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THE EFFECTS OF VARIOUS EXTERNAL MEDIA ON THE HAEMOLYMPH OF LARVAL *AESHNA CYANEA*

H.-U. HERZOG

Institut für Cytologie und Mikromorphologie der Universität Bonn, Ulrich-Haberland-Strasse 61a, 5300 Bonn 1, German Federal Republic

The transfer of *Aeshna cyanea* larvae from 5 mosmol sea salt solution, which is similar to freshwater and very hypo-osmotic to the haemolymph, to hyper-osmotic sea salt solution (400 mosmol) leads to a rapid increase in the osmolarity and the NaCl concentration of the haemolymph, which after 2 days exceed those of the external solution by about 60 mosmol and 20 mM, respectively. When the osmotic concentration of the external medium is raised to 400 mosmol by the addition of mannitol and the salinity left unchanged at 5 mosmol, the haemolymph concentration increases more slowly and becomes only iso-osmotic to the external medium after 5 days. In s o d i u m– or c h l o r i d e– substituted media the haemolymph concentration of the kind of ion, which is externally present, slowly increases, whereas the concentration of the externally substituted counter-ion slowly drops. The results suggest that sufficient concentrations of both s o d i u m and c h l o r i d e in the external medium are required for the proper osmoregulation.

INTRODUCTION

As hyperosmotic regulators the larvae of *Aeshna cyanea* are able to maintain their haemolymph concentration constant over a wide range of environmental salinities reaching from nearly salt-free water to about 90% iso-osmolarity (SCHOFFENIELS 1950, 1960; MOENS 1975; KOMNICK, 1977). The normal value of the haemolymph osmolarity is about 330 mosmol. The major components are sodium (145 mM) and chloride (130 mM) representing 83% of the total osmolarity. In hyperosmotic sea salt solutions, ie. solutions exceeding the normal value of 330 mosmol, the osmolarity and the sodium chloride concentration of the blood rise to a new constant level at about 110% of the external concentrations. Under these conditions sodium chloride constitutes about 90% of the total haemolymph os-

molarity.

The upper limit of the haemolymph osmolarity appears to be 660 mosmol, which is about twice the normal value and is reached in 600 mosmol sea salt solution. At this high concentration the larvae, depending on the body size, survive for only a few days.

MATERIAL AND METHODS

For the experiments unfed larvae between 30 and 40 mm in body length were preadapted in a freshwater equivalent (5 mosmol sea salt) for 5 days and then transferred to the experimental solutions. The osmotic pressure of haemolymph samples withdrawn after various time periods was determined with a semi-microosmometer (Knauer). The sodium and chloride concentrations were measured using an atomic absorption spectrophotometer (Perkin-Elmer model 360) and a "micro-chlor-o-counter" (Marius), respectively. The mean and standard deviation of the haemolymph concentrations are based on at least 6 individuals.

For the determination of the rate of drinking 1% of Amaranth was added to different experimental solutions and the volume of colored medium present in the fore- and midgut photometrically determined.

For the estimation of blood volume change the body weights of 10 individual larvae in each experimental solution were measured gravimetrically at intervals over a total period of 5 days.

RESULTS AND DISCUSSION

The transfer of the larvae from 5 to 400 mosmol sea salt solution leads to a rapid increase in the osmolarity and sodium-chloride concentration of the haemolymph, which after 2 days exceed the figures of the external solution by 60 mosmol and 20 mM, respectively (Fig. 1). When osmotic concentration of the external medium is raised to 400 mosmol by the addition of mannitol and the salinity left unchanged at 5 mosmol, the haemolymph concentration increases more slowly and becomes only iso-osmotic to the external medium after 5 days, whereas the sodium-chloride concentration drops to less than 70% of the total haemolymph concentration after 3 days (Fig. 1).

The determination of the drinking rate shows, that in 5 mosmol sea salt solution the larvae drink, though in very small quantities (Table I). When they are transferred to 400 mosmol sea salt solution

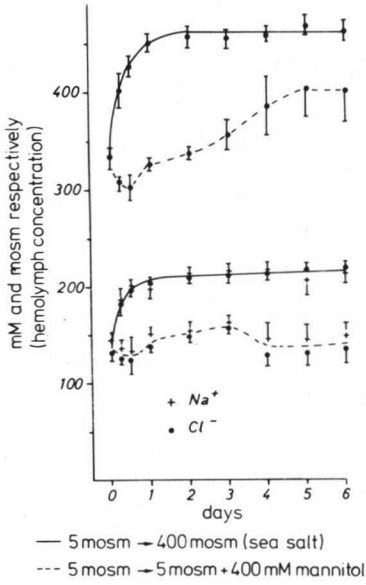

Fig. 1. Osmotic and sodium chloride concentration in the haemolymph of larval *Aeshna cyanea* after the transfer from 5 mosmol sea salt to 400 mosmol sea salt and 5 mosmol sea salt + 400 mM mannitol, respectively.

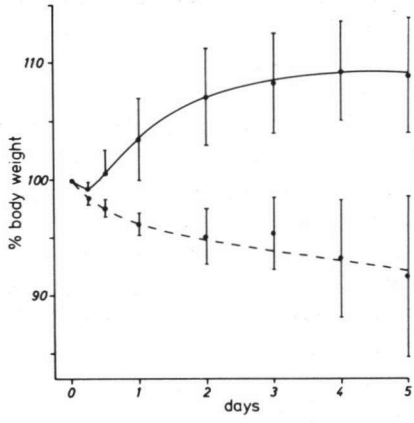

Fig. 2. Relative changes in body weight of larval *Aeshna cyanea* after the transfer from 5 mosmol sea salt to 400 mosmol sea salt (———) and 5 mosmol sea salt + 400 mM mannitol (- - - -) (Herzog & Komnick, unpublished results).

Table I

Drinking activity of larval *Aeshna cyanea* in different experimental media

Preadaptation (5 days)	Experimental medium + 1 % (60 mosmol) Amaranth	Time (h)	n	μl x h^{-1} x g^{-1} body weight x ± SD
5 mosmol sea salt	5 mosmol sea salt	6 + 12	13	0,3±0,06
5 mosmol sea salt	400 mosmol sea salt	3	14	11,7±2,29
400 mosmol sea salt	400 mosmol sea salt	3	13	2,0±0,40
5 mosmol sea salt	5 mosmol sea salt + 400 mM mannitol	6	14	0,7±0,27

the drinking rate increased markedly, dropping to nearly normal values however 5 days after the adaptation in this solution (see MOENS 1973, 1975). In hyperosmotic mannitol solution however, the drinking is scarcely higher than under normal conditions.

There seems to be a relation between the drinking activity and the change of blood volume. As Figure 2 shows the transfer of the larvae to hyperosmotic sea salt solution causes an increase in the haemolymph volume, which stabilises after 4 days at a higher level (see MOENS 1973, 1975), whereas in hyperosmotic mannitol solution the haemolymph volume decreases.

The osmotic loss of water in hyperosmotic sea salt solution seems to be initially overcompensated by the high drinking activity resulting in a rise of the blood volume. This effect is also known from other freshwater larvae exposed to salt solutions (SHAW 1955; SUTCLIFFE 1961; MOENS 1975; KAPOOR 1979). The passive influx and the active absorption of sodium and chloride lead as well to a rapid increase of the ionic concentration from the uptake of salt-rich drinking water. After the haemolymph concentration has exceeded the external concentration the drinking seems to be strongly reduced and the haemolymph volume stabilises at a higher level. This leads to the assumption that the hyperosmotic regulation in high salt concentrations serves for gaining water osmotically.

On the other hand the osmotic loss of water in hyperosmotic mannitol solution, which is apparently not conpensated by drinking, causes the slow increase of the osmotic pressure of the haemolymph merely leading to iso-osmolarity. Furthermore SCHMITZ & KOMNICK (1976) have shown that in hyperosmotic mannitol solution the absorption of chloride is strongly reduced. As a consequence the decrease of the sodium-chloride concentration 3 days after the transfer seems to be due to the passive efflux of these electrolytes. It must be suggested, that, when only the osmotic concentration of the external medium is increased and the salinity left unchanged at the freshwater equivalent, the larvae are not able to maintain their ionic regulation and water balance either by raising the drinking activity or by increasing the sodium chloride concentration of the haemolymph for hyperosmotic regulation.

In the sodium- or chloride-substituted media the increase of the haemolymph osmolarity and the concentration of the externally present ion is clearly reduced in comparison to the controls, whereas the concentration of the externally substituted counterion stays constant (sodium) or slowly drops (chloride) (Figs 3 and 4).

Since the volume of haemolymph which could be withdrawn was as small as that from larvae transferred to hyperosmotic mannitol solution, being only half of the volume obtained from larvae transferred to hyperosmotic salt solution, it appears that water and ionic balance in these substitute media is effected mainly by passive phen-

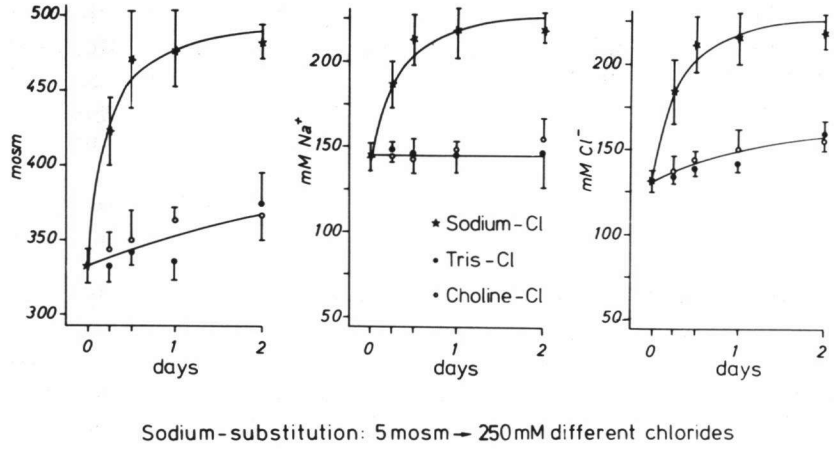

Fig. 3. External Na-substitution. Osmotic and sodium-chloride concentrations in the haemolymph of larval *Aeshna cyanea* after the transfer from 5 mosmol sea salt to 250 mM NaCl (control) and 250 mM Tris-Cl and Chol-Cl, respectively.

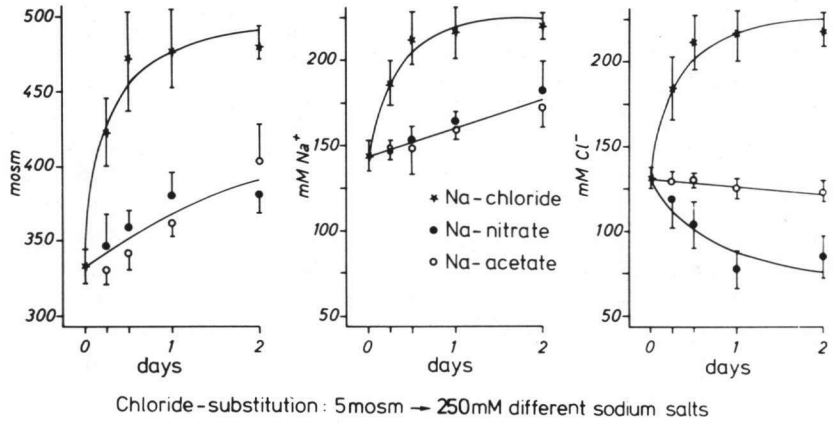

Fig. 4. External Cl-substitution. Osmotic and sodium chloride concentrations in the haemolymph of larval *Aeshna cyanea* after the transfer from 5 mosmol sea salt to 250 mM NaCl (control) and 250 mM Na-nitrate and Na-acetate, respectively.

omena, osmotic loss of water, passive influx of the non-substitute kind of ion and passive efflux of the substituted ion. This indicates, that both kinds of ions, sodium and chloride are required externally in appropriate concentrations for the normal operation of the active osmoregulatory ion-absorbing mechanism. This leads to the assump-

tion,that the absorption of the 2 kinds of ions is inter-dependent possibly in the sense of a coupled transport or possibly by the requirement of electroneutrality.

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