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WATER UPTAKE AND LOSS IN RELATION TO THE SALINITY OF THE MEDIUM IN THE AMPHIPOD CRUSTACEAN *GAMMARUS DUEBENI*

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INTRODUCTION

Except in the case of certain prawns, mysids, isopods and grapsoid crabs the osmotic gradient between the body fluids and the medium is very small in the majority of marine invertebrates so far studied. Specific means may therefore have to be employed by such almost isosmotic forms in order to take in the water which they subsequently excrete as urine. Burger (1957) has shown that anuric lobsters can be made to start producing urine again after injection of serum, and has postulated that blood colloid osmotic pressure is of importance in contributing to fluid uptake. Another alternative, adopted particularly by forms such as *Neomysis integer* and *Palaemon serratus* which are hypotonic to sea water, is to drink the medium and take up fluid and ions from the gut (Ralph, 1965; Parry, 1955). *Carcinus* too drinks the medium at moult and absorbs the fluid to increase its blood volume (Robertson, 1960).

Webb (1940) has suggested that fluid uptake by some isosmotic marine forms might be associated with an inward transport of ions, and support for this concept has been provided by experiments which show that if *Gammarus duebeni* moults in sea water the rate of active uptake of sodium rises some 20- to 40-fold during the period around the time of ecdysis when the principal intake of water occurs. This uptake of ions is not associated with drinking (Lockwood & Andrews, 1970). Intermoult animals of the same species have also been shown to increase their rate of active uptake of sodium when the blood volume is depleted in individuals acclimatized to sea water (Lockwood, 1970).

Smith (1970) has found that osmotic entry of water can account for the observed urine production by *Carcinus* when the animal is in 50–70% sea water; but in sea water, in the absence of an overt osmotic gradient, some other mechanism must be present to bring water into the body. This he also suggests could involve some form of water movement related to the transport of inorganic ions.

The present work has been undertaken to examine urine flow rates in relation to the water fluxes, osmotic gradient between blood and medium, and ion fluxes, with a view to establishing whether these parameters are compatible with the hypothesis that some of the fluid excreted as urine is taken into the body initially by a process dependent upon the active uptake of inorganic ions.

MATERIALS AND METHODS

Gammarus duebeni were collected from the salt marsh at Redbridge on the River Test and were maintained in the laboratory prior to use in 50% sea water. They were fed on a diet of 'Bemax' and *Enteromorpha*.

Water-flux measurements

Water fluxes across the body surface were measured with tritiated water obtained from the Radiochemical Centre, Amersham. During the course of experiments the vessels containing the animals were sealed, except when sampling, in order to minimize loss of specific activity by exchange of tritiated water with water vapour in the air.

Tritiated water samples were counted by means of a dioxane-based phosphor using Panax scintillation and scaling equipment.

Tests indicated that, over the salinity range used, there was no significant difference in quench values for the various media.

Water flux, as a percentage of total body water exchanged per minute, was calculated from the expression

$$100\,\frac{\ln 2}{t_{\frac{1}{2}}},$$

where $t_{\frac{1}{2}}$ is the time for half exchange. Putative osmotic water flow, when a concentration gradient was present between the blood and medium, was determined from the flux data on the basis of the difference in mole fraction of water.

$$\frac{M_m - M_a}{M_m} F = \mathrm{Os},$$

where M_m is the mole fraction of water in the medium, M_a is the mole fraction in the blood, F is the water flux and Os is the net water flow.

Counting of ^{131}I-labelled sodium diatrizoate and ^{14}C-labelled mannitol

Measurements of blood volume and urine production rate were made using the metabolically inert compound sodium diatrizoate (sodium 3,5-diacetyl-2,4,6-triiodobenzoate), which was obtained with the iodine as ^{131}I from the Radio Chemical Centre. ^{131}I counts were made by means of a standard well-type solid scintillation system.

Estimations of ^{14}C activity were made by means of a Tracer Lab. gas-flow proportional counter.

Osmotic pressure measurements

The micro-cryoscopic method of Ramsay & Brown (1955) was used for all determinations of the osmotic pressure of blood and medium samples. Measurements were made in either duplicate or triplicate on each sample.

All experiments were made at a temperature of 18 ± 1 °C.

The 100% sea water from which other dilutions were made had a salinity of 33 ± 1‰.

Table 1. *The hypertonicity of the blood of* Gammarus duebeni *acclimatized to* 100% *sea water*

(Each value is based on the mean of at least two measurements of blood concentration and two of the concentration of the medium.)

Animal nos.	[Blood] – [Medium] (Δ °C)	[Blood] – [Medium] (m-osmoles)
1	0·000	0·00
2	0·015	8·06
3	0·017	9·14
4	0·000	0·00
5	0·005	2·69
6	0·048	25·81
7	0·045	24·19
8	0·032	17·20
9	0·003	1·61
10	0·017	9·14
11	0·000	0·00
12	0·007	3·76
13	0·013	6·99
14	0·012	6·45
15	0·010	5·37
16	0·002	1·07
17	0·023	12·37
18	0·025	13·44
19	0·006	3·22
20	0·008	4·30
21	0·010	5·37
		7·63 ± 7·39

RESULTS

Osmotic pressure of the blood

Any attempt to elucidate the means by which water is caused to enter marine organisms demands an accurate knowledge of the osmotic relationship between blood and medium. Duplicate measurements of the osmotic pressure of the haemolymph of *Gammarus duebeni* after full acclimatization (at least 1 week) to 100% sea water suggest that the blood of the majority of individuals is maintained at a level slightly hypertonic to sea water. Of the 21 specimens studied 16 had blood less than 10 m-osmoles hypertonic to the medium and 11 individuals were less than 5 m-osmoles hypertonic. By contrast, three of the remaining five animals had blood more than 18 m-osmoles hypertonic to the medium, thus raising the mean (Table 1).

The sea-water acclimatization medium had a concentration somewhat in excess of 1000 m-osmoles so it appears that the majority of a population of *G. duebeni* acclimatized to this medium will be hypertonic to an extent of less than 1% of the concentration of the medium. It should be recognized, however, that even this small degree of apparent hypertonicity may perhaps exaggerate the true situation since the fact that the animals were blotted dry before sampling could have resulted in some evaporative water loss and hence minor concentration increase. Furthermore, rupture of blood cells could conceivably have added autolysis products to the blood.

Values for the blood osmotic pressure compiled from our measurements and those of Beadle and Cragg (1940) and of Haywood (1970) for sea water and other salinities are given in Fig. 1.

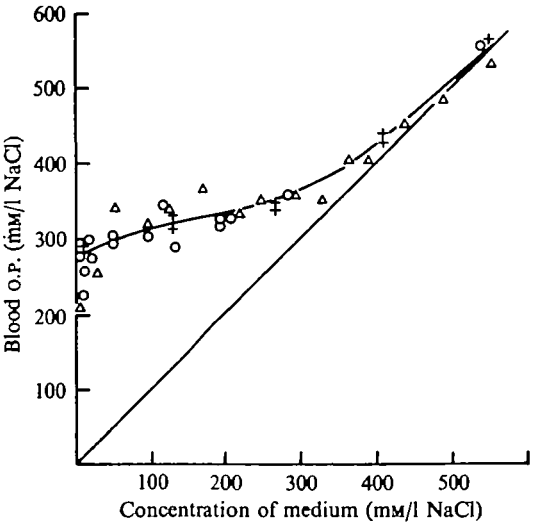

Fig. 1. The relationship between the osmotic pressure of the blood and the concentration of the medium in *Gammarus duebeni*. +, Beadle & Cragg (1940); △, Heywood (1970); ○, Lockwood (1961).

Table 2. *Presumptive urine flow rate based on water flux and osmotic gradient measurements in different media*

Medium	Osmotic gradient (m-osmoles)	$t_{\frac{1}{2}}$ outflux (min)	N	Flux/ day	Net flow (% body water/day)
2% sea water	540	$16{\cdot}8 \pm 2{\cdot}5$	16	5940	57·1
40% sea water	250	$12{\cdot}8 \pm 1{\cdot}5$	12	7827	34·8
100% sea water	10	$7{\cdot}3 \pm 1{\cdot}4$	16	13670	2·4

Calculated net water intake by osmosis in various media

Batches of animals which had been acclimatized for 1 week to 100%, 40% and 2% sea water were loaded to a steady state in ^3H_2O media of the same salinity as their acclimatization medium. They were then rinsed briefly in unlabelled medium, blotted to remove superficial fluid, and transferred to 10 ml of medium of the same concentration. Fifty μl samples of medium were withdrawn at intervals for counting, and a curve of activity against time was plotted from which the half-time of exchange could be read. The half-times for outflux observed in these three media were: 100% sea water, $7{\cdot}3 \pm 1{\cdot}4$ min, $N = 16$; 40% sea water, $12{\cdot}8 \pm 1{\cdot}5$ min, $N = 12$; 2% sea water, $16{\cdot}8 \pm 2{\cdot}5$ min, $N = 16$. Corresponding values for other salinities are discussed in more detail by Lockwood, Inman & Courtenay (1972).

Taking the flux data given above and the osmotic gradients read from Fig. 1, the expected osmotic inflow was calculated for each medium (Table 2). (In the case of 100% sea-water animals the osmotic gradient was arbitarily rounded up to 10 m-osmoles instead of the observed mean of 7·6 m-osmoles.)

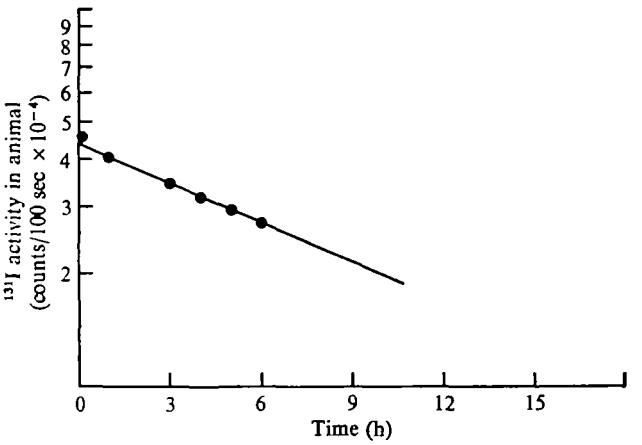

Fig. 2. Loss of ^{131}I-labelled sodium diatrizoate from an animal acclimatized to 2 % sea water.

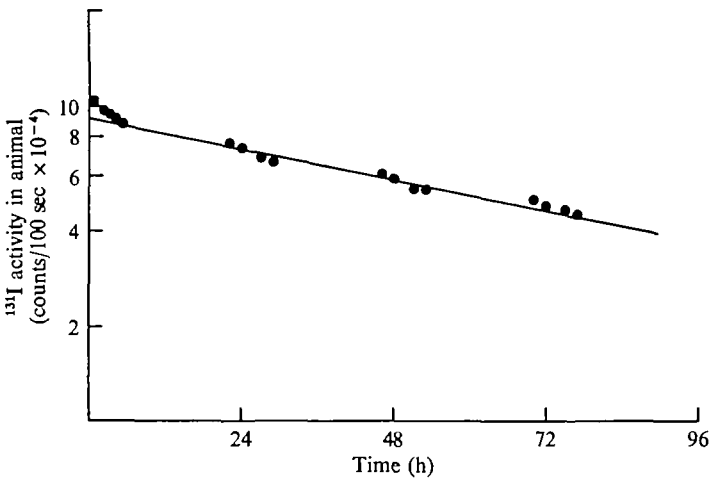

Fig. 3. Loss of ^{131}I-labelled sodium diatrizoate from an animal acclimatized to 100 % sea water.

Sodium diatrizoate clearance

Sodium diatrizoate, which in vertebrates has been found to be a suitable substitute for inulin in glomerular filtration rate studies (O'Dell, 1966), was used in clearance studies designed to estimate the rate of primary urine formation.

Animals were injected with 1·0–2·0 μl of diatrizoate solution containing 0·5 mCi/ml and 12 mg diatrizoate/ml and then returned to their acclimatization medium for 2 h to permit any bleeding to cease. The tracer activity of animals from 100 % sea water was recorded at hourly intervals for 5 h on the day of injection and at 2 h intervals on the three succeeding days. Animals from 2 % and 40 % sea water lose tracer more rapidly than those in 100 % sea water and were measured only for the 6 h following injection.

Table 3. *The clearance of sodium diatrizoate in different media*

Medium	N	$t_{\frac{1}{2}}$ (h)	σ	S.E.
2% sea water	19	8·0	±2·3	±0·5
40% sea water	18	12·5	±3·8	±0·9
100% sea water	22	133	±73	±16

In the case of animals acclimatized to 2% and 40% sea water a semi-logarithmic plot of activity remaining in the animal against time approximates to a straight line, after the first 2 h. This indicates that, after the initial diuresis and bleeding has stopped, a single rate constant dominates the loss over a considerable period of time (Fig. 2).

When the urine production rate was slower, as in animals acclimatized to 100% sea water, the loss rate of diatrizoate was more variable and three features were distinguishable: (1) a relatively rapid loss over the first few hours after injection, (2) a somewhat slower rate of loss during the night hours than during the day, and (3) a small but progressive decline in the rate of loss with time (Fig. 3).

During the first 6–7 h after injection the average rate of clearance of sodium diatrizoate from animals in 100% sea water was some six times as fast as that observed on the second and third days. This initial high rate of loss is presumed to indicate that a diuresis of some magnitude occurs after injection. If, as seems likely, the diatrizoate is being lost primarily in the urine, then the rate of primary urine formation over this period exceeds the calculated influx of water (from Table 2) by an order of magnitude. Such a rapid loss rate may perhaps be attributed to a combination of diuresis induced by shock and by the process of elimination of the excess fluid volume injected.

It is assumed that the loss rates on the second and third days after injection more nearly approximate to the normal rate of primary urine formation for animals in sea water. Clearance rates based on the loss during this period are given in Table 3 for 100% sea-water animals and over 6 h for 2% and 40% animals.

The relative concentrations of sodium diatrizoate in urine and blood

The value obtained for the clearance of diatrizoate is taken to approximate to the rate of production of primary urine. The definitive urine volume will differ from this if any reabsorption of water occurs in the excretory tubule. The magnitude of such reabsorption can be determined by comparison of the concentration of diatrizoate in the blood and in the definitive urine after correcting for the differing water content or urine and blood:

$$\% \text{ water reabsorbed} = 100 - \frac{100}{U/B} \quad \text{(Lockwood \& Riegel, 1968)},$$

where U and B are the concentrations of diatrizoate in urine and blood respectively. This estimation is potentially liable to error if the bladder volume is large as in crabs (Binns, 1969), but in the Gammaridae no bladder is present.

For measurement of U/B ratios animals weighing 60–80 mg were injected with 1–2 μl of diatrizoate solution and then replaced in their acclimatization medium for 1·5 – 6 h to permit equilibration of the injectant throughout the circulatory and urinary systems. Urine was then collected by a modification of the technique previously described (Lockwood, 1961), the animals being placed partially through a rubber

Table 4. The *U/B ratio for sodium diatrizoate in animals acclimatized to different salinities*

Medium	*N*	Mean *U/B*	σ	S.E.	Corrected *U/B*
2% sea water	7	1·25	±0·17	±0·06	1·15
40% sea water	3	1·09	±0·05	±0·03	1·01
100% sea water	8	1·12	±0·03	±0·01	1·05

membrane which was then clamped between two pieces of perspex so that the thorax and abdomen could be placed in an aqueous medium whilst the head was under liquid paraffin. Urine samples (1 μl) were collected directly into a dropping pipette which was then discharged into 1 ml of water in a test-tube for counting. Similar volumes of blood, taken from the cut end of an antenna, were counted for comparison.

The medium in which the posterior portion of the animal was placed during collection of urine was 2% sea water for animals initially acclimatized to 2% or 40% sea water. For animals initially acclimatized to 100% sea water 50% sea water was used in order to expedite urine collection. However, samples were only taken in the first 1–1·5 h after transfer to the medium and there would have been no possibility of the urine becoming hypotonic to the blood in this last group (Lockwood 1961, 1965).

The values of the *U/B* ratio for sodium diatrizoate in the three media are given in Table 4.

As a result of the differing protein contents of blood and urine the value of the diatrizoate *U/B* somewhat overestimates the apparent withdrawal of water during passage through the excretory duct. No measurements of the protein level of *Gammarus* blood are available but if the overall water content of the blood is comparable with that of other crustaceans given by Florkin (1960) the approximate correction factor will be 0·925 and the corrected *U/B* ratios using this factor are given in the final column of Table 3.

The results imply that a negligible proportion of the fluid passing down the excretory tubule is reabsorbed whether the urine be isotonic, as it will be in the animals acclimatized to 100% sea water, or hypotonic as in animals acclimatized to 2% sea water (Lockwood, 1961). This finding contrasts with that of Riegel & Kirschner (1960), who, working with crayfish, found the *U/B* for inulin to have a mean value of 3·4 when urine could readily be collected and 9·0 when little urine was being produced. However, the freshwater gammarid *G. pulex* also has an average *U/B* ratio for diatrizoate of approximately 3 (Lockwood & Inman, unpublished).

Blood volume

Blood volumes were determined by isotopic dilution of diatrizoate. About 1–2 μl of diatrizoate solution were injected into a number of animals which were then replaced in their medium for a period of $\frac{3}{4}$ h to allow mixing of the injectant within the circulatory system. The animals were then rinsed in unlabelled medium and counted individually. After counting, a blood sample of known volume was removed and its activity was measured. (A test indicated that there was no detectable difference in the proportional count given by tracer within the animal and that in isolated blood samples.) The value for the diatrizoate space (presumptive extracellular space) was

Table 5. *The diatrizoate space of animals acclimatized to* 100% *and to* 2% *sea water*

Medium	Diatrizoate space as % body wet weight	N
2% sea water	29·0 ± 4·5	20
100% sea water	29·6 ± 4·6	20

then obtained by simple proportion. No statistical difference was apparent in the diatrizoate space of animals acclimatized to 100% and to 2% sea water (Table 5).

The water content of the body

The measured water content of *Gammarus* which had been blotted dry, weighed and then dried to constant weight at 70 °C was 72·6 ± 1·6% (N = 20) for animals acclimatized to 100% sea water, 74·1 ± 2·2% (N = 10) for animals acclimatized to 40% sea water and 76·1 ± 3·41% (N = 20) for animals acclimatized to 2% sea water. In each case the animals had been allowed access to food until immediately before measurement. Sutcliffe (1971) gives values of 76·3, 76·2 and 78·1% for these three media on animals which had not been fed for a few days before analysis. Possibly the difference between our results and his are due to this short period of starvation since we have observed that individuals in 100% sea water which had not been fed for 4 weeks before measurement had the unusually high average water content of 79·6% of body wet weight.

Urine volume

Using the various data discussed above, the urine produced per day by animals in different media has been calculated from the expression:

$$\frac{24.V_b.100.\ln 2.V_f}{U/B.\ t_{\frac{1}{2}}.100^2} = U_v,$$

where V_b is the diatrizoate space as a percentage of the total fluid volume, V_f is the water content of the animal as a percentage of its wet weight, U/B is the diatrizoate concentration ratio between blood and urine corrected on the assumption that the blood contains 4% protein and that there is no protein in the urine, $t_{\frac{1}{2}}$ is the half-time (in hours) for the loss of diatrizoate from the body, and U_v is the definitive urine volume per day (fP_{O_8}) as a percentage of the wet weight.

The values obtained for animals previously acclimatized to 100%, 40% and 2% sea water are given in Table 6 and are there compared with the values (calculated from water flux and osmotic gradient) expected if all the water being excreted had entered the body by osmosis (fP_{diff}). It may be noted that the ratio fP_{O_8}/fP_{diff} is less than 1·5 when the osmotic gradient is large but that when the animals are near isotonicity in sea water there is a larger deviation from unity. In fact this deviation may be larger than that given in Table 6 since the result is somewhat weighted by assuming that animals in sea water are 10 m-osmoles hypertonic to their medium whereas 16 of the 21 animals measured were markedly less hypertonic than this.

Table 6. *Calculated definitive urine volumes in different media based on determinations of diatrizoate clearance (fP_{Os}) and on water flux and osmotic gradients (fP_{diff})*

Medium	Urine volume as % body wet weight/day		fP_{Os}/fP_{diff}
	fP_{Os}	$fP_{diff.}$	
2% sea water	50·2	43·4	1·16
40% sea water	37·2	25·8	1·44
100% sea water	3·4	1·8	1·97

Table 7. *The effect of thionine on the clearance of sodium diatrizoate*

	$t_{\frac{1}{2}}$ for loss of diatrizoate (h)	σ	S.E.	N
Thionine-treated*	260	±139	±44	10
Controls	137	±65	±14	22

* A thionine-treated animal with an unusually slow rate of loss ($t_{\frac{1}{2}}$ of 1094 h) was excluded from the above data in order not to weight the results unduly in a manner favouring the argument.

In view of this the result appears to add some additional support to the contention that when the animals are in sea water the intake of fluid by osmosis is supplemented by another process.

The possible role of ion transport as a driving force responsible for part of such a supplemented water intake has been investigated by exposing animals to sea water containing thionine.

The effect of thionine on clearance of sodium diatrizoate

The basic dye thionine was shown by Koch & Evans (1956) to inhibit the active uptake of sodium by crustacean gills. It has also been found to decrease markedly the rate at which sodium is actively taken up by *G. duebeni* moulting in sea water (Lockwood & Andrews, 1969). Treatment with thionine has now also been shown to influence the rate at which diatrizoate is cleared from the body.

Thirty-three animals from 100% sea water were injected with approximately 1 μl of [^{131}I] sodium diatrizoate and replaced in sea water for 20 h to recover from the shock of injection. After this period 11 were placed in 100% sea water saturated with thionine whilst the remainder were retained in untreated sea water as controls. The tracer activity remaining in the body was recorded at intervals during the period 46–77 h after the initial injection.

During this time the loss of diatrizoate from the animals in the thionine-containing medium was markedly slower than that from the controls (Table 7), implying that thionine was influencing the rate at which water was leaving the body and hence also the rate at which it was entering.

Urine production in mannitol solutions isotonic with sea water

If ion transport is important in contributing to water uptake then substitution of a non-electrolyte for the ion content of the medium should eventually result in a decrease in urine production.

A short-term experiment in which animals were transferred from sea water to isotonic mannitol and diatrizoate clearance measured over 7 h indicated, however, that there was a rise rather than a fall in urine production. Thus the mean clearance shown by control animals was 77 ± 46/h whilst that of animals (12) in isotonic mannitol was $47{\cdot}8 \pm 11{\cdot}0$.

More extended experiments in isotonic non-electrolyte cannot be carried out because of the limited survival of the animal in such media.

A probable explanation of the increase rather than decrease in clearance shown by animals in mannitol lies in the fact that mannitol does not behave as a perfect non-penetrating material. When [^{14}C] mannitol was placed in the medium it was found that the amount penetrating into the blood over a period of 4 h amounted to some 1·0 g/l or approximately 5·4 m-osmoles. Chromatography of blood samples indicated that the ^{14}C label was still primarily associated with mannitol. The penetration of mannitol into the blood could perhaps have produced a gradient sufficient to account for the anomalous clearance rate. The increased clearance occurring in the non-electrolyte medium is therefore insufficient grounds for abandoning the concept that part of the fluid excreted may be derived from fluid taken up in relation to the intake of inorganic ions.

DISCUSSION

The average urine production rate for *Gammarus duebeni* in sea water – 3·44 % of the body weight per day – lies within the range reported for various marine decapod crustacea (Parry, 1960) and is also similar to the value given by Werntz (1963) for *Gammarus oceanicus*, which is essentially a marine species. Urine production rate, as estimated from the clearance of the metabolically inert substance sodium diatrizoate, and also the sodium diatrizoate [U]/[B] ratios, increase as expected if the osmotic gradient between blood and medium is enlarged so that in 40 % and 2 % sea water at 18 °C *G. duebeni* is producing a urine volume equivalent to some 37 % and 59 % of its weight per day. Such rates are comparable with, though a little higher than, the flow observed in the freshwater species *G. fasciatus* (Werntz, 1963).

Apart from the opportunity such data offer for interspecific comparisons, they also provide a basis for discussion of the means by which *G. duebeni* and other isosmotic or near isosmotic marine animals obtain the water which they ultimately excrete.

When acclimatized to 100 % sea water the majority of individuals of *G. duebeni* have blood osmotic concentrations which are within 10 m-osmoles of the concentration of the medium. Whether or not this degree of hypertonicity is adequate to account for the urine production rate through uptake of water by conventional osmosis was examined by consideration of the water fluxes and urine flow rates. The interpretation of these studies naturally depends on the degree of reliability which can be placed on the evaluation of the flux data into terms of net water flow, and there is no means of determining this from the fluxes alone. However, study of the relationship between the calculated and actual urine flow in dilute media offers some possibility of resolving this difficulty.

Comparison of the urine flow (fP_{Os}) (as measured by the clearance of sodium diatrizoate and diatrizoate [U]/[B] ratio) and the osmotic uptake of water (fP_{diff}) (as calculated from the flux of tritiated water and the osmotic gradient between blood and

medium) give fP_{Os}/fP_{diff} ratios of 1·16 and 1·44 respectively for *G. duebeni* acclimatized to 2 % and 40 % sea water. No data are available on which to base direct comparison of these results with those obtained on other invertebrates, but such values compare reasonably closely with P_{Os}/P_{diff} ratios for the eel *Anguilla*, marine perch *Serranus* and flounder *Platichthys* (Motais *et al.* 1969). Levels such as these differ widely from those for fluid movement across anuran skins where the ratios (quoted from the literature by Motais *et al.*) range from 5·1 to 27·2. Ratios of P_{Os}/P_{diff} differing so markedly from unity are assumed to result either from the fact that water movement across the epithelium involves passage through long pores (Koefoed-Johnsen & Ussing, 1953) or alternatively that unstirred layers are present on either face of the epithelium (Dainty & House, 1966). Presumably no such interfering factors occur in *Gammarus duebeni* in these two media.

When *G. duebeni* is acclimatized to 100 % sea water a discrepancy appears between the urine flow rate and the calculated water intake. Thus the urine flow is 3·44 % of the body weight per day whereas the expected uptake of water by conventional osmosis, even assuming an osmotic gradient of 10 m-osmoles (a value in excess of that observed in two-thirds of the animals measured) is only 1·75 % of the body weight per day. Whilst changes in the morphology of the gill cells in relation to salinity (Lockwood *et al.* 1972) might perhaps be responsible for producing changes in the free diffusional flow of water in animals from 100 % sea water, the alternative proposition must be considered that not all the water taken up from sea water enters as a result of conventional osmosis.

Uptake of water in the food doubtless normally supplements that taken up by other means but such a contribution may be discounted in the present experiments since the animals were not fed. Similarly, metabolic water may be ignored since, on the basis of the values for oxygen consumption by *G. duebeni* given by Suomalainen (1956), the daily water formation would be expected to be less than 0·01 % of the body weight. Colloid osmotic pressure, causing an inward bulk flow of medium, and fluid intake driven by an active transport of inorganic ions remain as possible alternative mechanisms.

A relationship between water uptake and the transport of ions was first studied by Kalman & Ussing (1955), and they showed that when a toad was placed in isosmotic Ringer solution and stimulated by hormonal injection to take up sodium the rate of water uptake was also increased. No such increase in water uptake occurred after hormonal injection when the animals were in isosmotic sugar solutions, which implies that the water uptake in ionic media is associated with ion movements. No direct correlation occurred between the rate of sodium transport and the amount of water taken in, but the general relationship was such as to suggest a dependence of the water movement on the ion transport. Since then the hypothesis that there may be an interrelation of ion and water movement has been placed on a firmer basis, and various models by which this might occur have been proposed (Diamond, 1965; Diamond & Tormey, 1966; Curran & McIntosh, 1962).

The evidence that such a system may contribute to part of the water uptake necessary to provide the fluid for urine production in *Gammarus* in sea water is, at present, circumstantial. Nevertheless we feel that it is sufficiently compelling to merit some consideration. Three lines of argument may be considered. (1) The osmotic gradient

across the body surface is inadequate to account for the volume of water produced as urine in most individuals. It is therefore necessary to postulate an alternative to osmosis for some of the water taken in. (2) It has already been shown that *G. duebeni* increases the rate of ion transport into the body when its fluid volume is caused to fall (Lockwood, 1970) and at the time of ecdysis when the fluid volume has to be increased (Lockwood & Andrews, 1969). In both of these cases it was suggested that fluid uptake under the influence of the ion-transport system was responsible for re-establishing the 'normal' fluid content of the body. (3) Lockwood & Andrews (1969) also showed that the dye thionine (3,5-diamino phenthiazine) inhibits some 90% of the active sodium uptake in freshly moulted animals though a lower degree of inhibition was obtained in intermoult animals. The observation in the present study that animals exposed to a saturated solution of thionine in sea water show a reduced rate of urine production is taken to indicate that the partial blockage of sodium uptake may be affecting the volume of fluid available for excretion. Perhaps fortuitously, the reduction in urine flow caused by treatment with thionine is approximately the same as the difference between the actual urine flow in 100% sea water and the flow calculated on the basis of the average osmotic gradient. If, however, this similarity is not mere coincidence, then it would appear possible that some form of water transfer driven by ion uptake is responsible for part of the fluid inflow when the animals are in sea water.

In presenting this suggestion it is not intended to imply that ion-related water movement is necessarily the only mechanism utilized by near-isosmotic marine forms. A few of the *G. duebeni* measured were sufficiently hypertonic to their medium to account for water uptake by osmosis. Also, in some cases the colloid osmotic pressure of the blood may be adequate, as postulated by Burber (1957) for the lobster. Nevertheless, if the fine control of fluid uptake were regulated by the process responsible for active transport of inorganic ions it would doubtless be of some assistance in relieving the animal of the need to regulate the colloid osmotic pressure exactly. In view of the fluctuations in non-ionic components of the blood in relation to the stages of the moulting cycle (Robertson, 1960) and also in relation to blood concentration (McLusky, 1968; Sharma, 1968; Dehnel, 1966), this would not be without its advantages.

The postulate that water uptake and ion uptake are directly related can clearly only be sustained if it can be demonstrated that the amount of inorganic ions taken in could account for the water uptake. The value for sodium influx by *G. duebeni* from 10 m*M*/l NaCl plus sufficient sucrose to make the medium isotonic with sea water is 0·14 μ-equiv Na/animal/h (Lockwood & Andrews, 1969). This influx, if solely due to active transport, would be sufficient (if accompanied by equivalent anion) to account for fluid uptake of 7 μl/day or twice the observed rate. Sutcliffe (1967) and Lockwood & Andrews (1969) have suggested that some exchange diffusion of ions occurs even in media as dilute as 10 m-equiv/l, so the actual value for net intake of sodium is likely to be lower than that quoted above. Consequently, if water is taken in as a result of the ion movements, the volume expected to be transferred will be closer to that observed to be excreted.

The urine of *G. duebeni* in sea water is isosmotic with the blood (Lockwood, 1961; Haywood, 1970) and it may be presumed that a large part of the urine concentration is due to the presence of inorganic ions. Ions must therefore be brought into the body

continuously to replace those excreted. It is perhaps worth stressing that, if the uptake system is so regulated that the total haemolymph concentration is somewhat higher than that of the medium, water will enter the body by osmosis. However, if the permeability to water is sufficiently high, or the concentration gradient is localized in channels, the overall gradient maintained between blood and medium is likely to be negligible, and in effect isosmotic fluid transfer will occur. The precise causative agent for ion-dependent fluid uptake is therefore perhaps best regarded as one of degree rather than kind and could conceivably vary according to circumstance in the individual. The range of osmotic gradient between blood and medium, 0–25 m-osmoles, implies that this could indeed be the case in *G. duebeni* in 100% sea water.

No evidence is available as to the route by which ions and water enter the body when *G. duebeni* is in sea water, though the ultrastructure of some of the cells in the gills bears a resemblance (Lockwood *et al.* 1972) to cells, such as those of the proximal renal tubule in mammals, which are known to transport fluid isosmotically. However, as intermoult *Gammarus* have also been shown to drink up to 1·06 μl/h, either orally or anally (Lockwood & Andrews, 1969), uptake of fluid from the gut might contribute to, or be wholly responsible for, the intake.

Finally, it is interesting to speculate as to whether a mechanism evolved initially in isosmotic marine organisms to transport fluid into the body might not have provided the necessary unidirectional transport system on which selection could act to produce transport mechanisms capable of maintaining the blood strongly hypertonic to dilute media. The presence of such a pre-adapting process as a general feature in marine forms would then go some way towards explaining the frequency with which the capacity to regulate hypertonically has evolved in fresh-water and brackish-water species.

A necessary requirement for an animal able to maintain its blood hypertonic to dilute media and also to effect isosmotic water transport when isosmotic with its medium is to be able to alter the relative permeability of the surface to water and inorganic ions. One of the possible ways of achieving this would be the ability to vary the permeability to water, and indeed such changes in permeability to water in relation to the salinity of the medium have been shown to occur in *G. duebeni* (Lockwood *et al.* 1972).

SUMMARY

1. The water fluxes across the body surface and the rate of urine production have been studied in the euryhaline amphipod *Gammarus duebeni*.

2. Urine flow rates (fP_{Os}) have been determined from measurements of loss of [^{131}I]sodium diatrizoate from the body, and the expected urine flow (fP_{diff}) has been calculated from determinations of the osmotic gradient between blood and medium and the flux of tritiated water.

3. For animals in 2% and 40% sea water the ratio of fP_{Os}/fP_{diff} are 1·16 and 1·44 respectively, and thus approximate fairly closely to unity. This implies that in these media the water subsequently excreted as urine enters the body by osmosis and that there is little interference with the free diffusion of water at the body surface due to passage through long pores or across unstirred layers.

4. In sea water the ratio fP_{Os}/fP_{diff} is normally (assuming an osmotic gradient of

10 m-osmoles) almost twice unity but urine production is approximately halved when the animals are exposed to sea water saturated with an inhibitor of active sodium uptake (thionine).

5. It is suggested that there is a *prima facie* case for assuming that part of the fluid subsequently excreted by this species, when in sea water, is taken into the body initially by a process dependent upon active ion transport.

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