Aspects of sodium regulation in a brackish-water and a marine species of the isopod genus *Sphaeroma*

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Abstract

Sodium and chloride concentrations of the haemolymph were determined in *Sphaeroma rugicauda* Leach and *S. serratum* Fabricius acclimatized to a range of salinities. Sodium loss and uptake (using $^{24}$Na) were measured in salinities below 1.1 % for *S. rugicauda* and 7.2 % for *S. serratum*. Potential differences between haemolymph and medium indicate active uptake of both ions in certain salinities. The active-uptake component of total sodium-influx was found to be related non-linearly to the sodium concentration of the medium. Curves of $\frac{N}{Km + C}$ were fitted to the active-uptake data. $Km$ values indicate that, in *S. rugicauda*, the sodium uptake system has a greater affinity for sodium than in *S. serratum*. These findings are discussed in relation to the distribution of each species.

Introduction

Brackish-water and fresh-water crustaceans are believed to have evolved from marine ancestors. Shaw (1961a) suggested that the adaptation of the Crustacea to fresh water involved a reduction in the permeability of the body surface to ions, and a lowering of the external concentration necessary to saturate the ion-uptake systems. Thus, ion-uptake mechanisms were developed which operated maximally in low external-medium concentrations. These mechanisms were necessary to maintain ion concentrations of the haemolymph above those of the dilute media (see review by Lockwood, 1962). Since inorganic ions are the major osmotic effectors in crustacean haemolymph, this maintenance is necessary to contain overall haemolymph concentration changes within the capability of intracellular osmotic-pressure regulatory mechanisms (see review by Florkin and Schopfenters, 1965). Cellular function also requires that haemolymph ion-concentrations are maintained at defined levels (Lockwood, 1962).

Comparison of sodium-loss rates, sodium being the predominant cation in many crustacean haemolymphs (Robertson, 1949, 1953), over a range of marine, brackish-water and fresh-water species indicates that there is a reduction in permeability to this ion in the latter two groups. Similarly, those fresh-water crustaceans which have been studied show sodium-ion uptake mechanisms which are saturated at low external-sodium concentrations. Comparisons between fresh-water and brackish-water species of the same genus, *Gammarus* (Shaw and Sutcliffe, 1961; Sutcliffe and Shaw, 1967), and various races of *Mesidotea entomon* (Croghan and Lockwood, 1968) have also been made. No comparative studies of sodium balance in both a marine and a brackish-water species of the same genus have been made. In the present work, sodium uptake and loss in the two isopod species *Sphaeroma rugicauda* Leach and *S. serratum* Fabricius are studied in the low salinity range.

Methods and materials

*Sphaeroma rugicauda* were collected from tidal creeks and salting pools at Totton Marshes, near Southampton, England. They were kept in 25 % sea water (100% = 35.8 %o S) at room temperature (16° to 20°) and supplied with detritus from the pool and creek bottom. They survived well in these conditions. Specimens of *S. serratum* were obtained from the Marine Biological Laboratory at Plymouth, England, and some were also collected under stones and on juvus from Lulworth Cove, Dorset. They were kept in 100 % sea water.

Groups of 20 isopods were rinsed in deionised water and transferred into 1 l of the various medium concentrations. The media were changed daily and fatalities were recorded. Salinities in excess of 35.8 % were prepared by evaporating sea water gradually at 16° to 20°C. The time required for 50 % mortality (LD50) was noted and, in cases where this exceeded 14 days, the species were considered to tolerate that salinity. Controls were kept in the original salinities. Groups of isopods were transferred from the lowest and highest salinities, where LD50 exceeded 14 days, into slightly lower or higher concentrations to determine the effect of gradual acclimatization.

Haemolymph was collected by micropipette and stored under liquid paraffin in a siliconised watch glass. Sodium was determined by flame photometry using a Unicam SP900 flame spectrophotometer. Replicate determinations of a standard were accurate to...
standard deviation (SD) ±2%. This value includes pipetting and instrument errors. Salinity was measured using an Electronic Switchgear Temperature-Salinity Bridge type M.C.5.

Chloride space was determined using wet-weighted isopods which had been dried to a constant weight at 70 °C. After ashing in a muffle furnace at 450 °C, the finely ground ash was taken up in 2 ml of 1/5 H2SO4. A 50 µl aliquot of standard NaCl was treated similarly to detect any loss of chloride. The chloride content of both sample and standard was determined by the first method of Ramsay et al. (1965). Thus, chloride space =

\[
\text{chloride concentration in the total body water} \times 100.
\]

The electrical potential difference (PD) between the haemolymph and the medium was measured using glass micro-electrodes filled with 3M KCl containing 1% agar. The micro-electrodes had a tip diameter of about 15 µ and were attached to PYE calomel electrodes. The potential difference was recorded on a Beckman Research pH meter of 1012 ohms input impedance. Subsequent measurements on Sphaeroma serratum were made using an E.I.L. Vibron Electrometer Model 33B-2. All determinations were made at 18° ± 2 °C. Initially, the PD was measured with both electrodes in the experimental medium to determine any electrode asymmetry. The isopods were then sealed in a pierced polythene lid, using a 50/50 mixture of violin rosin and beeswax, so that the gills were immersed in the experimental medium. One electrode was inserted intersegmentally into the haemocoel, the other clamped with the tip in the medium. The PD was recorded as soon as the reading was stable.

Measurements of sodium influx were made using 22Na. The loading medium was circulated between two small polythene chambers (animal and counting chambers). These were connected by polythene tubing. The total volume of the system was 10 ml. The counting chamber was inserted into the well of an EKCO scintillation counter.

The counts of the medium were recorded on an EKCO N810 scaler for 1 h at the start of the experiment. This ensured that the volume of medium within the counting chamber remained constant. Forty similar-sized individuals were acclimatized to the lowest medium concentration in which they survived. This was 0.7% S (10 mM/1 Na) in the case of Sphaeroma rugicuada and 5.8% S (80 mM/1 Na) in S. serratum. After weighing, they were placed in the animal chamber and a perforated polythene disc positioned to prevent them crawling out of the medium. The fall in counts of the medium was measured every 5 min over a period of 60 min. The total internal sodium of the isopods always exceeded that of the medium, thus, the increase of 22Na in the haemolymph was never great enough for large back-flux of 22Na to occur. The uptake of 22Na under these conditions was assumed to be proportional to the influx of sodium.

In external concentrations below 0.7% S in Sphaeroma rugicuada, or 5.8% S in S. serratum, net loss of sodium occurs. Since the internal sodium exceeds the external sodium by a large margin, this will result in a marked increase in the medium concentration even over a 60 min period. The mean of initial and final medium concentrations, measured by flame photometry, was taken, and rates of influx were plotted against this. The rates of influx of sodium per isopod were converted to mM Na/1 haemolymph/h using the mean weights of the isopods and values of chloride space determined previously in the two species. Although chloride space only approximates blood volume, there was reasonable agreement between the rates of influx measured using this method and an alternative method which was carried out on isopods acclimatized to the initial medium. In this latter method, individual isopods were placed in a loading medium of 0.7% S (10 mM/1 Na) containing 22Na (5.8% S 80 mM/1 Na for S. serratum). The rise in count of the animal was measured by placing it, after rinsing off tracer, into a test tube with a small amount of inactive medium and counting in the scintillation counter. The rate constant for the influx was determined from the relation:

\[
k = \frac{1}{t} \frac{2.303 \log_{10} \frac{C_0}{C_t}}{C_0 - C_t}
\]

where \(C_0\) is the count of the fully-loaded animal, and \(C_t\) the animal count after time \(t\). \(C_0\) can be either measured after complete exchange has taken place, or calculated from the haemolymph sodium concentration.

The former method was used where, if the animals were not in sodium balance, \(C_0\) values could be neither measured nor calculated. Rates of sodium efflux were measured by washing, with inactive medium, fully-loaded animals in the circulation system. Under these circumstances, the animal chamber was counted in the scintillation counter. A two-way tap in the system allowed a change of the medium flowing through the system. The effluent was discarded, not recirculated as in the influx experiment. The animal counts were recorded at 5 min intervals. The rate constant \((k)\) was obtained from the relation:

\[
2.303 \log_{10} \frac{C_0}{C_t} = kt.
\]

The loss rate \((R)\) of sodium as mM/Na/h haemolymph is the product of \(k\) and the haemolymph sodium concentration corrected for the potential difference present. The effect of a potential difference was calculated according to the approach of Horsa (1963):

\[
JE_1 = \frac{\left(\frac{(FE_i)RT}{C_t} \exp (FE_iRT)\right) \left(1 - \exp FE_iRT\right)}{(FE_i)RT}.
\]

If \(E_1\) is set at zero and measured values of \(E_2\) substituted, \(JE_1/JE_2\) can be calculated, thus giving