

THE RELATIVE LOSSES OF SODIUM IN THE URINE AND ACROSS THE BODY SURFACE IN THE AMPHIPOD, *GAMMARUS DUEBENI*

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The suggestion has previously been made (Lockwood, 1961) that the rapid change from isotonic to hypotonic urine production which occurs when *Gammarus duebeni* is transferred from a high concentration (100-175 % sea water) to fresh water may subserve the function of slowing the loss of ions from the body. Any such slowing would decrease the rate of fall of the blood concentration and hence allow additional time for the necessary concomitant changes in cellular osmotic pressure to be brought about. The ability to slow the rate of ion loss for this purpose might be of functional significance to this species as it is often found in environments such as salt-marsh pools which may be subject to sudden and extensive variations of salinity.

If a change in urine concentration is to have any substantial effect on the rate of ion loss from the body the loss across the body surface must represent only a small part of the total loss. Potts (1954), when calculating the minimum thermodynamic energy that must be expended on osmoregulation by various animals, made the assumption that the body surfaces were semi-permeable and hence that all the salt leaving the body was in the urine. If this were the case in *G. duebeni* then the switch from isotonic to hypotonic urine on dilution of the medium would clearly exert a marked effect on the rate of sodium loss. However, Shaw (1959*a*) has pointed out that in all three of the Crustacea for which detailed information is available the urinary losses form only a small part of the total loss. Thus in *Eriocheir sinensis* the surface salt loss accounts for 86 % of the total (Krogh, 1938), and in *Austropotamobius* (*Astacus*) surface loss is 90 % of the total (Shaw, 1959*b*) or 92 % Bryan (1960*a*). Blocking the excretory pores of the freshwater crab, *Potamon niloticus*, made no apparent difference to the salt loss over a 3 hr. period indicating that in this animal too the major part of the loss is via the surface (Shaw, 1959*a*). Both *Potamon* and *Eriocheir* produce urine isotonic with the blood, but the surface loss forms such a large part of their total loss that even if these animals were to produce hypotonic urine it would contribute little to decreasing the sodium loss from the body. If *G. duebeni* were to have a surface to excretory loss ratio as high as that in these three forms then clearly changes in the concentration of the urine could have only a comparatively small effect on the rate of loss from the body. In this case the hypothesis suggested in Lockwood (1961) would be untenable.

This paper describes experiments undertaken to determine the relative salt losses via the body surface and excretory organ in *G. duebeni*. It will be shown that when this animal is producing urine isotonic with the blood it more closely approximates

to the semi-permeable condition envisaged by Potts than to the condition of the decapods mentioned above.

MATERIALS AND METHODS

The *G. duebeni* used in these experiments have been obtained from brackish water sites at the estuary of the River Stour, Suffolk, at Plymouth and at the estuary of the River Test, Hampshire. Prior to use they have been kept for days or weeks in the laboratory in *c.* 20% sea water, and fed on a mixture of *Enteromorpha* and 'Bernax'. Survival is good, the animals breeding in captivity and appearing normal in every way.

The analytical methods used were the same as those described in Lockwood (1961) with the addition that during some experiments a well-type scintillator was used for counting ^{22}Na instead of an end-window Geiger-Muller tube, and a Unicam S.P. 900 flame-spectrophotometer has been used for certain of the sodium determinations.

RESULTS

There are two types of evidence which suggest that the loss of sodium in the urine accounts for the major part of the loss of this ion from the body in conditions where the urine is isotonic with the blood. (1) Direct determination indicates that the loss of sodium from the head and first thoracic segment (and thus including the openings of the excretory organs) is markedly greater than that from the remainder of the body. Such a result could conceivably have been found if losses from the mouth and head surface were large, but the major participation of the urine in the loss is confirmed by the observation (2) that the loss of sodium is very small when the animal is bathed by a sucrose solution isotonic with the blood by comparison with that when it is bathed by deionized water. The gradient down which sodium diffuses from the body would not be expected to differ whether the animal was in sucrose or in deionized water, but the volume of urine produced in the former medium would be small.

(1) *Direct comparison of urinary and surface sodium loss*

The animals were acclimatized to 150% sea water to which ^{22}Na had been added. A small hole was then burnt in a rubber membrane and an animal was positioned through this so that it was gripped in the region of the first or second thoracic side plate. The head and excretory openings were thus on one side of the membrane and the gills and remainder of the thoracic and abdominal body surface on the other. The head was placed in a polythene tube containing 2 c.c. of deionized water and 1 g. of Amberlite Monobed III mixed cation-anion exchange resin; and the posterior part of the body was placed in a tube similarly filled. After 2 hr. the animal was removed and the tracer was eluted from the resin in each tube with an excess of N/1 HCl. The HCl was evaporated to dryness and the eluted tracer was counted. Five such experiments gave the results listed in Table 1. On average the loss in the urine is some 4 times as fast as that across the body surface thus suggesting that the major part of the sodium loss is in the urine. On the basis of previous results (Lockwood, 1961) the urine would be expected to remain isotonic with the blood throughout the course of this experiment.

Table 1. *Direct determination of the ratio of sodium loss in the urine to sodium loss across the body surface*

	Urine loss Surface loss
1	4.4
2	5.0
3	4.7
4	4.3
5	1.8

$$\bar{m} = 4.05 \pm 1.28$$

(2) *Loss to isotonic sucrose*

The animals used were acclimatized as before to 150% sea water to which ^{22}Na had been added. When equilibration was complete they were transferred into a sucrose solution (25 g. in 50 c.c.) having a molality close to that of 150% sea water. A small chamber containing the animal was placed immediately below a Geiger tube so that a continuous record could be kept of the tracer present in its body. The sucrose solution was circulated through the chamber and then passed down a column of Amberlite resin to remove any sodium before being again passed through the chamber. After some hours the sucrose solution was replaced by deionized water and the rate of loss of sodium to this medium was also recorded.

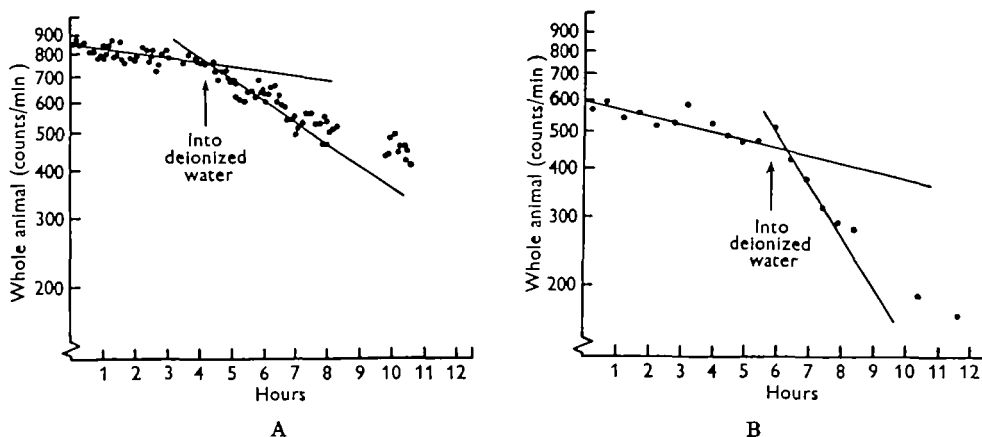


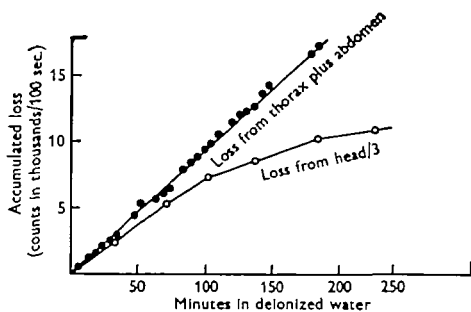
Fig. 1 A, B. The rate of loss of ^{22}Na from two animals initially acclimatized to labelled 150% sea water and then transferred first to sucrose approximately isotonic with 150% sea water and subsequently to deionized water. The solid lines indicate the average rate of loss in sucrose and the initial rate of loss to deionized water.

The results of two such experiments, illustrated in Fig. 1, indicate that the loss of sodium is some four to five times faster to the deionized water than to the sucrose solution. Urine production would be expected to be minimal in the sucrose solution as this is approximately isotonic with the body fluids of the animal at the start of the experiment. The fact that the total loss is very greatly increased in deionized water therefore suggests that the loss of sodium in the urine constitutes a large proportion of the total loss in these circumstances. It should be noted that in the conditions

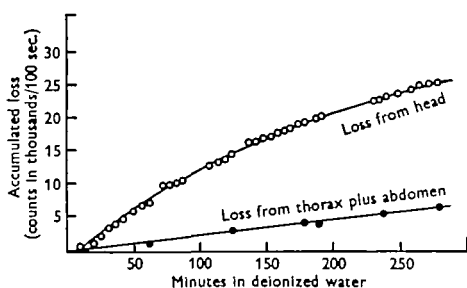
under which the experiment was performed the urine will be isotonic with the blood initially though it would be expected to become hypotonic some 2 hr. after transfer to the deionized water. This change in urine concentration may in part contribute to the decrease in the rate of loss to deionized water which occurs in the later part of the experiment.

The effect of changes in urine concentration on the total loss rate

A modification of the first technique was used to study the changes in the rate of total loss under conditions where the concentration of the urine is changing. The tracer-labelled animals were placed in membranes, as before, and a continuous stream of deionized water was circulated past the region of the body on one side of the membrane. The part of the body on the other side was placed in a tube containing deionized water and Monobed III resin. The effluent from the circulated side was taken to the base of a column of the resin mounted in the well of a scintillation counter. By counting the gamma activity of this column a continuous record was obtained of the rate of loss of sodium from that region of the animal. Periodically the static tube was detached and counted in order to give a measure of the loss from the other side of the membrane. In successive experiments the anterior and posterior parts of the animal were exposed to the circulated water; the results, however, were similar whichever side was irrigated.



A



B

Fig. 2A. Loss of ^{22}Na from the head region and from the thorax plus abdomen of animals, previously acclimatized to labelled 100% sea water, during washing with deionized water (see text).

Fig. 2B. Loss from the head region and from the thorax plus abdomen of an animal, previously acclimatized to labelled 120% sea water, during washing with deionized water.

The rate of loss of sodium from the thoracic and abdominal regions was found to remain relatively constant for many hours when the animals were transferred from 100 to 125% sea water to deionized water, thus implying that there is little or no decrease in the permeability of the surface to sodium after transfer to a dilute medium. The loss from the head region on the other hand declined markedly with time. The results of two such experiments are illustrated in Fig. 2A, B.

In the experiment shown in Fig. 2A the loss from the head region, immediately following transfer from 100% sea water to deionized water, accounted for some 70%

of the total sodium loss from the body. This proportion remained almost constant for the first 100 min. of the experiment but subsequently there was a fairly rapid decline in the rate of loss from the head region. As a result, the loss from the head region after $3\frac{1}{2}$ hr, was only some 20 % of the initial level and its proportion of the total loss had declined to 33 %. In the second experiment (Fig. 2B) the rate of loss from the head was down to 44 % of its original value after 4 hr., and its proportion of the total loss at this time was down to half the initial level. Again the rate of loss from the thorax and abdomen did not change appreciably.

The influx of sodium at various blood concentrations

As a large part of the sodium loss is in the urine the animal's ability to control the urine concentration makes it possible for it to regulate the rate of sodium loss from the body. Consequently, no very large increase in the rate of uptake of sodium at the body surface is necessary in order to support the increasing gradient between blood and medium if the latter is diluted. This has been shown as follows.

The influx of sodium into animals previously acclimatized for 5 days to media ranging in concentration from 2 to 50 % sea water has been tested by placing them in 2 % sea water labelled with ^{22}Na to which enough sucrose has been added (17.1 g. in 100 c.c.) to make the medium approximately isotonic with the blood. The animal's count rate was observed after an exposure of 1 hr. to the medium at 15° C. This particular medium was chosen because 2 % sea water contains more sodium than the level required to saturate the transporting sites of *G. duebeni* (Shaw & Sutcliffe, 1961), but is not so concentrated that ion exchange diffusion constitutes any appreciable part of the influx (see below). Since the same loading solution is used for all animals, differences in the influx can be assumed to be due to differences in the rate of active uptake of sodium. The presence of the sucrose ensures that there is no rapid change in blood concentration during the loading period. Hence the rate of uptake observed can be related to the rate of uptake from the acclimatization medium immediately prior to loading.

Marked individual variation is observed in the influxes, particularly in the case of animals from the more dilute media. The average influx of those animals previously acclimatized to 10, 15, 25, 30 and 40 % sea water is not, however, very different from that of animals from 50 % sea water. The animals from 2 % sea water have an average influx about twice that of the animals from 50 % sea water (Fig. 3A, B). This difference is nevertheless very small in comparison with the eightfold difference in the concentration gradient between blood and medium of animals in 2 % sea water as compared with animals in 50 % sea water. (The difference in sodium concentration is about 30 mM./l. when the animals are in 50 % sea water and 250 mM./l. when they are in 2 % sea water (Lockwood, 1964).

The possibility must be considered that the technique imposes some limit on the rate of uptake thus causing the general similarity in the rates of uptake by animals from widely differing media. It seems, however, that this suggestion is unlikely to be true in view of the exceptionally high rates of uptake shown by two individuals from 2 % sea water (Fig. 3A).

Abnormally high rates of uptake have been found to occur in animals which are within a few hours of ecdysis; but the high count of these two individuals could also

be accounted for if, for some reason, they were not forming a urine as dilute as that appropriate to the medium. Considerable variation was in fact found in the urine concentrations at any one salinity (Lockwood, 1961).

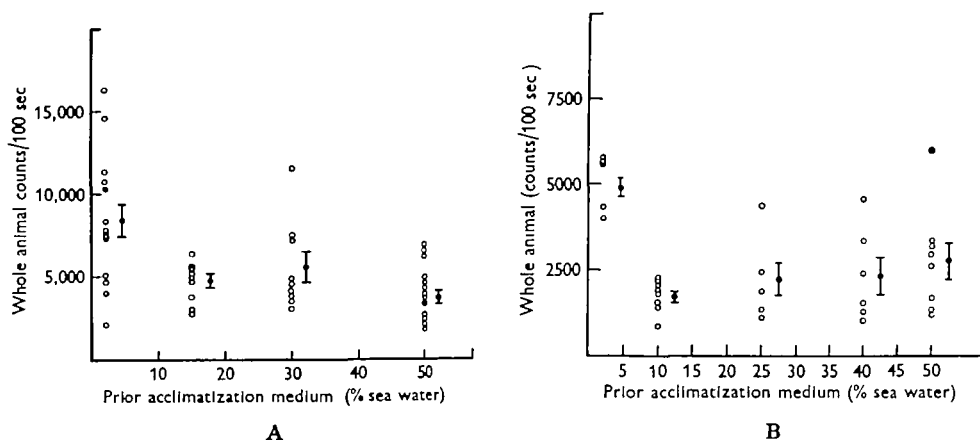


Fig. 3 A, B. The influx of ^{22}Na from a solution of labelled 2% sea water containing 17.1 g. sucrose in 100 c.c. by animals previously acclimatized to various salinities in the range 2% to 50% sea water. The solid circle and vertical lines indicate the mean and standard error of the mean of the points immediately to their left.

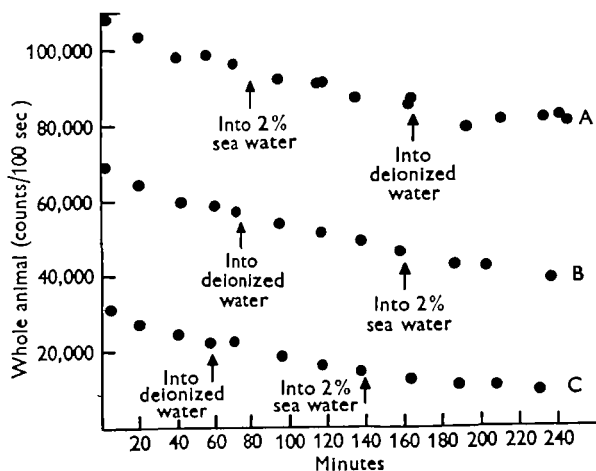


Fig. 4. The loss of ^{22}Na from three animals placed successively into 2% sea water and deionized water.

Tests for interference by ion exchange diffusion

The assumption made above, that the rate of active uptake of sodium by animals acclimatized to 2% sea water is, on average, about twice that of animals acclimatized to 50% sea water, is only valid provided that it can be shown that ion-exchange diffusion makes a negligible contribution to the influx when the animals are being loaded in 2% sea water. The rate of efflux of sodium from individuals initially acclimatized to sodium-labelled 2% sea water and then successively washed with unlabelled 2% sea water and deionized water has been measured (Fig. 4).

The period in deionized water was kept short so as to limit the net loss of ions from the body. The loss was therefore probably largely recovered during the periods in 2% sea water. Each successive washing can therefore be regarded as a separate experiment. Direct comparison of the rates of loss in deionized water and in 2% sea water indicate that there is no gross difference between the two (Fig. 5).

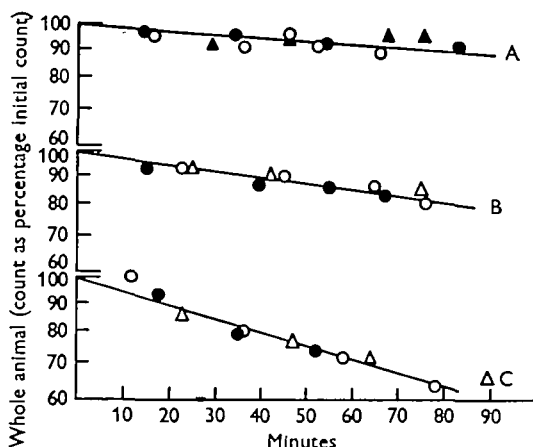


Fig. 5. Comparison of the rates of loss of tracer sodium in deionized water and in 2% sea water from the animals shown in Fig. 4. \blacktriangle , \triangle : periods in deionized water; \circ , \bullet : periods in 2% sea water.

Ion exchange diffusion or increased loss during active uptake, such as that observed by Bryan (1960b) on the crayfish, may hence be presumed to account for only a small part of the sodium exchange. The influxes observed in previous experiments can therefore be directly equated with the rate of active uptake of sodium.

DISCUSSION

The experiments described above indicate that when *Gammarus duebeni* is forming urine isotonic with the blood only some 20% of the total sodium loss from the body takes place across the general body surface. In this respect it differs from most other Crustacea which have been studied. Some 86% of the salt loss from *Eriocheir sinensis* (Krogh, 1938) and over 99% of the loss from *Potamon niloticus* (Shaw, 1959a) occurs across the body surface, even though the urine is always isotonic with the blood in these two crabs. Such forms would effect little in the way of conservation of salt by producing urine hypotonic to the blood. *G. duebeni*, on the other hand, is able to exert a considerable measure of regulation of the rate at which sodium is lost from the body by controlling the concentration of the urine. This animal maintains an increasing gradient of concentration between the blood and medium in the range of media 50% sea water to fresh water. The present experiments indicate that the rate at which sodium is taken up from the medium varies relatively little when the animals are previously acclimatized to media in the concentration range 2–50% sea water and so suggests that the reduction of the concentration of the urine over this range has the effect of keeping the total sodium losses from the body almost constant. For example, when the animals are in 2% sea water the uptake of sodium is on average

only about twice the rate present in animals in 50 % sea water, though the difference in sodium concentration between blood and medium is 250 mM./l. in the former and 30 mM./l. in the latter. Animals from 10, 15, 25, 30 and 40 % sea water all have influxes very similar to that of animals from 50 % sea water.

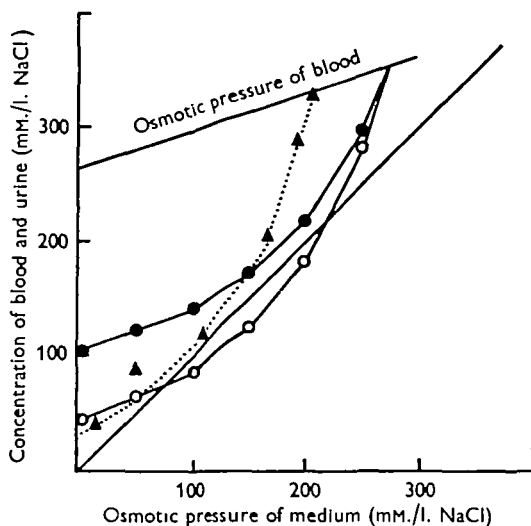


Fig. 6. The osmotic pressure of the blood and minimum observed concentrations (▲) at different salinities (from Lockwood, 1961) with superimposed curves indicating the urine concentrations necessary to keep (a) the total loss constant over the range of salinity (○), or (b) the urine loss constant over the same range (●).

If it is assumed: (1) that the urine volume is dependent on the difference of concentration between blood and medium (Werntz (1962) has shown that this is a reasonable assumption in the case of other species of *Gammarus*); and (2) that the permeability of the body surface does not change in different media, then it is possible to calculate the urine concentration necessary in various media to keep the total salt losses constant at the level found in 50 % sea water. Fig. 6 illustrates a comparison of the minimum observed urine concentrations in media of different concentrations (Lockwood, 1961) and also the calculated curves of the urine concentrations necessary if: (1) the total salt losses are to be kept at the same level as in 50 % sea water; and (2) the urinary losses are to be kept at the same level as in 50 % sea water. Naturally the construction of such curves depends on an accurate knowledge of the gradient between blood and medium in 50 % sea water. As this is somewhat variable the curves confirm only in general terms the fact that if the urine is produced at the minimum concentration actually observed then the overall loss of sodium from the body will not be very dissimilar in dilute media and in the 50 % sea water. Hence, if *G. duebeni* is able to transport ions at an adequate rate to maintain the gradient between blood and medium when it is in 50 % sea water it will also be able to maintain the gradient when in 10 % sea water provided that the energy can be produced for the dilution of the urine. Even in 2 % sea water only a doubling of the rate of uptake in 50 % sea water is required.

The ability to move from a high to a low salinity without the necessity of greatly increasing the rate of active transport at the body surface when the gradient between blood and medium is increased must be of considerable advantage to an animal living in brackish water. The likelihood of a fatal loss of salts occurring if the rate of active uptake is temporarily decreased for any reason is thereby reduced, a factor which may be of particular importance in animals which have a high water turnover. As all small animals tend to have higher rates of water turnover than larger comparable forms it would not be surprising if the systems outlined above were of fairly general occurrence amongst the species of small body size.

The fact that the rate of active uptake from the medium is not necessarily increased when *G. duebeni* moves from 50 to 10% sea water does not of course absolve the animal from performing additional osmotic work. It is not clear, however, precisely what this increase will be. Potts (1954) using the classical thermodynamic approach has given equations from which the minimum work which must be done by animals in various conditions may be calculated. He has shown that the minimum work necessary to maintain the blood concentration is less if hypotonic urine is produced than if the same volume of isotonic urine is formed and the lost ions are replaced by active transport at the body surface. The difference is large when the medium is very dilute, but some, though a lesser, advantage is achieved when the medium is of higher concentration. Croghan (1962) has, however, pointed out that the classical thermodynamic equations are not applicable to this steady-state situation, as the transporting processes are likely to be operating well away from the equilibrium position of the reactions involved. Hence the energy utilized in transport is likely to be dependent on the nature of the mechanisms transporting the ions and not on the gradient against which the ions are moved. This conclusion seems to be justified for at least some transporting systems, as Zerahn (1956*b*) has found that there is a stoichiometric relationship between the oxygen consumption and the sodium transported by the frog skin. The oxygen utilized is dependent on the amount of salt transported and not on the gradient through which it is moved. If this is also the case in *Gammarus* and if the transporting mechanisms are the same in the excretory organ and at the body surface then no energetic advantage can be derived from the production of dilute urine. Any advantage in such a case would be limited to the greater inherent safety of a system which tends to conserve ions already in the body. However, study of the number of sodium ions transported per molecule of oxygen utilized in a variety of vertebrate tissues indicates that some of those which normally transport sodium across a small gradient utilize less oxygen in moving a given number of sodium ions than do those transporting sodium across a large gradient. Thus in the dog kidney on average 28.5 sodium ions are transported for each molecule of O_2 utilized (Thaysen, Larsen & Munck, 1961), whilst the frog skin and toad bladder only transport 16–20 sodium ions per molecule of O_2 (Zerahn, 1956*a, b*; Leaf & Renshaw, 1959). If there were a comparable difference between the O_2 utilization and sodium transported at the body surface and in the excretory organ in *G. duebeni* then it is apparent that the system producing hypotonic urine might after all be potentially capable of conferring an energetic advantage by comparison with a system recovering lost ions from the medium. This might offer an explanation of the observation by Suomalainen (1956) that *G. duebeni* acclimatized to various salinities in the range 2.8% sea water to 57%

sea water (0.1–2 % salinity) show little difference in their rates of O_2 consumption despite the big increase in concentration gradient between blood and medium over this range. The mean rates of uptake were 0.1 c.c./g. animal/hr. at these two extremes and 0.08 c.c./g. animal/hr. in 11.5 and 20 % sea water. Further speculation on the possible energy relations of transport at this time would be premature especially as Whittam & Willis (1963), reviewing the literature on the relationship between sodium transport and O_2 utilization, point out that a number of tissues such as muscle and nerve have much lower Na/O_2 ratios than those already cited.

The means by which the division of labour between the transporting sites at the body surface and in the excretory organ is controlled poses an intriguing problem. The same stimuli result in production of hypotonic urine and increase in the rate of active uptake in *G. duebeni* (Lockwood, 1961, 1964) suggesting that both systems are controlled by a common mechanism. On the other hand, it would seem likely from the present work that if the urine is very hypotonic when the animal is in a dilute medium, then little increase is necessary in the rate of uptake at the body surface. Conversely, if the urine is not very dilute a faster rate of pumping will be required at the surface in order to maintain the gradient between blood and medium. The reciprocal nature of this effect would seem to imply that independent control of kidney operation and transport at the body surface is also possible.

SUMMARY

1. The relative contributions of urine production and diffusion across the body surface to the loss of sodium from the body of the amphipod *Gammarus duebeni* have been investigated.
2. When the urine is isotonic to the blood some 80 % of the total sodium loss is via the urine.
3. As the gradient between blood and medium is increased in dilute media production of urine hypotonic to the blood counteracts the tendency for sodium loss to increase.
4. In consequence, the average rate of sodium uptake at the body surface by animals acclimatized to 2 % sea water needs to be only about twice that of animals acclimatized to 50 % sea water.
5. It is suggested that the conservation of ions within the body by the production of hypotonic urine is likely to be found to be a common feature of the smaller brackish water crustacea, especially those with a high rate of water turnover.

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