ACTIVATION OF THE SODIUM UPTAKE SYSTEM AT HIGH BLOOD CONCENTRATIONS IN THE AMPHIPOD GAMMARUS DUEBENI

By A. P. M. LOCKWOOD

Department of Zoology, University of Southampton

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INTRODUCTION

In an earlier paper (Lockwood, 1961) it was shown that the brackish-water amphipod, *Gammarus duebeni*, begins to form urine hypotonic to the blood about 2 hr. after the animal is transferred from a highly concentrated medium (100–175% sea water) to fresh water. The osmotic concentration of the blood at the time the urine first becomes hypotonic may be as much as 1 M./l. NaCl, a value twice as high as that at which hypotonic urine would normally be produced by an animal in a steady state with its medium. It was suggested in the same paper that the capacity to produce hypotonic urine in these circumstances might be of adaptive significance to the species.

*G. duebeni* is usually found in small streams and pools near the top of salt marshes, rock pools above the neap high tide mark and similar brackish-water sites (Kinne, 1959). One feature of such habitats is that they are liable to sudden and possibly large changes in salinity as a result of inundation by fresh water or sea water. The loss of sodium in the urine accounts for a major part of the total loss from the body. Hence the switch from isotonic to hypotonic urine production shortly after the medium is diluted will have the effect of slowing the rate of salt loss. This slowing of the loss will in turn allow more time for osmotic adjustments at the cellular level to be made, and there will consequently be less liability to cellular swelling or circulatory crisis as the blood concentration falls.

An additional factor which could further slow the drop in blood concentration when the medium is suddenly diluted would be an increase in the rate of active uptake of ions. Activation of the ion transport system of animals has been studied mainly on freshwater species: Krogh (1938) on *Eriocheir*, Shaw (1959a) on *Austropotamobius*, Bryan (1960) on *Austropotamobius* and Lockwood (1960) on *Asellus*; and in these forms an increase in the rate of active transport is correlated with a fall in the concentration of the blood below its 'normal' level. However, it seems likely that in the invertebrates, as well as in the vertebrates, the systems responsible for the active transport of sodium are influenced by other factors in addition to the concentration of the blood. The work described in this paper shows that an increase in the rate of uptake of sodium can be elicited at high blood concentrations if the concentration of the medium is suddenly lowered.
MATERIALS AND METHODS

Materials

The *Gammarus duebeni* used were collected on various occasions between October 1961 and July 1963 from Flatford on the River Stour, from Chisholm Meadows, Plymouth, and from Totton Marshes, Southampton. They were maintained in the laboratory in about 20% sea water and fed on 'Bemax' and *Enteromorpha*. In such conditions the animals live and breed apparently normally.

Methods

The osmotic pressures of blood and urine were measured cryoscopically by the method of Ramsay & Brown (1955). Sodium was determined by flame photometry and $^{22}$Na activity was determined by the use of a well-type scintillator in conjunction with an EKCO scaler. When whole animals were counted they were placed in 2 c.c. of water in a test tube. Movements of the animals within this limited volume did not disturb the geometry sufficiently to cause appreciable differences in count.

RESULTS

The main technique used in the study of the activation of the system responsible for the uptake of sodium has involved the sudden lowering of the blood concentration of animals previously acclimatized to sea water, or to higher concentrations, followed by comparison of the sodium influx and that of control animals acclimatized to a lower concentration. Before experiments of this type could be interpreted it was first necessary to establish the normal sodium concentration of the blood of animals acclimatized to various concentrations and also to determine the relationship between a fall in the tracer sodium count of an animal washed in deionized water and the concomitant fall in the concentration of sodium in its blood.

The steady-state sodium concentration of the blood

A curve illustrating the relationship between the steady-state concentrations of sodium in the blood and in the medium is given in Fig. 1. Sodium is about 20–30 m-equiv./l. more concentrated in the blood than in the medium when the latter is in the range 50–100% sea water; but it is markedly more concentrated in the blood than in the medium when the latter is less concentrated than 50% sea water. Comparable figures for osmotic pressure and chloride concentrations of the blood are given by Beadle & Cragg (1940).

Sodium concentration of the blood and total sodium loss on washing out

The animals used in this experiment were acclimatized to concentrations in the range 120–170% sea water to which $^{22}$Na had been added. When they had come to a steady state they were placed in deionized water and their count was monitored until they had lost 50–60% of their total sodium. A blood sample was then taken and the sodium concentration was determined. In Fig. 2 the results are plotted as counts lost (expressed as a percentage of the initial count) against the percentage of the initial sodium concentration of the blood. If the total sodium in the body and the sodium concentration of the blood fell at the same rate then the values for blood should lie
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along the solid line. It can be seen that the sodium concentration of the blood falls less rapidly than the total sodium. This result may be due to loss of sodium from tissues other than the blood, to movement of water into the tissues when the blood is rapidly diluted or to a combination of these factors.

![Graph of sodium concentration](image)

**Fig. 1.** The relationship between the concentrations of sodium in the blood and in the medium.

![Graph of percentage of initial counts lost](image)

**Fig. 2.** The relationship between the initial concentration of sodium in the blood and the loss of sodium from the body.

*Increase in the sodium influx after washing the animals with deionized water*

The general method used to stimulate an increase in active transport of sodium at high blood concentrations is based on the following four stages: (1) Control animals are
acclimatized to a concentration of about 50% sea water and experimental animals to a higher concentration. (2) The experimental animals are washed with deionized water until their blood concentration is at a level similar to, but a little above, that of the controls. (3) Both controls and experimental animals are placed in a similar tracer-labelled medium and their respective influxes are compared. (4) The final sodium concentrations of the blood in both experimental and controls are determined.

The medium used for the measurement of influx consisted of 5% sea water plus sucrose labelled with $^{22}$Na. This solution will be referred to as 'the loading medium'. The concentration of sucrose used is given in the table or figure appropriate to each experiment.

5% sea water contains sodium at a concentration more than adequate to ensure that the active transport system is fully saturated and that the rate of transport is not therefore limited by the concentration of the medium (Shaw & Sutcliffe, 1961). It is not, however, so concentrated that the interpretation of the results is complicated by the ion-exchange component of the influx. The sucrose was added to decrease the osmotic inflow of water into the animal during the determination of influx and so to decrease the loss of sodium in the urine. The presence of sucrose in the solution has no effect on the influx of animals in such experiments.

Two batches of animals were acclimatized for 4 days to 110% and 66% Plymouth sea water respectively. Both batches were then placed in the loading medium for 1½ hr. At the end of this period they were rinsed and counted to determine the sodium influx. The controls from 66% sea water were replaced in 66% sea water and the experimental animals from 110% sea water were put into deionized water. Four hours later both batches were re-counted to determine the change in their tracer content and they were then replaced in the loading medium for a second 1½ hr. period. At the end of this second period of influx blood samples were taken and their sodium concentrations were measured. The results are given in Fig. 3.

The period in deionized water resulted in an average loss of 42% of the sodium initially in the animals which had been in 110% sea water. The blood concentration of these animals after washing out would therefore be expected to be close to that of the controls in 66% sea water allowing for the observation, from the previous experiment, that the fall in blood concentration is somewhat lower than the drop in total count. Despite this expected similarity in blood concentration the experimental animals had an average sodium influx in the second period of loading more than three times that of the controls in the first period of loading, and more than four times that of the controls in the second period (Fig. 3). The influx during the second period of uptake by the experimental animals was $4.86 \pm 1.47$ times that found during the first period. Determination of the blood concentration after the second period of loading indicates that the sodium concentration in the blood of the experimental animals is higher than that of the controls. It is not possible therefore that the higher influx of the experimental animals is due to their having a lower blood concentration than the controls.

In another similar experiment the experimental animals showed a rise in influx of 3.5 times the initial level whilst the controls again showed a small fall.

The observation that the average influx into the animals exposed to deionized water for some hours is 3–4 times that of the controls in 66% sea water is remarkable in view of the fact that there is a much smaller difference in the influxes when animals main-
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taining small and large gradients of concentration between blood and medium are compared. Thus comparison of the influx (from an identical loading medium) into animals previously acclimatized for 40 hr. to 50% sea water with the influx into animals previously acclimatized to 2% sea water indicates that the influx into the latter is only some $1\frac{1}{2}$ times that into the former (Table 1).

![Graph](image)

Fig. 3. The effect of treatment with deionized water on the subsequent influx of sodium into animals initially acclimatized to 110% sea water. A, Count of animals from 110% sea water after 1 hr. in the loading medium; B, count of the same animals after 4 hr. in deionized water; C, net increase in count after a second period of 1 hr. in the loading medium; D, count of controls from 66% sea water after 1 hr. in the loading medium; E, count of controls after an additional 4 hr. in 66% sea water; F, net increase in count of controls after a second period of 1 hr. in the loading medium. The loading medium was 5% sea water plus 34.2% sucrose. The final blood sodium concentrations were:

- **Experimentals**
  - 84% sea water (2 pooled)
  - 78% sea water (2 pooled)

- **Controls**
  - 70% sea water (2 pooled)
  - 68% sea water (4 pooled)

| Table 1. Comparison of the influx of sodium into animals acclimatized to 50% sea water and to 2% sea water when both are placed in the same loading medium |
|---------------------------------|---------------------------------|
| **Count/300 sec. of animals**   | **Count/300 sec. of animals**   |
| from 2% sea water after 1 hr. loading | from 50% sea water after 1 hr. loading |
| 4975                            | 1747                            |
| 2037                            | 3842                            |
| 3174                            | 2242                            |
| 4356                            | 1900                            |
| 3346                            | 2357                            |
| Mean 3574 ± 1160               | Mean 2376 ± 500                 |

The loading medium was 5% sea water plus 20.5% sucrose labelled with $^{22}\text{Na}$. 


An increase in the rate of sodium uptake can also be elicited at blood concentrations higher than those mentioned above. Animals acclimatized to 161% sea water and then placed in deionized water for 3 hr. prior to loading showed a subsequent influx markedly in excess of that of untreated controls from 100 and 50% sea water (Fig. 4). 

![Diagram](image-url)

Fig. 4. Activation of sodium uptake in animals transferred from 161% sea water to deionized water prior to loading. A, Count of animals from 161% sea water after 1 hr. in the loading medium; B, count after 3 hr. in deionized water; C, net increase in count in second period of 1 hr. in the loading medium; D, count of controls from 50% sea water after 1 hr. in the loading medium; E, count of controls from 100% sea water after 1 hr. in the loading medium. The sodium concentrations in the blood of the experimental animals after the second period of loading were equivalent to: 97% (2 pooled), 90%, 96% (2 pooled) and 87% sea-water sodium. The loading medium was 5% sea water plus 20.5% sucrose.

However, when animals from 161% sea water have their blood concentration lowered by being placed in 50% sea water instead of deionized water they show no increase in influx on subsequent loading. The influx measured following 4 hr. in the 50% sea water is similar to that of controls initially acclimatized to 50% sea water. Additional control animals used in this experiment, which had been acclimatized to 100% sea water and then put into deionized water for 4 hr. prior to loading, showed the high influx to be expected from the previous results (Fig. 5). These results are comparable with those obtained in studies on the control of urine concentration in *G. duebeni* (Lockwood, 1961), where it was found that animals changed from producing isosmotic urine to producing hypotonic urine some 2 hr. after they were transferred from 110% sea water (or more saline medium) to fresh water, but did not do so if transferred from higher concentrations (150–175% sea water) to 50% sea water. Furthermore, if the animals are transferred from a similar high concentration to a sucrose solution isosmotic with 50% sea water for a few hours, there is no increase in influx of sodium when they are subsequently placed in the loading medium (Fig. 5).
Likewise animals transferred from a high salinity into a sucrose solution isosmotic with 50% sea water do not produce hypotonic urine (Fig. 6).

An increase in the influx of sodium is not itself proof of an increase in the net uptake of sodium even if the same medium is used for uptake, since it is just conceivable that the prior treatment of the animals might affect the rate of the ion-exchange diffusion component of the total flux. However, study of the total sodium in the animal during an experiment of a similar type indicates that the changes in the fluxes are a measure of the net sodium movements. The animals were divided into two batches and acclimatized for 3 days to 116% and 56% sea water to which $^{22}$Na had been added. The specific activity was the same in both media. The half-time for sodium exchange in these media at room temperature (c. 18°C.) is between 5 and 8 hr. (unpublished results) so in 3 days exchange should be more than 99% complete. After loading, the animals were counted and those from 116% sea water were transferred to deionized water. The controls were replaced in their 56% sea-water medium. After 8 hr. in deionized water the experimental animals were re-counted and then placed in 5% sea water (the original loading medium diluted) plus enough sucrose to make the medium

![Graph](attachment:image.png)
osmotically equivalent to 95% sea water. The controls were also put in this medium. After 3 hr. both sets of animals were re-counted. The experimental animals all showed a rise in count above the level found after the régime in deionized water. The controls, on the other hand, showed a slight fall (Table 2). Determination of the sodium concentration of the blood at the end of the experiment indicated that the concentration was higher in the experimentals than in the controls. Since the specific activity is the same in the animals and the medium during loading the observed changes in the animals' count are a measure of corresponding changes in their total sodium content.

![Osmotic pressures (as mm./l. NaCl) of blood and urine after transfer from a highly saline medium to 17.1% sucrose. Blood: solid circles and rings; urine: solid and hollow triangles.](image)

In a supplementary experiment the events subsequent to activation of the uptake system were followed for a longer period. An animal was loaded to a steady state in 150% sea water plus ^{22}Na (50 hr.) and then washed with deionized water until its count had fallen to 40% of the initial value. It was then transferred to a loading solution consisting of 1 part of the original medium plus 19 parts of a solution of 17.1 g. of sucrose in 100 c.c. of distilled water. After 3 hr. in this medium the count was found to have risen some 30% above the level at the end of the time in deionized water. After 10 hr. the increase in count was still maintained but after 22 hr. it was declining (Fig. 7). At this time the animal was transferred to 50% sea water (made by diluting
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the original tracer-labelled 150% sea-water medium). The count continued to fall over the next 48 hr., despite the nearly sevenfold increase in the sodium concentration of the medium.

Table 2. Net uptake of sodium by animals initially acclimatized to 116% sea water and then exposed to deionized water for 8 hr. prior to being put into the loading medium

<table>
<thead>
<tr>
<th>Animals from 116% sea water</th>
<th>Count after 8 hr. in deionized water</th>
<th>Count after loading</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21,700</td>
<td>8,490</td>
<td>11,370 (3 hr.)</td>
<td>+34</td>
</tr>
<tr>
<td>22,020</td>
<td>11,198</td>
<td>12,310 (2½ hr.)</td>
<td>+10</td>
</tr>
<tr>
<td>23,030</td>
<td>12,780</td>
<td>13,050 (2 hr.)</td>
<td>+2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Controls from 56% sea water</th>
<th>Count after 8 hr. in deionized water</th>
<th>Count after loading</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4,720</td>
<td>—</td>
<td>4,630 (3 hr.)</td>
<td>-7.7</td>
</tr>
<tr>
<td>15,690</td>
<td>—</td>
<td>14,370 (3½ hr.)</td>
<td>-9.0</td>
</tr>
<tr>
<td>4,750</td>
<td>—</td>
<td>3,790 (3½ hr.)</td>
<td>-20.0</td>
</tr>
<tr>
<td>9,390</td>
<td>—</td>
<td>8,910 (3½ hr.)</td>
<td>-5.0</td>
</tr>
<tr>
<td>5,310</td>
<td>—</td>
<td>5,080 (3½ hr.)</td>
<td>-4.0</td>
</tr>
</tbody>
</table>

Sodium concentration of the blood after loading:

<table>
<thead>
<tr>
<th>Sea water (%)</th>
<th>Experimental animals</th>
<th>Controls from 56% sea water, pooled blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>78</td>
<td>75.3</td>
<td>54</td>
</tr>
<tr>
<td>75.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Loading medium was 5% sea water with the same specific activity as the original acclimatization media, plus 17.1% sucrose.

A repetition of this experiment gave a similar result though with a smaller increase in the count whilst in the loading medium subsequent to washing out. The later decline in count occurred as before.
On the basis of experiments carried out on freshwater crustacea it has come to be accepted that the rate of active transport of sodium is related to the concentration of the blood provided that the external concentration is adequate to saturate the transporting sites (Shaw, 1959a, b; Shaw & Sutcliffe, 1961; Bryan, 1960; Lockwood, 1960. If the blood concentration falls below the normal level the rate of active uptake is increased above the usual maintenance rate but gradually returns to normal as the loss is made good. The experiments described in this paper indicate, however, that the concentration of the blood cannot be the only factor influencing the rate of active uptake of sodium. By acclimatizing Gammarus duebeni to a high salinity (100-161% Plymouth sea water) and then lowering the blood concentration in deionized water a situation has been created in which animals with a higher blood concentration than control animals nevertheless show an influx of sodium several times greater than that of the controls when both sets of animals are subsequently placed in the same loading medium. The mechanism responsible for activation of the system transporting sodium must, in such circumstances, be responding to some factor other than the absolute concentration of the blood. A feature of the activation by treatment with deionized water is the extent of the effect. It might be expected that animals maintaining themselves very hypertonic to their medium, as do those acclimatized to 2% sea water, would have a very much greater influx of sodium than animals only slightly hypertonic, e.g. those acclimatized to 50% sea water. Measurements of the influx into animals from these concentrations in an identical loading medium indicate that the uptake by animals from 2% sea water is some 50% higher than that by animals from 50% sea water. Such a difference is, however, small in comparison with the influx into animals from high salinities after treatment with distilled water for a few hours. This influx may be as much as 300-400% higher than the influx into controls from 50-66% sea water.

Rapid loss of sodium from the body may be expected to result in a faster dilution of the blood than can be matched by adjustment of cellular osmotic concentration. Water is therefore likely to pass by osmosis from the blood to the cells. A possible interpretation of the observation that the fall in total body sodium is more rapid than that of the sodium concentration of the blood, when animals are placed in deionized water, is that such water shifts do occur. Factors, other than the blood concentration, to which monitoring systems ultimately responsible for determining the rate of transport might be responding could therefore include changes in the cell volume of certain cells or variations in the blood volume. Precedents for both such mechanisms are known in the vertebrates. Verney (1947) found that injections of small amounts of isosmotic solutions of sucrose and of NaCl into the internal carotid artery of dogs had almost equal effects in stimulating the output of the anti-diuretic hormone. He concluded that the receptor cells were responding to variations in their volume resulting from changes in the osmotic balance across their surface. More recent evidence, reviewed by Farrell & Taylor (1962), suggests that stretch receptors in the auricles and great veins may detect changes in blood volume and initiate appropriate adjustments to the release of ADH and the glomerulotrophic hormones from the brain. However, though comparable systems may be present in the crustacea, one of the experiments which has
been described suggests that they are not of critical importance in the present circumstances. It was found that animals transferred from 100% sea water to deionized water for 4 hr. showed a higher influx when later put into the loading medium. Animals removed from 161% sea water into 50% sea water or sucrose solution isosmotic with 50% sea water showed no such increase. It seems therefore that the extent of the gradient between blood and medium at the start of the rapid drop in blood concentration is of no importance in determining whether or not there will be an increase in the rate of sodium uptake. This gradient would be expected to determine the rate of fall in blood concentration. It would also determine the rate of cellular swelling and the rate of decrease in blood volume if the cells were unable to adjust their internal osmotic pressure sufficiently rapidly to match the change in blood concentration. It thus seems likely that none of these factors is the one monitored prior to the change in rate of sodium uptake. Another factor which might be monitored is the concentration of the medium. Lagerspetz & Mattila (1961) have shown that species of \textit{Gammarus} are able to select a suitable salinity when placed in a salinity gradient. They may therefore be presumed to have the ability to detect the concentration of the medium. Whether or not the sense organs responsible for detecting the concentration of the medium are able to form the basis of the system regulating changes in the rate of active uptake of sodium remains to be proved. The present experiments do not rule out such a possibility.

The analogy between the control of the production of hypotonic urine (Lockwood, 1961) and of the influx of sodium is striking. Both are stimulated by the transfer of the animals from a high concentration to deionized water for a few hours but neither system is activated by transfer from even higher concentrations to 50% sea water or to sucrose isosmotic with 50% sea water. As the production of hypotonic urine may be expected to involve the active withdrawal of sodium from the tubule of the antennary gland it seems possible that both hypotonic urine production and the rate of active transport at the body surface are controlled by the same mechanism.

The means by which the control of the effector cells responsible for sodium transport is achieved has not been specifically investigated, but two observations suggest that a humoral mechanism may be involved: (1) The uptake of sodium from the loading medium goes on for many hours after the stimulus causing the increased uptake (deionized water) has been removed. Such a time lag after the removal of the stimulus would seem a priori unlikely if control of uptake were directly mediated by the nervous system but more probable if a hormone were responsible. The latter could be expected to act for some hours, especially if the high concentration of sucrose in the medium slowed its elimination by diminishing the rate of urine production. (2) There is a decline in the total body sodium some hours after the initial increase caused by activation of the uptake mechanism. This result would be compatible with the eventual slow removal of a hormone. Nevertheless, it must be stressed that though these points are suggestive neither represents proof of hormonal control of ionic regulation.

**SUMMARY**

1. Some factors responsible for eliciting an increase in the rate of active uptake of sodium by \textit{Gammarus duebeni} have been studied.
2. Animals previously acclimatized to high salinities (100–161% sea water) had their blood concentration suddenly lowered by treatment with deionized water to a level similar to, but a little above, that of animals kept in 50–66% sea water. Both groups were placed in the same tracer medium, i.e. 5% sea water labelled with \(^{22}\)Na and with sucrose added. The animals treated with deionized water showed an influx, on average, of 4 times that of the controls from 50 to 66% sea water.

3. No increase in influx followed treatment of animals from 161% sea water with 50% sea water or with sucrose solution isosmotic with 50% sea water, despite the fact that the osmotic gradient between 161 and 50% sea water is greater than the gradient between 100% sea water and deionized water.

4. It is concluded that in these experiments the rate of uptake is not influenced primarily by the absolute concentration of the blood, the rate of change of blood concentration, the rate of swelling of the tissues or the extent of the blood volume.

5. The possibility is considered that both the concentration of the urine and the rate of uptake of sodium may, in some circumstances, be controlled by an extero-receptor which monitors the concentration of the medium and mediates its effect via a humoral system.

I would like to thank the Director of the Marine Laboratory and Dr G. W. Bryan for the use of facilities and for their help during my visit to Plymouth in October 1962. Part of this work was supported by a Research Equipment Grant from the Department of Scientific and Industrial Research.

REFERENCES


