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REGULATION OF WATER AND SOME IONS IN GAMMARIDS (AMPHIPODA)

II. *GAMMARUS PULEX* (L.)

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INTRODUCTION

Gammarus pulex was investigated by Beadle & Cragg (1940) in their analysis of the process of osmoregulatory adaptation to fresh water. This study was centred on a comparison of the blood and tissue chloride levels in several species of *Gammarus* which were exposed to a wide range of external salinities. Beadle & Cragg concluded that in the euryhaline species *G. duebeni* and *G. locusta* an important part of adaptation to variations in salinity was the ability to regulate or maintain the tissue chloride content at a fairly constant level in the face of large changes in the blood concentration. In contrast the inability of *G. pulex* to tolerate high salinities was associated with its inability to prevent chloride from entering the tissues when the blood concentration was raised above the normal level. This aspect of osmoregulation in crustaceans was later studied in more detail on the large muscles of decapods (Shaw, 1955*a*, *b*, 1958*a*, *b*, 1959; Bryan, 1960*a*, *b*). However, there is still relatively little information about water and ion regulation in the tissues of invertebrates (see reviews by Potts & Parry, 1964; Potts, 1968; Lange, 1968).

The 'tissue' studied by Beadle & Cragg (1940) was in fact the whole animal cut into two parts and pressed between filter papers to remove the blood. Derouet (1952) apparently used the same technique and she obtained very similar results on *G. pulex*. Some blood would undoubtedly remain in this tissue preparation (Sutcliffe, 1971*a*). In the present series of studies on salt and water regulation in gammarids the intracellular concentrations of sodium, potassium and chloride were estimated by an indirect method based on the assumption that the distribution of potassium and chloride in the animal conforms to a Donnan equilibrium between the extracellular and intracellular spaces (Croghan & Lockwood, 1968; Sutcliffe, 1971*a*, *b*). This method is justified in this paper by comparison with the results obtained by Beadle & Cragg and by Derouet, and also by comparison with results based on the extracellular inulin space in *G. pulex* measured by Butterworth (1968).

MATERIAL AND METHODS

Large male specimens of *G. pulex* were obtained locally. The experiments were carried out at a temperature of 9 ± 1 °C. The experimental procedures and methods of analysis were as described previously (Sutcliffe, 1971*a*). That paper also gives the

method employed for the calculation of the blood space, defined as the proportion of the body water in the extracellular space.

RESULTS

Water content

The mean water content remained constant at 79·0–80·3% body wet weight in animals acclimatized to salinities ranging from 0·06 mM/l NaCl to 50% sea water (Table 1). Inspection of the standard errors given in Table 1 shows that variability in the water content of the six animals in each group did not increase at high external salinities. This is surprising in view of the fact that *G. pulex* drinks when in sea-water media (Sutcliffe, 1967) and it was expected that imbibition of salt water might influence water balance in the animal, especially at salinities between 30 and 50% sea water where mortality is high. In this particular case there were no deaths in 10% and 30% sea water at 9 °C, and all of the animals were active and normal in appearance in

Table 1. *Wet weight, water content and blood ion concentrations at various external salinities*

(Mean results from six animals ± 1 standard error.)

Medium	Wet weight (mg)	Water content (% wet wt.)	Blood ions (mM/l) Na_o	Cl_o	K_o	Ratio: body sodium/ chloride
0·06 mM/l NaCl	66·1 ± 0·99	79·8 ± 0·62	106	126	5	1·61
0·25 mM/l NaCl	66·9 ± 1·85	79·3 ± 0·60	131	124	5	1·48
0·25 mM/l NaCl	61·7 ± 1·56	79·4 ± 0·97	—	—	—	1·70
2% SW	72·8 ± 0·97	79·3 ± 0·89	—	—	—	1·45
10% SW	67·1 ± 1·75	79·0 ± 0·65	132	130	6	1·30
10% SW	57·8 ± 1·19	80·3 ± 0·70	—	—	—	1·51
30% SW	65·5 ± 1·99	78·9 ± 0·30	179	188	7	1·08
40% SW	58·4 ± 1·11	79·2 ± 0·67	[200]*	[230]	[8]	1·04
50% SW	54·7 ± 1·99	79·8 ± 0·42	[250]	[280]	[10]	1·00

* [ ] blood concentrations assumed for calculation of blood space and sodium space.

30% sea water. When transferred to 40% sea water they became less active and did not adopt the characteristic attitude of *G. pulex* when at rest, where the telson is curled round to lie beneath the abdomen. Instead the uropod and telson were extended in line with the thorax and abdomen. This is the attitude normally adopted when swimming, and also after death. During a period of 6 days in 40% sea water one-half of the animals died. The remainder were moved to 50% sea water for 24 h. The six animals used for analysis were barely active. The rest were either dead or in a semi-comatose condition.

Sodium and chloride

A few analyses on large pooled samples of blood are given in Table 1. These supplement previous analyses given in the literature (Beadle & Cragg, 1940; Derouet, 1952; Lockwood, 1961; Sutcliffe, 1967; Vincent, 1967). No attempt was made to obtain blood from animals in 40–50% sea water as most of the animals were in a semi-comatose condition. It was assumed that in these animals the sodium concentration in the blood was slightly higher than the sodium concentration in the medium, and that the chloride concentration in the blood was equal to the chloride concentration in the

medium (Table 1). Beadle & Cragg, and also Derouet, found blood chloride concentrations of about 200–220 mM/l in *G. pulex* from 40% sea water. In *G. pulex* from 50% and 60% sea water Derouet (1952) gives values for the blood chloride concentration of approximately 230 and 260 mM/l respectively. These blood concentrations are well below the external chloride concentrations normally found in 50 and 60% sea water.

The concentrations of total body sodium and chloride in the body water are shown in Fig. 1. The body sodium and chloride concentrations were constant in animals from 0·25 mM/l NaCl up to 10% sea water, both were lowered in animals acclimatized to

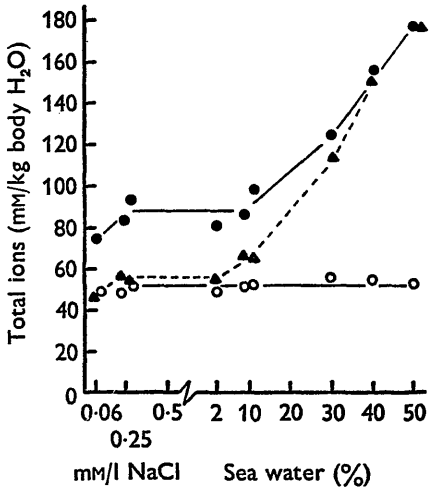

Fig. 1. The total body concentrations of sodium (●), potassium (○) and chloride (▲) in *Gammarus pulex*.

0·06 mM/l NaCl, and both rose markedly at salinities above 10% sea water. The variability in body sodium and chloride was not noticeably correlated with the external salinity. Over the salinity range studied the standard errors in groups of six animals varied between 1·3 and 4·0% of the mean body sodium concentrations and 1·9–5·0% of the mean body chloride concentrations. Changes in the total sodium and chloride content presumably reflect the changes in the blood concentration which occur at external salinities above 10% sea water and below 0·25 mM/l NaCl. A large increase in the sodium and chloride content of the blood at high salinities results in a change in the relative proportions of the two ions in the whole animal. Thus the ratio body sodium/chloride fell from 1·3 to 1·5 in 10% sea water to about 1·1 in 30% sea water and to 1·0 in 50% sea water (Table 1). At salinities below 10% sea water the ratio was 1·45–1·70, comparable with the ratios of body sodium/chloride found in *G. duebeni* and *G. zaddachi* at low salinities (Sutcliffe, 1971*a*, *b*). This large excess of sodium relative to chloride in the whole animal under normal conditions (i.e. at low salinities) indicates that 30–40% of the body sodium must be located in the tissues, since the blood contains approximately equal amounts of sodium and chloride.

Potassium

Values for the blood potassium concentration are given in Table 1. They are practically the same as the blood potassium concentrations found in *G. duebeni* and *G. zaddachi*. The total body potassium concentration was constant over the entire salinity range studied (Fig. 1). The mean values in groups of six animals ranged between 47·5 and 55·2 mM–K/kg body H_2O, and the standard errors varied between 1·0 and 5·2% of the mean concentrations. Since these values for body potassium are very similar to the values for body potassium in *G. duebeni* and *G. zaddachi* (Sutcliffe, 1971*a*, *b*) it may be inferred that the intracellular concentration of potassium in *G. pulex* is more or less the same as in the brackish-water gammarids.

Some preliminary experiments were carried out to determine the potassium loss rate and the external potassium concentration necessary for the animal to achieve a temporary steady state with respect to potassium. In *G. pulex* starved for 24 h in Windermere lake water the mean loss rate into de-ionized water was 0·035 μM/h/animal at 9 °C. This rate is faster than the potassium loss rates in *G. duebeni* and *G. zaddachi*. Potassium balance in starved animals was maintained at an external concentration of 0·010–0·015 mM/l potassium for periods of up to 4 days. However, current experiments with fed animals show that *G. pulex* can survive for at least 6 weeks in natural water containing only 0·005 mM/l potassium. Thus potassium balance is maintained at a lower external concentration when the animals are fed. This suggests that either some potassium is obtained directly from the food, or more energy is available to drive the transporting system involved in potassium uptake from the medium.

Blood space

The proportion of the body water in the blood space was calculated from the data for total body chloride and potassium (Fig. 1) and the blood chloride and potassium concentration (Table 1). The results are given in Fig. 2, together with values for the chloride and sodium spaces. These were calculated on the assumption that all of the chloride and sodium in the animal were at the concentrations found in the blood.

In animals from 0·25 mM/l NaCl and 2% sea water the blood space was equivalent to 38–42% body H_2O. This may be regarded as the normal water content of the blood space in *G. pulex* living in fresh water. In animals from 0·06 mM/l NaCl the blood chloride and potassium concentrations remained unaltered, but the fall in body chloride (Fig. 1) is associated with a shift in water from blood to cells, so that the blood space now represents only 31% body H_2O.

The values for the blood space in animals from the above media were calculated on the assumption that a Donnan equilibrium exists between potassium and chloride in the blood and cells. This assumption is unlikely to be a reasonable one in the case of animals exposed to salinities greater than about 10% sea water, where the blood concentration and the total concentration of sodium and chloride rise steeply. However, it is of interest to examine briefly the situation where a Donnan equilibrium is assumed to exist between the blood and cells in animals from salinities up to 50% sea water. The blood space would be increased to about 60% body H_2O and practically all of the body chloride and sodium would be held in the blood space (Fig. 2). Now it

seems very unlikely that the mean intracellular chloride concentration would remain at the low (Donnan) concentration found in animals from fresh water (see next section) and if the cell chloride concentration is raised then the blood space must be less than 60% body H_2O. As a convenient point of reference it is therefore assumed that the blood space did not increase above 50% body H_2O in animals from 30 to 50% sea water.

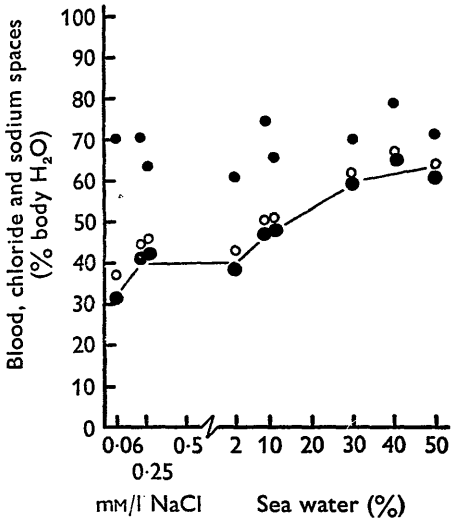

Fig. 2

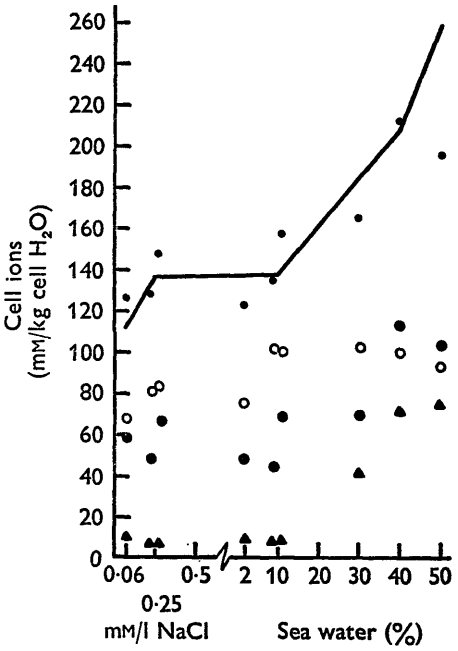

Fig. 3

Fig. 2. The mean blood space (●—●), chloride space (○) and sodium space (●) in *Gammarus pulex*.

Fig. 3. The mean intracellular concentrations of sodium (●), potassium (○) and chloride (▲) in *Gammarus pulex*. The solid line represents the concentrations of Na+K (mM/l) in the blood; (●) represents intracellular Na+K (mM/kg cell H_2O).

Cell ions

The mean concentrations of sodium, potassium and chloride in the intracellular water are shown in Fig. 3. The values for animals in 30 to 50% sea water are based on a cell water content equivalent to 50% body H_2O. The values for animals at lower salinities are based on the assumption that a Donnan equilibrium exists between potassium and chloride in the blood space and the intracellular space. At low salinities the cell concentrations of potassium and chloride are very similar to the calculated cell concentrations of the same ions in *G. duebeni* and *G. zaddachi* from low salinities. The cell sodium concentrations, 40–70 mM/kg cell H_2O, are slightly lower compared with the estimates of cell sodium in the two brackish-water gammarids. In *G. pulex* from 30 to 50% sea water there is a small increase in the cell potassium and sodium concentrations, and a large increase in the chloride concentration which is raised from the normal level of 7–10 up to 70–75 mM/kg cell H_2O. The cell chloride concentration would be even higher in animals from 40 to 50% sea water if (1) the blood space was less than 50%

body H_2O or (2) the blood chloride concentration was below that of the external medium.

With the exception of animals in 50% sea water the concentrations of cell sodium and potassium when summed together are very close to the cumulative concentrations of the same ions in the blood (Fig. 3). This agreement between the observed blood concentrations and the calculated mean cell concentrations may be fortuitous but it is, nevertheless, valid to note that the cell concentrations are at least of the right magnitude and do not exceed the blood concentrations to any significant extent.

Table 2. *Calculation of the proportion of the extracellular tissue space assumed to be excluded from the inulin space measured by Butterworth* (1968)

		g H_2O/100 g animal
Body water	79·5% wet wt.	79·5
Blood space	40·0% body H_2O	31·8
Inulin space	32·7% body H_2O	26·0
Intracellular space (ICS)	60·0% body H_2O	47·7
Extracellular tissue space (ECTS)	20·0% tissue H_2O	
Tissue water	ICS+ECTS (75% body H_2O)	47·7+11·9 (59·6)
Proportion of ECTS not available to inulin	(blood space – inulin space) (9·7% tissue H_2O)	31·8−26·0 $\left(\frac{5·8}{59·6}\times 100\right)$

DISCUSSION

The values for the body water content in *Gammarus pulex* are in very close agreement with the value of 79·55% wet weight found by Butterworth (1968). By injecting ^{14}C-labelled inulin into animals from fresh water Butterworth estimated that the inulin space was equivalent to 26 ± 5% body wet weight. This gives a mean value for the inulin space equivalent to 32·7% body H_2O, compared with the estimates made here for the blood space equivalent to 38–42% body H_2O. These values for the blood space were calculated from the assumption that a Donnan equilibrium exists between the blood space and the intracellular space. The small difference (7·3% body H_2O) between the above two estimates of the extracellular space in *G. pulex* may therefore indicate that the distribution of potassium and chloride ions does not strictly conform to a Donnan equilibrium. On the other hand, although the injection of inulin into whole animals clearly provides a reasonably good estimate of the extracellular tissue space (Robertson, 1961, 1965, 1970) the inulin molecule may not completely penetrate this space (e.g. Flemister, 1958). Now it is clear that the intracellular space in *G. pulex* must contain at least 60% of the body water, and in a variety of crustaceans the extracellular tissue space represents approximately 20% of the tissue water (Robertson, 1961, 1970; van der Kloot, 1966; Hays, Lang & Gainer, 1968; Lang & Gainer, 1969; Mackay & Prosser, 1970). If the extracellular tissue space in *G. pulex* is also equivalent to 20% of the tissue water then it is possible to calculate the minimum water content of the tissues. This is done in Table 2, where it is seen that the tissue water content must be equivalent to approximately 75% of the body water. Furthermore, if the difference

between the estimates for the blood and inulin spaces is in fact entirely due to the inability of inulin to penetrate part of the extracellular tissue space, then the space unavailable to inulin would represent about 10% of the tissue water, i.e. one-half of the extracellular tissue space (Table 2). The alternative conclusion, based on Butterworth's value for the inulin space, is that in *G. pulex* the total extracellular tissue space does not exceed 10% of the tissue water. Bearing in mind these points, it is concluded that the estimated blood space, equivalent to 40% body H_2O, is very close to the real value for the extracellular space in *G. pulex* from fresh water. Hence the assumption that the distribution of potassium and chloride between the blood and cells conforms to a Donnan equilibrium is justified. This of course is still only an approximation made with respect to the mean intracellular content of the animal. The concentrations of individual ions are known to vary in different tissues in other animals (e.g. Bryan, 1960*a*, 1963; Bryan & Ward, 1962).

Beadle & Cragg (1940) and Derouet (1952) found approximately 25–28 mM-Cl/kg wet tissue in *G. pulex* from fresh water. The intracellular chloride concentration of this 'tissue' was estimated by assuming that 80% of the tissue weight was due to water. It is also assumed that 20% of the tissue water was held in the extracellular tissue space with a chloride concentration equal to that of the blood (*c.* 120 mM/l chloride). The extracellular tissue space would then contain about 19 mM chloride, leaving 6–9 mM-Cl/640 g cell H_2O or 9–14 mM-Cl/kg cell H_2O. The 'tissue' probably also contained a residual amount of blood equivalent to about 7–10% of the blood space (Sutcliffe, 1971*a*). When an allowance was made for the chloride content of this blood it was calculated that the intracellular chloride concentration would lie between 2 and 9 mM-Cl/kg cell H_2O. This is an excellent agreement with the present estimates of an intracellular chloride concentration of 7–9 mM/kg cell H_2O (Fig. 3). Similar estimates were also made of the intracellular chloride concentrations of the 'tissues' obtained by Beadle & Cragg and by Derouet from *G. pulex* in 50% sea water. The calculations indicate that the mean cell chloride concentration was 40–60 mM/kg cell H_2O, compared with the estimate of 75 mM/kg cell H_2O given in Fig. 3. From Derouet (1952) it was calculated that the cell chloride concentration increased to about 225 mM/kg cell H_2O in animals exposed to 60% sea water for 24 h. In this case the intracellular chloride concentration would be close to the blood concentration (*c.* 260 mM/l chloride) which suggests that a massive movement of chloride had occurred from the blood into the cells.

The present investigation confirms Beadle & Cragg's analysis of the main features of chloride regulation in the tissues of *G. pulex*, in particular the rapid penetration of chloride into the tissues when the blood concentration is raised above the normal level. Beadle & Cragg also made the point that this increase in tissue chloride is far greater than the increase expected if there was a simple proportional relationship between the concentrations of chloride in the blood and tissues. A proportional relationship of this kind does occur in the muscle fibres of *Carcinus* with respect to both chloride and sodium over a wide range of blood concentrations (Shaw, 1955*b*). There is also a proportional relationship between sodium in the blood and muscle fibres of *Astacus* at low blood concentrations, but at blood concentrations above 200 mM/l sodium there is an increased penetration of sodium into the fibres (Bryan, 1960*b*). This is illustrated in Fig. 4, where the mean intracellular concentrations of chloride and sodium in

G. pulex (from Fig. 3) are plotted against the blood concentrations of the same ions. It is seen that the increases in the mean intracellular concentrations of both chloride and sodium in *G. pulex* closely parallel the increases in muscle sodium found in three freshwater decapods when the blood sodium concentration is raised. In contrast the mean intracellular sodium concentration in *G. duebeni* does not rise steeply at high blood concentrations, and the intracellular chloride concentration shows a proportional relationship with the blood chloride concentration, as in *Carcinus* (Fig. 4).

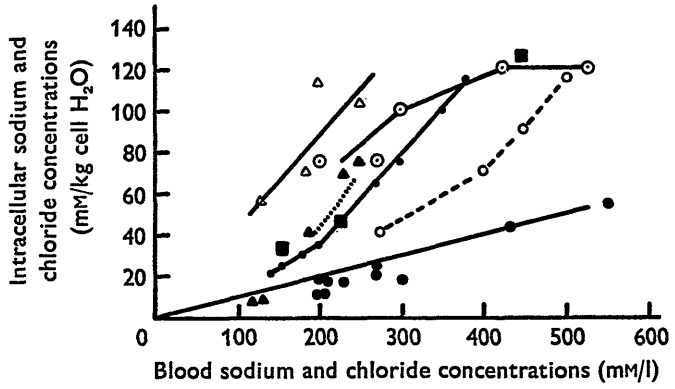

Fig. 4. A comparison of the intracellular concentrations of sodium and chloride in relation to the blood concentrations in some arthropods. △, Na, ▲, Cl in cells of *Gammarus pulex*. ⊙, Na, ●, Cl in cells of *G. duebeni* (Sutcliffe, 1971*a*). ■, Na in muscles of *Limulus* (Robertson, 1970). ●—●, Na in muscles of *Astacus* (Bryan, 1960*b*). ○- - -○, Na in muscles of *Potamon* (Shaw, 1959). The short dotted line represents Na in muscles of *Pacifastacus* (Kerley & Pritchard, 1967). The solid line drawn through the origin of the Figure represents Na and Cl in muscle fibres of *Carcinus* (Shaw, 1955*b*).

In *G. pulex* from fresh water the sodium space is much larger than the chloride space, and 30–40% of the body sodium is probably located in the intracellular space. From Fig. 4 it is clear that the mean intracellular sodium concentration is high in relation to the blood concentration when compared with the sodium concentrations of the muscle fibres in decapods and in the arachnid *Limulus*. This raises the possibility that a large proportion of the intracellular sodium in *G. pulex* may not be located in the muscles. In *Asellus aquaticus* Lockwood (1959) found that the sodium space was 27% greater than the chloride space and he suggested that a considerable proportion of the excess sodium was held in the form of crystalline sodium urate in the cells of Zenker. *Asellus* stores unusually large amounts of uric acid in these cells. But the uric acid content of *A. aquaticus* was approximately one hundred times greater than the uric acid content of *G. pulex* (Dresel & Moyle, 1950) so it is unlikely that the excess sodium in *G. pulex* is specifically associated with the storage of uric acid. In fact the accumulation of excess sodium relative to chloride may be a common feature of crustaceans. The few analyses made so far on freshwater crustaceans are collected together in Table 3. *G. duebeni* closely resembles *G. pulex*. *Asellus* has the largest excess of sodium relative to chloride, and the other isopod *Mesidotea* is peculiar in that the chloride space is practically equal to the sodium space. Both spaces are very large in the freshwater race of *Mesidotea* from L. Mälaren and this is associated with an unusually high proportion of water in the blood space (Croghan & Lockwood, 1968). In the amphipod *Crangonyx*

Table 3. *Sodium and chloride spaces in some fresh water crustaceans*

Species	mM/l		Body H_2O (%)		Remarks	Source
	Blood Na	Blood Cl	Na space	Cl space		
Astacus pallipes	169	—	54–56*	—	Mean of 5 animals	Shaw (1959)
	200	—	57–60*	—	Mean of 12 animals	Bryan (1960*a*)
	[200]†	[200]	61	50	1 animal, 2·2 g wt.	Original
	[200]	[200]	72	60	1 animal, 1·0 g wt.	Original
Crangonyx pseudogracilis	95	91	71	62	30 animals pooled	Original
Gammarus pulex	131	124	63–71	44–46	Means of 6 animals	This paper
G. duebeni	185–240	195–240	52–72	34–50	Means of 6 animals	Sutcliffe (1971*a*)
Asellus aquaticus	137	125	80	53.	Means of 14–18 animals	Lockwood (1959)
Mesidotea entomon	170–248	167–266	68–86	67–76	4 animals from L. Mälaren	Croghan & Lockwood (1968)
	183–261	185–324	50–62	45–69	6 animals from Askö (brackish water)	Croghan & Lockwood (1968)

* Assuming body H_2O = 70–73 % body wt.
† [ ] assumed blood concentrations.

and the crayfish *Astacus* the sodium spaces are approximately 10% larger than the chloride spaces. Bryan (1960*a*) estimated that in *Astacus* the mean intracellular sodium concentration was equal to 51 mM/l, whereas the muscles contain about 35 mM/kg H_2O. Bryan suggests that the digestive gland in *Astacus* might contain about 100 mM-Na/kg H_2O. This gland certainly has a high potassium concentration (Bryan & Ward, 1962).

SUMMARY

1. The water content, and the concentrations of sodium potassium and chloride in the blood and body water were determined in *Gammarus pulex* acclimatized to external salinities ranging from 0·06 mM/l NaCl up to 50% sea water.

2. The mean body water content remained constant at 79·0–80·3% body wet weight. The total body sodium and chloride concentrations were lowered in 0·06 mM/l NaCl and increased markedly at salinities above 10% sea water. The normal ratio of body sodium/chloride was 1·45–1·70, decreasing to 1·0 at 50% sea water.

3. The total body potassium concentration remained constant at 47·5–55·2 mM/kg body H_2O. The rate of potassium loss across the body surface was relatively fast. Potassium balance was maintained at an external potassium concentration of 0·010–0·015 mM/l by starved animals, and at 0·005 mM/l by fed animals.

4. The proportion of body water in the blood space was calculated from the concentrations of potassium and chloride in the blood and in the body water. The blood space contained 38–42% body H_2O in animals from fresh water. The blood space decreased to 31% body H_2O in animals from 0·06 mM/l NaCl. The sodium space was equivalent to about 70% body H_2O.

5. The mean intracellular concentrations of sodium, potassium and chloride were estimated and the results were compared with previous analyses made on the tissues of *G. pulex* and other crustaceans. It was concluded that in *G. pulex* from fresh water the distribution of potassium and chloride ions between the extracellular blood space and the intracellular space approximately conforms to a Donnan equilibrium. 30–40% of the body sodium is apparently located in the intracellular space.

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