* (Pyralidae: Lepidoptera) were reared on their natural food, mango leaves (*Mangifera indica*) under laboratory conditions. Chronologically comparable final instar larvae were separated from the colony and reared separately with limited numbers. The sixth instar period is divided into six periods [5].

For extracting haemolymph, the larvae were anaesthetized slowly with diethyl ether. One of the thoracic legs was amputated and the oozed out haemolymph was immediately drawn into a capillary tube. To ensure complete extraction, the larva was gently pressed from the anterior and posterior ends simultaneously until no more haemolymph was extracted. The haemolymph was deproteinized with equal volumes of 2/3 N sulphuric acid and 10% sodium tungstate. The precipitated protein was centrifuged off and the supernatant was analysed for uric acid according to Brown [6]. The standard uric acid treated in the same way was used for calibration. The food, mango leaves was also analysed for uric acid.

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3. RESULTS

Haemolymph volume at different periods of the sixth instar development is presented in table 1. The volume of haemolymph was increased from 0 h (15.60 µl/larva) and reached the highest volume at 72 h (41.80 µl/larva). Then the volume was decreased and 120 h recorded the lowest value (10.33 µl/larva). The concentration of uric acid based on the unit volume of haemolymph was initially high at the beginning of the instar (4.81 µmole/larva at 0 h) but decreased to the lowest value at 72 h (table 2). Concentration of 3.84 µmole/ml at 72 h was followed by an increase in the value (5.73 µmole/ml) which was followed by a slight decrease in concentration at 120 h (5.59 µmole/ml). However, based on the total volume of haemolymph, the concentration increased gradually from 0 h (0.08 µmole/total haemolymph) to 96 h (0.20 µmole/total haemolymph) followed by a decrease recording the lowest value at 120 h (0.06 µmole/total tissue) (table 2).

Table 1. Volume of the haemolymph

Larval periods (h)	Volume of haemolymph µl/larva
0	15.60 ± 1.95
24	18.33 ± 3.27
48	32.20 ± 5.02
72	41.80 ± 3.83
96	34.80 ± 5.07
120	10.33 ± 1.30

The results are the mean of five determinations with standard deviations.

Table 2. Uric acid content of haemolymph

Larval period (h)	Uric acid	
	µmole/ml	µmole/total haemolymmph
0	4.81 ± 0.47	0.08 ± 0.01
24	4.76 ± 0.40	0.09 ± 0.01
48	4.27 ± 0.48	0.14 ± 0.02
72	3.84 ± 0.18	0.16 ± 0.01
96	5.73 ± 0.92	0.20 ± 0.03
120	5.59 ± 0.45	0.06 ± 0.005

The results are mean of five independent determinations with standard deviations.

4. DISCUSSION

Uric acid is synthesized mainly in the fat body [2], [3], [7]. The synthesized uric acid might be sequestered in to the haemolymph for removal by way of excretion. Compared to other nitrogenous end products like ammonia or urea, uric acid is less toxic and allows considerable build up concentration. Due to this nontoxic effect, the larva produces more uric acid at post-maturation period, when the larva stops excretion (table 2). The uric acid produced may be used as a storage metabolite [8]. Uric acid is known to contribute to osmotic pressure of the medium and involves in the acid-base balance and buffering capacity [9], [10]. Further, the variation in the concentration of the uric acid observed during the development of the larva suggests that uric acid plays an important role in the maintenance of the homeostatic equilibrium of the internal environment.

The occurrence of uric acid in the haemolymph of the larva suggests that it is a true metabolite. Analysis of the food of the larva showed that it contains no uric acid. Also the compound was found to be important ingredient of excreta [11]. This suggests the synthetic nature of the uric acid by the animal.

The uric acid from the haemolymph may be removed by way of excretion and stored in the fat body. The larva of *O. exvinacea* is uricotelic excreting 62.47 µmole/g to 123.15µmole/g uric acid [11]. Kuzhivelil and Mohamed [8] also showed that this larva can store considerable amount of uric acid in the fat body.

5. CONCLUSION

The present study shows that hameolymph of the larva show high titre of the uric acid. Uric acid is synthesized as a nitrogen metabolite and it is sequestered into the haemolymph for removal by way of excretion or may be stored in the fat body.

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REFERENCES

[1] Florkin M. and Jeuniaux C. (1974) Haemolymph: composition. In: *The Physiology of Insecta*, 2nd ed. (Ed. Rockstein M.), 5, 255-307, Academic Press, London.

[2] Cochran D.G. (1975) Excretion in Insects. In: *Insect Biochemistry and Function* (Eds. Candy D J and Kilby B A), 177-281, Chapman and Hall, London.

[3] Cochran D. G. (1985) Nitrogenous Excretion. In: *Comprehensive Insect Physiology and Biochemistry and Pharmacology* (Eds. Kerkut G. A. and Gilbert L. I.), 4, 467-506, Pergmon Press, Oxford.

[4] Lazar K. V. and Mohamed U. V. K. (1988) Changes in the levels of uric acid in the developing larval haemolymph of *Spodoptera mauritia* Boisduval. *J. Sci. Res.*, 10, 19-20.

[5] Kuzhivelil B. T. and Mohamed U. V. K. (1998) Allantoin and allantoic acid titer in the faeces and tissues of the developing larva of the moth, *Orthaga exvinacea* Hampson. *Insect Biochem. Mol. Biol*. 28, 979-986.

[6] Brown H. (1945) The determination of uric acid in human blood. *J. Biol. Chem.*, 158, 601-608.

[7] Klowden M. J. (2013) *Physiological Systems in Insects*. 3rd ed. Academic Press, London.

[8] Kuzhivelil B. T. and Mohamed U .V. K. (1999) Uric acid levels in the developing fat body

[9] of the sixth instar larva of the moth, *Orthaga exvinacea* Hampson. *Asian J. Zool. Sci.*, 8, 1-4

[10] Florkin M. and Morgulis S. (1949) *Biochemical Evolution*. Academic Press, London.

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

[12] Kuzhivelil B. T. (1991) Studies on Nitrogen Metabolism in *Orthaga exvinacea* Hampson (Pyralidae: Lepidoptera). Ph.D. Thesis, University of Calicut, Kerala, India.