

**CHANGES IN THE FREE AMINO ACID CONCENTRATIONS OF THE
HAEMOLYMPH DURING DEVELOPMENT IN *ORTHETRUM SABINA*
(DRURY) (ANISOPTERA: *LIBELLULIDAE*)**

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The total free amino acid concentration in the haemolymph is similar with a very narrow range of variation in the different larval instars as well as in the imago. The basic amino acids are found in higher quantities in the early larval instars and they decrease in the later instars to reach their minimum values in the imago. The reverse is the case with the acidic amino acids. The sulphur-containing amino acids are totally absent in the adults while they are present in the larvae.

INTRODUCTION

FLORKIN (1949) observes that a very high titer of free amino acids in the haemolymph is characteristic of insects. The values range from 275 to 2340 mg/100 ml (BISHOP et al., 1925; USSING, 1946; WYATT et al., 1956; DUCHATEAU & FLORKIN, 1958; STEVENS, 1961). All these studies are on neopteran insects and the Palaeoptera have received much less attention. DUCHATEAU & FLORKIN (1958) record 399 mg of free amino acid per 100 ml of plasma in the larvae of *Aeshna grandis*. SUTCLIFFE (1962) is of the opinion that the concentration of the free amino acids of the haemolymph in Odonata may vary in different larval instars. But this has not been experimentally studied hitherto. The present investigation has been conducted to throw some light on this problem.

MATERIAL AND METHODS

The materials used are larval and adult *Orthetrum sabina* (Drury). These are available in plenty throughout the year in and around the Coimbatore city. The eggs, collected from the natural ponds, were maintained in the laboratory in large tanks under conditions similar to those in the natural habitat. The hatched larvae thrived well on mosquito larvae.

By repeated trials it was found that only from the sixth instar onwards sufficient quantities of haemolymph could be obtained for analyses. The method of obtaining the samples of haemolymph and the procedure for the estimations have been given elsewhere (SUNDARA RAJULU, 1970). The individuals in intermoult condition were used for free amino acid estimations. The individuals selected for estimations were kept isolated and unfed for 24 hrs to avoid variations in the free amino acid content due to differential feeding.

RESULTS

Sixth instar larvae have 284.4 ± 13.2 mg of free amino acids per 100 ml of haemolymph. It is interesting that the imago has a value not very much different from that found in the larval stage, i.e. 287.3 ± 14.2 mg/100 ml. The values in instars 7 to 13 are also more or less similar to those measured in young larvae and in the imagoes; they range from 275.5 to 285.1 mg/100 ml (Tab. 1).

Table I

Total concentration of free amino acids in the haemolymph of *Orthetrum sabina* (Drury) in the various instars (mg/100 ml)

Instars	Total amino acids *
6	284.4 ± 13.2
7	285.1 ± 9.7
8	276.7 ± 11.6
9	281.4 ± 13.4
10	286.8 ± 12.5
11	280.2 ± 12.8
12	275.5 ± 10.3
13	280.6 ± 14.1
Imago	287.3 ± 14.2

* Average for values in ten individuals in every instar.

Qualitative analyses of the haemolymph free amino acids reveal that the sixth instar larvae have 17 amino acids (Tab. II). Of these, glycine, histidine, proline, threonine and tyrosine are found in higher concentrations; the amino

Table II
Differential concentration of free amino acids in the haemolymph of *Orthetrum sabina*
(Drury) in the various instars (in mg/100 ml)

Amino acids	Larval instars								Imago
	6	7	8	9	10	11	12	13	
Alanine	11.0 ± 2.4	14.6 ± 2.6	12.1 ± 2.1	12.9 ± 2.4	13.8 ± 1.9	11.5 ± 2.5	12.7 ± 1.7	14.2 ± 2.7	13.5 ± 0.9
Aspartic acid	3.7 ± 0.6	4.8 ± 0.7	5.4 ± 1.1	7.2 ± 0.8	8.7 ± 0.8	8.4 ± 2.4	11.5 ± 1.8	15.3 ± 1.9	16.1 ± 1.7
Arginine	9.4 ± 2.5	9.6 ± 2.2	8.3 ± 1.6	7.8 ± 0.9	7.3 ± 0.9	6.2 ± 1.3	5.2 ± 0.7	3.7 ± 0.8	2.6 ± 0.6
Cystine	3.2 ± 0.2	3.2 ± 0.4	2.6 ± 0.3	2.7 ± 0.2	2.4 ± 0.3	2.1 ± 0.4	1.3 ± 0.2	0.9 ± 0.1	—
Glutamic acid	6.2 ± 1.2	7.5 ± 0.9	8.4 ± 1.3	9.6 ± 1.1	10.4 ± 1.2	11.7 ± 1.6	18.3 ± 2.1	19.2 ± 2.2	23.0 ± 2.9
Glycine	61.4 ± 6.2	48.6 ± 5.1	52.7 ± 5.6	48.9 ± 4.2	57.6 ± 5.5	60.5 ± 5.9	56.6 ± 4.9	59.3 ± 4.4	62.4 ± 2.8
Histidine	22.3 ± 4.4	22.1 ± 3.1	21.8 ± 4.4	21.2 ± 3.2	20.4 ± 2.7	20.1 ± 2.7	12.2 ± 1.9	10.4 ± 2.1	10.9 ± 1.4
Isoleucine	9.8 ± 1.7	11.2 ± 2.2	11.6 ± 2.1	10.5 ± 1.9	9.9 ± 1.4	11.4 ± 1.8	12.3 ± 1.3	10.9 ± 1.6	11.3 ± 2.6
Leucine	10.2 ± 1.6	12.5 ± 1.9	12.1 ± 1.3	11.6 ± 1.8	10.2 ± 1.4	12.4 ± 1.6	11.7 ± 0.9	12.6 ± 1.7	12.7 ± 1.3
Lysine	13.5 ± 2.0	12.8 ± 1.4	12.1 ± 1.3	12.4 ± 1.1	11.8 ± 1.2	11.4 ± 1.6	6.7 ± 0.8	4.9 ± 0.8	3.7 ± 0.4
Methionine	4.7 ± 0.7	4.2 ± 0.5	4.1 ± 0.6	4.3 ± 0.7	4.1 ± 0.6	3.2 ± 0.4	2.7 ± 0.2	1.4 ± 0.4	—
Phenylalanine	14.6 ± 2.1	11.5 ± 1.6	12.7 ± 1.8	13.6 ± 0.9	13.9 ± 2.2	14.7 ± 2.5	13.4 ± 1.7	14.2 ± 1.6	13.6 ± 1.8
Proline	42.8 ± 5.9	43.7 ± 6.6	42.6 ± 4.8	43.2 ± 4.2	43.4 ± 3.3	42.5 ± 4.1	41.8 ± 3.5	42.2 ± 4.7	43.5 ± 3.2
Serine	12.6 ± 1.9	14.4 ± 1.4	13.9 ± 1.7	14.6 ± 1.8	13.6 ± 2.1	12.7 ± 2.2	13.9 ± 1.5	12.8 ± 1.7	13.5 ± 2.1
Threonine	22.1 ± 3.2	24.5 ± 3.2	20.6 ± 2.6	23.5 ± 2.7	22.7 ± 2.8	19.8 ± 1.8	21.6 ± 3.1	20.7 ± 2.0	23.5 ± 3.6
Tyrosine	21.2 ± 3.7	21.7 ± 3.5	22.4 ± 3.5	20.5 ± 3.1	22.3 ± 2.3	21.2 ± 2.2	23.1 ± 3.8	22.6 ± 2.8	21.5 ± 3.6
Valine	13.6 ± 1.6	14.1 ± 1.6	14.7 ± 1.3	12.8 ± 0.9	13.4 ± 1.1	14.2 ± 1.6	11.8 ± 1.8	13.0 ± 1.6	12.6 ± 1.3

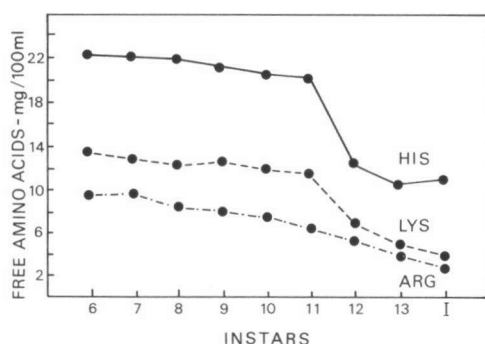


Fig. 1. Variations of the free basic amino acids in the haemolymph of *Orthetrum sabina* Drury during the various instars. (ARG, arginine; — HIS, histidine; — I, imago; — LYS, lysine).

case with the acidic amino acids (Fig. 2). Both acidic amino acids are in lower concentration in the sixth instar. The values rise gradually up to the 11th instar. Thereafter the increase is steep.

Apart from these amino acids the sulphur-containing ones also show variations. They are found in very low concentration in the sixth instar itself, but decrease gradually in the later instars and finally disappear completely (Fig. 2).

DISCUSSION

DUCHATEAU & FLORKIN (1958) reported that the total molar concentration of the haemolymph free amino acids is roughly 33 mM for *Aeshna* larvae. The values for *A. grandis* and *A. cyanea* are 34 mM and 39 mM, respectively (SUTCLIFFE, 1962). For the latter species RAPER & SHAW (1948) and MOENS (1975) record very high values, up to 127 mM and 77 mM, respectively. SCHOFFENIELS (1960) gives very low values, equivalent to 8-9 mM, in *Libellula* larvae, and 10 to 11 mM, in *Aeshna* larvae. Such varying results led

acids found in least quantities are aspartic acid, glutamic acid, cystine and methionine.

A careful study of the differential concentration of the free amino acids in the various instars may disclose that the basic amino acids histidine, lysine and arginine, are in higher concentration in the sixth instar, while they are continuously on the decline in the later larval stages (Fig. 1). This fall is gradual in the case of arginine. But the concentrations of histidine and lysine drop steeply in the 12th instar. The reverse is the

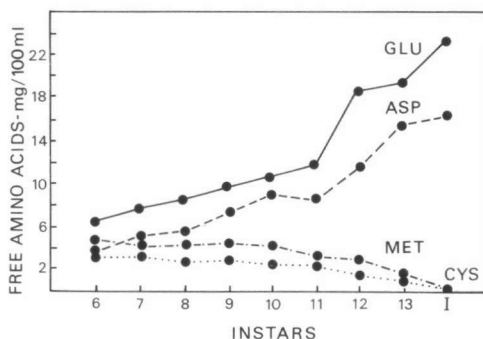


Fig. 2. Variations of the free acidic and sulphur-containing amino acids in the haemolymph of *Orthetrum sabina* Drury during the various instars. (ASP, aspartic acid; — CYS, cystine; — GLU, glutamic acid; — I, imago; — MET, methionine).

SUTCLIFFE (1962) to suggest that there may be steep variations in the haemolymph free amino acid concentration in the different larval instars of dragonflies.

The results recorded in the present investigation do not support the suggestion of SUTCLIFFE (1962). There are undoubtedly variations in the free amino acid content of the haemolymph in the different larval instars of *Orthetrum sabina*. Such fluctuations however are minor, the minimum value being 275.5 mg/100 ml in the 12th instar and the maximum is 287.3 mg/100 ml in the adult stage. In the light of these observations it is suggested that, in dragonflies, the physiological pattern may vary from species to species.

Though the total concentration of the free amino acids in the haemolymph does not fluctuate very much in the different larval stages of *Orthetrum sabina*, the acidic, basic, and the sulphur-containing amino acids show marked variations during the different instars. While there are higher quantities of the basic amino acids in the early instars and lower in the later stages, the acidic amino acids follow a reverse pattern in their fluctuation. The instar 11 seems to be an important stage in these changes. Histidine and lysine, which are gradually decreasing from the sixth to the tenth instar, drop down steeply in their quantity in the eleventh instar. Exactly the reverse is the case with the glutamic acid and aspartic acid. The significance of such conspicuous variations in the eleventh instar deserves further investigation. Similarly, the presence of cystine and methionine in the larval stage and their absence in the adult stage also merit attention.

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