Odonatologica 9 (4): 321-324 *December 1, 1980*

CHEMICAL COMPOSITION OF HAEMOLYMPH IN THE LARVAE OF THE DRAGONFLY *ORTHETRUM CHRYSIS* (SELYS) (ANISOPTERA: LIBELLULIDAE)

V.K. THAKARE, D.B. TEMBHARE, M.W. KHAN and V. PENDHARKAR
Department of Zoology, Nagpur University, Nagpur-440010, India

Received July 17, 1979 / Accepted October 1, 1979

A quantitative analysis of the chemical composition of the haemolymph is provided. Sodium and chloride appear to be the major electrolytes primarily responsible for maintaining the osmotic balance. Besides a high concentration of trehalose, haemolymph also contains glucose and glycogen in low concentration. Neutral lipids, phospholipids and cholesterol are present in varying amounts. A high concentration of proteins and a low concentration of inorganic phosphorus seem to be peculiar features of the haemolymph of dragonfly larvae.

INTRODUCTION

The reviews at present available summarize the work done on the chemical composition of haemolymph in a large number of insects during post-embryonic development and also in the adult condition. Since no information is available on the chemical composition of dragonflies except on larvae of *Aeshna cyanea* (TIMON-DAVID, 1930; SUTCLIFFE, 1962; MOENS, 1975) and *Uropetala carovei* (BEDFORD & LEADER, 1975), an attempt was made to determine the organic and inorganic composition of the haemolymph in the larvae of the libellulid *Orthetrum chrysis.*

MATERIAL AND METHODS

Last instar larvae were collected from ponds near Nagpur and acclimatized in the laboratory in dechlorinated water at a constant photoperiod (10L : 14D) and temperature (20°±2°C). They were fed on mosquito larvae for a week. Newly moulted or pharate larvae were discarded at the time of collecting the blood.

The haemolymph was pooled in chilled centrifuge tubes and immediately subjected to centrifugation at 2000 rpm in order to sediment the haemocytes. The supernatant was used for the determination of various metabolites (cf. Tab. I). The unpublished method of Professor J.E. Steele for the estimation of trehalose is described below.

Five μl insect blood was added to 0.995 ml of 5% TCA, mixed and centrifuged. About 0.5 ml of the clear supernatant was taken to a test tube, and standards were prepared by dissolving 0.04 M trehalose. All tubes were chilled in ice before addition of about 2.5 ml of anthrone reagent and mixed vigorously. The tubes were placed in a boiling water bath for 15 min. Optical density was recorded at 620 nm on spectromic 20 (Bausch & Lomb).

RESULTS AND DISCUSSION

The composition of various metabolites in the haemolymph is given in Table I. Each value represents a mean of at least five determinations after collecting blood for each sample from at least three insects.

Table I

Chemical composition of the haemolymph of *Orthetrum chrysis* larvae

Determination	Method	Results
Na^+	Flame photometry	129.00 m eq/l
K^+	Flame photometry	4.80 m eq l
Ca^{++}	Flame photometry	12.00 m eq/l
Mg^{++}	Flame photometry	9.50 m eq/l
Cl^-	Potentiometric titration (*Sigma techn. Bull.* 830)	102.00 m eq l
Trehalose	STEELE unpublished	950.00 mg 100 ml
Glucose	HAWK et al., 1954	35.00 mg 100 ml
Glycogen	CARROLL et al., 1956	10.000 mg 100 ml
Total lipids	FOLCH et al., 1957	1.50 g 100 ml
Phospholipids	BARTLETT, 1959	43.58 mg 100 ml
Cholesterol	PLUMMER, 1971	83.78 mg 100 ml
Proteins	LAYNE, 1957	2.50 g 100 ml
Inorganic phosphorus	FISKE & SUBBAROW, 1925	6.00 mg 100 ml

As in other dragonflies, in *O. chrysis* the concentration of Na^+ and Cl^- is higher than that of other ions; they appear to be responsible for maintaining the ionic balance in the haemolymph of dragonfly larvae (cf. SUTCLIFFE, 1962; BEDFORD & LEADER, 1975; MOENS, 1975).

A survey of the haemolymph ionic concentration of various insects reveals the striking presence of Na^+ and Cl^- in high and that of K^+, Ca^{++}, Mg^{++} and

PO_4^- in lower concentrations in hemi- and heterometabolic exopterygotes than in holometabolic endopterygotes and also higher in the various forms adapted to fresh water habitats with carnivorous feeding habits (BONÉ, 1944; SUTCLIFFE, 1962; STOBBART & SHAW, 1964). The high sodium/potassium molar ratio of *O. chrysis* resembles that found in some lower arthropods on one hand and in some orthopteroid taxa on the other.

Trehalose measured about 95.4% of the total carbohydrates in the haemolymph, which suggests that it is a major blood sugar in the larvae of *O. chrysis*. The presence of trehalose in high concentration in haemolymph has been reported for the majority of insects, particularly for larval forms, including *Aeshna cyanea* and *Uropetala carovei* (cf. WYATT, 1961; FLORKIN & JEUNIAUX, 1964; BEDFORD & LEADER, 1975). Glucose, as a reducing sugar, constitutes 3.5% of the total haemolymph sugars in *O. chrysis*, and glycogen 1%.

The concentration of total haemolymph lipids determined amounts to some 1.5%. The phospholipids and cholesterol were also analysed and are reported here for the first time. The low concentration of lipids in *Orthetrum* seems to be related to the ancient phylogenetic origin of dragonflies. In most of the higher insect orders lipids occur in high concentrations (FAST, 1964).

It has been reported (FLORKIN & JEUNIAUX, 1964) that the concentration of total proteins varies from 1 g/100 ml in Orthoptera to 4 g/100 ml in Hymenoptera. In the larvae of *Orthetrum chrysis* total proteins determined are about 2.5%.

The free amino acids from the haemolymph of the same dragonfly have been determined earlier (THAKARE et al., 1975).

Inorganic phosphorus was estimated to amount to about 6 mg/100 ml. The presence of low concentrations of inorganic phosphorus might be a general feature of the insect haemolymph (e.g. TERRA et al., 1974).

ACKNOWLEDGEMENTS

Thanks are due to Prof. J.E. STEELE, University of Western Ontario, London, Canada, for his valuable help in the trehalose estimation and to B.D.H. and Sigma Chemicals, for generously supplying trehalose and some other chemicals.

REFERENCES

BARTLETT, G.R., 1959. Phosphorus assay in column chromatography. *J. biol. Chem.* 234: 466-468.

BEDFORD, J.J. & J.P. LEADER, 1975. Haemolymph composition in the larvae of the dragonfly, Uropetala carovei. *J. Ent.* (A) 50: 1-7.

BONÉ, G.J., 1944. Le rapport sodium/potassium dans le liquide coelomique des insectes. *Annls. Soc. r. Zool. Belg.* 75: 123-132.

CARROLL, N.V., R.W. LANGLEY & J.H. ROC, 1956. The determination of glycogen in liver

and muscles by use of anthrone reagent. *J. biol. Chem.* 220: 583-593.

FAST, P.G., 1964. Insect lipids, a review. *Mem. ent. Soc. Can.* 37.

FISKE, C.H. & Y. SUBBAROW, 1925. The colorimetric determination of phosphorus. *J. biol. Chem.* 66: 375-400.

FLORKIN, M. & C. JEUNIAUX, 1964. Haemolymph: composition. *In*: M. Rockstein, [Ed.], The physiology of Insecta, Vol. 3, pp. 190-152. Academic Press, New York.

FOLCH, J., M. LEE, & G.H. SLOANE-STANLEY, 1957. A simple method for the the isolation and purification of total lipids from the animal tissue. *J. biol. Chem.* 226: 497-509.

HAWK, P.B., B.L. OSER & W.H. SUMMERSON, 1954. *Practical physiological chemistry*. 13th ed. McGraw-Hill, London.

LAYNE, E., 1957. Spectrophotometric and turbidometric methods for measuring proteins. *In*: S.P. Colowick & N.C. Kaplan, [Eds.], Methods in enzymology, pp. 447-454. Academic Press, New York.

MOENS, J., 1975. Ionic regulation of the haemolymph in the larvae of the dragonfly, Aeshna cyanea (Müller) (Odonata, Anisoptera). *Archs int. Physiol. Biochim.* 83: 443-451.

PLUMMER, D.T., 1971. Libermann-Burchard reaction for cholesterol estimation. *In*: An introduction to practical biochemistry. McGraw-Hill, London.

STOBBART, R.H. & J. SHAW, 1964. Salt and water balance: excretion. *In*: M. Rockstein, [Ed.], The physiology of Insecta, Vol. 3, pp. 189-258. Academic Press, New York.

SUTCLIFFE, D.W., 1962. The composition of haemolymph in aquatic insectes. *J. exp. Biol.* 39: 325-343.

TERRA, W.R., A.G. De BIANCHI & F.J.S. LARA, 1974. Physical properties and chemical composition of the haemolymph of Rhynchosciara americana (Diptera) larvae. *Comp. Biochem. Physiol.* (B) 47: 117-129.

THAKARE, V.K., D.B. TEMBHARE & K.B. DESHMUKH, 1975. Free amino acids in the haemolymph of the last instar nymph of the dragonfly, Orthetrum chrysis (Selys) (Odonata: Libellulidae). I. Effects of centrifugation and starvation. *Experientia* 31: 1472-1473.

TIMON-DAVID, J., 1930. Recherches sur les matières grasses des insectes. *Annls Fac. Sci. Marseille* (II) 4: 29-207.

WYATT, G.R., 1961. The biochemistry of insect haemolymph. *Ann. Rev. Ent.* 6: 75-102.