THE WATER BALANCE OF A SERPULID POLYCHAETE, 
MERCIERELLA ENIGMATA (FAUVEL) 

II. ION CONCENTRATION 

BY HELEN LE B. SKAER

Department of Zoology, University of Cambridge*

(Received 17 December 1972)

INTRODUCTION

Mercierella enigmatica (Fauvel, 1923), a serpulid polychaete, lives in an unusually wide range of salinities (< 1–55‰) (Seurat, 1927; Heldt, 1944; Tebble, 1953). The concentration of its blood has been shown to change with that of the external medium over almost the entire range (Skaer, 1974). The blood of Arenicola marina, another polychaete, shows a similar response to changes in the concentration of the bathing medium but the animal lives in a very much more restricted salinity range (Schlieper, 1929). In both investigations the concentration measurements are expressed in terms of the freezing points of the test solutions and body fluids. The results therefore give no information about the concentrations of individual ions in the body fluids.

The simplest interpretation of measurements of freezing-point depression of the blood of Arenicola or Mercierella equilibrated in media of different concentrations would be that the blood was diluted or concentrated as a result of osmosis and ion loss, and that the relative concentrations of ions in the blood remained the same throughout. There is evidence that this is not so in Arenicola. In animals from dilute sea water the concentration of potassium and calcium in the blood are found to be higher than would be expected by dilution (Robertson, 1949). It was therefore of interest to investigate the ionic composition of the blood of Mercierella enigmatica from media of different salinity.

MATERIALS AND METHODS

The collection and culturing of animals, their treatment for equilibration in different media and the extraction of blood from the operculum were as described previously (Skaer, 1974). The volume of the drop of blood collected was computed from its diameter, measured with the drop suspended in heavy liquid paraffin. Blood samples were either diluted for cation analysis or used directly for anion analysis. Sufficient blood could be obtained from a single animal for analysis of one ion species.

Analysis of cations

Sodium and potassium concentrations were measured by emission spectrophotometry on a Unicam SP 900 flame spectrophotometer and calcium and magnesium by...
absorption spectrophotometry with a Unicam SP 90 spectrophotometer. Interference between elements in the blood can prevent quantitative recovery of individual ions. Analyses of potassium and calcium are particularly susceptible to errors from this source, but tests indicated that there was no significant interference in the analysis of these ions in *Mercierella* blood.

**Analysis of anions**

Chloride concentrations were measured by electrometric titration against 0.01 N silver nitrate solution (Ramsay, Brown & Croghan, 1955).

**Analysis of free amino acids**

The free amino acid content of the blood was measured using the Technicon Automatic Analyser. Animals equilibrated in three different media (glass-distilled water, aquarium sea water, and hypersaline – 150% – sea water) were used. Samples of 3–4 µl were collected from a total of 50–100 animals for each reading.

**Microfiltration**

To estimate unbound ion concentrations in the blood, samples were filtered through a fine Millipore filter (Millipore 1000 molecular weight cut-off, PSAC 09005). The fluid can be forced through the filter by centrifugal force or hydrostatic pressure (Riegel, 1968, and personal communication). Microfiltration by hydrostatic pressure was carried out under liquid paraffin, the filter being mounted so that the filtered sample was forced through on to the upper surface of the membrane where it could be collected with a silica-glass pipette. As the filter clogged fairly rapidly, filtrates from many samples were needed for each ion analysis. The analysis of the ions in the filtrates was carried out as described above.

**RESULTS**

**Ion content after equilibration**

The concentration of different ions in the blood after equilibration of animals in media of various salinities are shown in Fig. 1. The concentrations of all five ions vary over a wide range with changes in the salinity of the external medium. The concentrations found for each ion are given in Table 1. These results are based on measurements of the total concentrations of the ions. However, the effective concentration of an ion in the blood can be much lower than the total concentration, since salts in solution do not behave as ideal electrolytes except at very low concentrations and larger impermeant molecules present in the blood may bind ions. This can be investigated by microfiltration. Filtered blood contains higher concentrations of calcium and potassium than unfiltered blood after equilibration with media of high salinity. This is because a significant proportion (ca. 20–5 %) of the volume of unfiltered blood is made up by the blood pigment, chlorocruorin (Skaer, 1974). Ion concentrations were therefore corrected for pigment volume and the corrected values are shown in Table 2 as well as in Figs. 1B and C as open circles.
Water balance of M. enigmatica. II

Fig. 1. The concentration of different ions in the blood after equilibration of animals in media of various salinities. Each point represents the arithmetic mean of readings from between five and ten animals, and the bars represent ± the standard error. The slope and the intercept on the ordinate of the straight lines drawn were calculated by the method of least squares. The spread of results for potassium is probably due to experimental error – the concentrations of potassium in the diluted samples were 0.5–5 μM/l and, at the gain necessary to read these values on the Unicam SP 900, the signal-to-noise ratio was unfavourable. Subscript i, concentration in the blood. Subscript o, concentration in the external medium. Closed circles, concentration in unfiltered blood. Open circles, concentration in filtered blood.
Table 1. The concentrations of ions in the blood of Mercierella enigmatica after equilibration for 4 days with media of different salinity

<table>
<thead>
<tr>
<th>Ion concentration in the blood (mM/l)</th>
<th>External medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na</td>
</tr>
<tr>
<td>Distilled water</td>
<td>50.5</td>
</tr>
<tr>
<td>Aquarium sea water</td>
<td>47.8*</td>
</tr>
<tr>
<td>Hypersaline sea water</td>
<td>66.32</td>
</tr>
</tbody>
</table>

- The abnormally high levels of these ions in the blood probably reflect the unusually high concentrations of K⁺ and Ca²⁺ present at that time in the laboratory aquarium.

Table 2. Analysis of calcium and potassium in blood and filtrates from animals equilibrated in three different media

<table>
<thead>
<tr>
<th>Medium</th>
<th>Blood</th>
<th>Ca²⁺(mM/l)</th>
<th>K⁺(mM/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>Unfiltered</td>
<td>25.74</td>
<td>13.04</td>
</tr>
<tr>
<td></td>
<td>Filtered</td>
<td>4.92</td>
<td>8.16</td>
</tr>
<tr>
<td>Aquarium sea water</td>
<td>Unfiltered</td>
<td>29.81</td>
<td>44.90</td>
</tr>
<tr>
<td></td>
<td>Filtered</td>
<td>17.44</td>
<td>39.33</td>
</tr>
<tr>
<td>Hypersaline sea water</td>
<td>Unfiltered</td>
<td>38.36</td>
<td>59.99</td>
</tr>
<tr>
<td></td>
<td>Filtered</td>
<td>30.91</td>
<td>54.98</td>
</tr>
</tbody>
</table>

Rate of equilibration

The concentrations of individual ions in the blood at set intervals after the beginning of equilibration in two different salines (hypersaline – approx 150%–aquarium sea water and glass-distilled water) are shown in Fig. 2. These two salines were chosen to represent extreme, but not lethal, conditions (Skaer, 1974). The rates of equilibration are comparable to those found for the depression of freezing point (Skaer, 1974), the half time being about ½–1 h and equilibration being almost complete after 6 h.

Amino acid content

Analysis of the free amino acid content of the blood indicates that glycine is the predominant amino acid in the blood, there being about ten times as much glycine as alanine, glutamic acid, aspartic acid or serine. Quantitative estimates were possible only for glycine due to the small size of the samples. The values are shown in Table 3.

DISCUSSION

Ion concentrations in the blood

Mercierella enigmatica tolerates very large variations in the osmotic and ionic strength of its blood. None of the slopes of the lines relating internal ion concentration to external ion concentration (Fig. 1) is very greatly different from the slope of the isionic line, except in very low salinities (< 100 mOsm).

The concentrations of sodium, chloride and unbound calcium in the blood are almost the same as the external concentrations, those of potassium (even in filtrates) and magnesium being significantly higher in the blood than in the external medium. It is quite likely that magnesium is bound to anions in the blood and it is possible that potassium is further bound to small molecules that passed through the Millipore
Fig. 2. The changes in concentration of ions in the blood (subscript i) of animals taken from the aquarium and placed in environments of altered salinity (at \( t = 0 \)). The bars represent \( \pm \) the standard errors of the means.
Table 3. Glycine content of the blood after 4 days equilibration in different media

<table>
<thead>
<tr>
<th>Equilibration medium</th>
<th>Glycine (mM/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass-distilled water</td>
<td>3.06</td>
</tr>
<tr>
<td>Aquarium sea water</td>
<td>19.10</td>
</tr>
<tr>
<td>150% sea water</td>
<td>27.02</td>
</tr>
</tbody>
</table>

filter. In addition, the elevated levels of potassium and magnesium may be due to a Gibbs-Donnan effect. Large anions in the blood cannot diffuse freely between the blood and external medium. If these anions are neutralized by inorganic cations there will be a tendency for the internal cation concentration to exceed the external concentration. The blood of Mercierella contains at least one large molecule, chlorocruorin. A higher internal concentration of cations might therefore be predicted, raising the osmotic pressure of the blood above the external medium as found (Skaer, 1974). It is however, difficult to explain why cation retention should be restricted to magnesium and potassium.

It seems likely that, with the possible exception of potassium and magnesium, ion exchange between the blood and the medium occurs passively.

Comparison with other species

Specimens of Arenicola marina from dilute sea waters (50–70 % sea water) have concentrations of chloride, magnesium and sodium in the coelomic fluid similar to those in the outside medium, while calcium and potassium levels are higher and sulphate levels are lower than in the environment (Robertson, 1949). It is not clear, however, whether these discrepancies result from active regulation or from differences in diffusion rates – calcium and potassium being lost from cells to the coelomic fluid faster than can be offset by diffusion of these ions between coelomic fluid and sea water. One mechanism of active regulation in Arenicola marina, Abarenicola pacifica and other polychaetes has been suggested. Clark (1968a, b) claims that direct transfer of amino acids from tissues to the body fluids slightly increases the osmolality of the latter. My amino acid analyses of the blood show that this phenomenon is unlikely to occur in Mercierella, since the amino acid concentrations decrease in dilute media very much more than would be predicted by Clark’s theory.

Nereis diversicolor can survive in salinities of 0.5 %, but it has been shown, with the use of 24Na and 36Cl, that active uptake of sodium and chloride occurs in dilute media (Fretter, 1955; Jorgensen & Dales, 1957; Smith, 1970a). Chloride is concentrated against both electrical and chemical concentration gradients over the lower third of the environmental salinity range, the coelomic fluid being negative to the outside by approximately 3.7 mV in 10 % sea water (Smith, 1970a) and falling sharply to — 50 mV in 0.2 % sea water (Fletcher, 1970). It has also been found that calcium and magnesium concentrations in the coelomic fluid are maintained relatively constant in lower salinities and it has been suggested that this results passively from active sodium and chloride regulation (Fletcher, 1970). In addition, Nereis diversicolor produces hypo-osmotic urine in dilute media and the ‘apparent permeability’, of the body wall
Water balance of M. enigmatica. II

337
to water (as D₂O) decreases (Smith 1970b, c). The osmoregulatory powers of _Nereis diversicolor_ are comparable to those of some crustacea and molluscs (e.g. _Carcinus maenas_ (Webb, 1940; Shaw, 1961)), and are in sharp contrast with those of _Mercierella_, which appears to be the most extreme case yet described of an osmoconformer.

**SUMMARY**

1. _Mercierella enigmatica_, a serpulid polychaete, lives in water ranging in concentration from fresh water to 150% sea water (< 1-55 %o).

2. The concentrations of five inorganic ions (Na⁺, K⁺, Ca²⁺, Mg²⁺ and Cl⁻) in the blood have been measured both during and after equilibration of the animals with media of altered salinity.

3. The concentrations of calcium and potassium have also been measured in filtrates of the blood from animals equilibrated in three media of differing salinity.

4. Concentrations of all the ions measured vary linearly with the concentration of the external medium. The levels of sodium, calcium (in filtered blood) and chloride are near the isionic line, while those of magnesium and potassium (even in filtered blood) are slightly higher in the blood over the whole range.

This work was supported by a Science Research Council grant and a studentship from Girton College, Cambridge. I am very grateful to my supervisor, Dr J. E. Treherne for his help and encouragement. I thank him, Dr J. Oschman, Dr R. J. Skaer and Dr B. Wall for reading and criticizing the manuscript. Dr J. A. Riegel very kindly allowed me to use his microfiltration apparatus and Dr C. Little his S.P. 90 flame spectrophotometer. I am grateful to Mr J. Rodford for drawing the graphs for Figs. 1 and 2.

**REFERENCES**


