Odonatologica 4 (4): 219-224 *December 1, 1975*

CHANGES OF HAEMOLYMPH VOLUME IN LARVAE OF *ANAX IMPERATOR* LEACH AND *AESHNA CYANEA* (MÜLLER) DURING STARVATION IN DIFFERENT EXPERIMENTAL MEDIA (ANISOPTERA: *AESHNIDAE*)

J. MOENS*

Laboratory of Zoophysiology, University of Louvain,
Naamsestraat 59, B-3000 Louvain, Belgium

Received May 7, 1975 / Accepted May 14, 1975

When larvae of anisopteran dragonflies are kept in hypertonic external media of diluted sea water different observations lead to the supposition that the haemolymph volume changes. It is shown in this paper that the blood volume increases markedly in larvae, kept in hypertonic media.

INTRODUCTION

In our investigations on the ionic and osmotic regulation and the water balance in larvae of *Aeshna cyanea* (MOENS, 1973, 1975a, 1975b) some observations led us to suppose that the haemolymph volume increases in individuals kept in hypertonic media. The first indication for this supposition was the fact that, although the same blood sampling technique was always used, the amount of haemolymph obtained from larvae in hypertonic media was always significantly higher than that from those placed in hypotonic media. Other evidence for this supposition came from the observation that a marked increase in body weight occurs in larvae starved in hypertonic media (MOENS, 1973).

The results of our experiments, related below, confirm clearly the supposition that the blood volume increases if larvae are kept in hypertonic media.

* Present address: S.B.M. Department, Limburg University Centre, University Campus, B-3610 Diepenbeek, Belgium.

MATERIAL AND METHODS

Last instar larvae of *Anax imperator* and *Aeshna cyanea* were used. The experiments were carried out as described elsewhere (MOENS, 1973, 1975a). The method used to measure the haemolymph volume was based on that elaborated by LEE (1958). It is a so called dilution method. An exactly known amount of amaranth dye is injected. The blood volume can be calculated from the measured dilution of the dye.

As it is essential that no haemolymph is lost between the injection of the dye and the withdrawing of the haemolymph sample, special precautions were taken. The larva was first anaesthetised in fresh soda water for 7 min. The larva was then dried with filter paper and immobilised on its back in a special trough. It was then covered with a thin perforated perspex plate so as to make one of its legs pass through a perforation. The femur was cut with a pair of fine scissors. By means of a self-made micro injection apparatus (a micro-hematocrit tube was pulled out and a thin bar of perspex coated with silicone grease was used as plunger) 10 µl of a 2% amaranth solution was injected. To prevent any loss of blood or dye solution the injection was carried out as follows. First a control haemolymph sample (30 µl) was taken after the leg had been cut. This sample was centrifugated. 20 µl of the supernatant fluid was then diluted in 2 ml of distilled water and used for the measurement of the absorption at 517 nm. Two more little drops from the supernatant fluid were used for filling of the injection apparatus.

As it was impossible to deliver quantitatively the dye solution from the syringe, a small drop of haemolymph was introduced before the dye solution was sucked up in the capillary. To avoid loss of dye during the insertion of the injection apparatus into the orifice another little drop of haemolymph was added in the top of the syringe. The injection apparatus was then fixed to the stand in which the larva was placed. The point of the syringe was ligatured in the leg. Now the stand, containing the larva with the syringe fixed into its leg, was placed in fresh aerated tap water to reactivate the contraction of its heart. The dye solution was then injected very slowly. After removal of the syringe the leg was ligatured and the insect, which was taken from the stand kept for 3 min in fresh water. The cuticle of the larva being transparent the mixing of dye with the blood could be easily observed. The larva was finally anaesthetised again and a new haemolymph sample was taken. After centrifugating this blood, 20 µl of the supernatant fluid were diluted in 2 ml distilled water. The difference between the absorption of this sample and of the control sample enabled us to calculate the dilution of the dye and the blood volume.

RESULTS

As it is impossible to measure twice the blood volume of the same individual we first determined the blood volume/body weight ratio of five normal larvae. Table I shows the results for the larvae of *Anax imperator*.

Table I

Determination of the blood volume/body weight ratio in normal larvae of *Anax imperator*. (W: weight in g; V: blood volume in µl).

Larva No.	W	V	V/W
1	1,138	383	337
2	1,203	417	347
3	1,155	396	343
4	1,245	421	338
5	1,011	355	351
mean			*343*

Table II

Changes in body weight and haemolymph volume during starvation of larvae of *Anax imperator* in different media of diluted sea water. (Larva 1-3: 48 h in 1/2 sea water; – 4-6: 72 h in 1/2 sea water; – 7-9: 96 h in 1/2 sea water; – 10-11: 96 h in 2/3 sea water).

Larva No.	W_o	W_x	ΔW	V_o	V_x	ΔV
1	1020	1143	123	350	477	127
2	1318	1394	76	459	523	71
3	1162	1219	57	399	488	89
4	1186	1305	119	407	536	129
5	1108	1167	59	380	416	36
6	1246	1323	77	434	516	82
7	1179	1265	86	404	478	74
8	1196	1294	98	410	470	60
9	1184	1259	75	401	494	88
10	1182	1218	36	405	457	52
11	1058	1096	38	363	410	47

W_o: initial weight W_x: weight after the time indicated
V_o: initial blood volume V_x: blood volume after the time indicated
ΔW and ΔV: change of the weight respectively blood volume.
The weight is expressed in mg, the blood volume in µl.

As can be seen from Table I, the V/W ratio is very constant. Advantage was taken from this fact to carry out other experiments as it was now possible to calculate the initial body volume from the body weight of the animal.

Table II summarises the results of two different experiments. In the first experiment nine larvae were placed in 1/2 sea water (osmotic pressure 242 mM NaCl). In the second, three larvae were kept in 2/3 sea water (osmotic pressure 304 mM NaCl). In the second experiment one larva died.

It is clear, from the above data, that the body weight of these larvae increases markedly. Comparing the changes of body weight (ΔW) to the changes of blood volume (ΔV) it becomes evident that the changes of haemolymph volume can account for the increase of body weight.

This assessment can be applied to larvae of *Aeshna cyanea* in which similar changes of body weight have been reported (MOENS, 1973). Therefore we only need the V/W ratio of normal larvae. From the early reported changes in body weight, the change of haemolymph volume can be calculated.

Table III shows the results obtained by the determination of the V/W ratio of 4 normal larvae.

Table III

Determination of the blood volume/body weight ratio in normal larvae of *Aeshna cyanea*. (For further explanation cf. Table I).

Larva No.	W	V	V/W
1	0,869	282	325
2	0,881	287	326
3	0,856	286	334
4	0,856	268	319
mean			*326*

Finally Table IV summarises the changes of blood volume as calculated from earlier reported experiments in which only the change in body weight was studied (MOENS, 1973). The first three larvae were kept in 1/2 sea water (osmotic pressure 235 mM NaCl) for 96 h, the other three in 2/3 sea water (osmotic pressure 304 mM NaCl).

DISCUSSION

The changes in body weight of larvae of *Anax imperator*, which were observed in our experiments (Tab. II), compare favorably with those already described for the larvae of *Aeshna cyanea* (MOENS, 1973). The body weight of the larvae of both species increases markedly when the animals are kept in hypertonic media.

Table IV

Change of body weight and haemolymph volume in larvae of *Aeshna cyanea* when kept in hypertonic media. (For further explanation cf. text and Table II).

Larva No.	W_o	W_x	ΔG	V_o	V_x
1	806	948	142	263	405
2	756	839	83	246	329
3	699	894	195	228	423
4	794	851	57	259	313
5	826	873	47	269	316
6	825	898	73	269	342
mean				*256*	*355*

As suggested for larvae of *Aeshna cyanea* the changes of body weight in larvae of *Anax imperator* are due to the effect of drinking (personal unpublished observation). The fact that the body weight stabilises at a higher level can be explained by the supposition that the animals stop drinking as the osmotic influx of water, caused by the increased haemolymph osmotic pressure, is compensated by the urine production.

As pointed out by SHAW (1955) for *Sialis* larvae and by SUTCLIFFE (1962) for larvae of *Limnephilus affinis,* it is also possible that urine production in our larvae depends upon the influx of water. We have no information, however, about the existence of a mechanism as proposed by STOBBART (1970) for larvae of *Aedes aegypti,* in which urine production is regulated by stretch receptors.

Finally, another conclusion can be drawn from our evidence. As reported elsewhere (MOENS, 1975b), the increase of the haemolymph osmotic pressure in larvae of *Aeshna cyanea* was lowered by the decrease of the concentration of free amino acids in the blood. Our observations lead us to suppose that the decrease of free amino acids is due to a dilution effect caused by the increase of the blood volume. Indeed, the concentration of free amino acids in normal larvae is 77 mM. The mean blood volume of normal larvae is 256 µl. If kept in hypertonic media the haemolymph volume becomes 355 µl. If amino acids are neither removed nor added by any mechanism in the insect blood, the concentration of free amino acids in the haemolymph would be $77 \times 256/355 = 55$ mM. This figure fits with that obtained in our earlier experiments (MOENS, 1975b) where an average concentration of 50 mM free amino acids was found. Thus, one may conclude that the increase of haemolymph volume of these larvae can account for the decrease of the free amino acid content of the blood if the larvae are kept in hypertonic media of diluted sea water.

ACKNOWLEDGEMENT

Our sincere thanks are due to Prof. Dr. H.J. KOCH, Laboratory of Zoophysiology, University of Louvain, Belgium, for his advice and encouragement, which have done so much to make this work possible.

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