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THE SODIUM BALANCE MECHANISM IN THE FRESH-WATER AMPHIPOD, *GAMMARUS LACUSTRIS* SARS

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INTRODUCTION

Osmoregulation in aquatic animals is effected by the co-ordinated activities of a number of mechanisms controlling the movements of ions and water in various parts of the body, and a considerable amount is known about some aspects of these regulatory mechanisms in Crustacea (reviewed by Robertson, 1960; Lockwood, 1962; Potts & Parry, 1964). One aspect which has received particular attention since the publication of Krogh's researches (Krogh, 1939) is the ability to absorb ions from very low external concentrations, and this has been studied intensively in the fresh-water crayfishes *Astacus pallipes* and *A. fluviatilis* (Bryan, 1960*a*, *b*, *c*; Shaw, 1959, 1960*a*, *b*, 1964). In the crayfish the excretory organs and the gut have only a minor role in regulating the blood sodium concentration when in fresh water, and the blood concentration is maintained at a steady level by ion-transporting mechanisms situated in the gills. In the case of the sodium uptake mechanism, this is saturated at an external concentration of 0·5–1 mM/l. NaCl, where the rate of uptake balances the rate of sodium loss from the animal. When the external concentration is reduced to below the saturation concentration the uptake rate declines, and as it is now exceeded by the loss rate there is a net loss of sodium. However, a small reduction in the blood sodium concentration is sufficient to activate the sodium transporting system so that, by raising the maximum (saturation) rate of uptake, the rate of sodium uptake at lower external concentrations is increased to balance sodium loss. In this way *Astacus* can, for short periods at least, maintain sodium balance at an external concentration of only 0·04 mM/l. sodium.

Thus, in *Astacus*, sodium regulation at external concentrations below 1 mM/l. is brought about largely by activation of the uptake mechanism. In contrast, sodium regulation in *Gammarus pulex* is also due to activation of the uptake mechanism, but this is associated with a considerable reduction in the rate of sodium loss at external concentrations below about 0·2 mM/l. NaCl (Shaw & Sutcliffe, 1961; Sutcliffe, 1967*a*). This reduction is brought about by the combined effects of a fall in the blood sodium concentration and the elaboration of a very dilute urine.

This paper is concerned largely with changes in the rate of sodium uptake and the overall rate of sodium loss in *G. lacustris*. This species, like *G. pulex*, is a true fresh-water animal, confined to lakes in northern parts of the British Isles (Hynes, 1955),

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so that a direct comparison between sodium regulation in the two species is of considerable interest. In fact, it is shown that sodium regulation in the two species is virtually identical, at least over the range of external sodium concentrations found in fresh water.

MATERIAL AND METHODS

G. lacustris was obtained from a small quarry pond at Oxford, near Berwick, in Northumberland. A few observations were made with animals collected from Talkin Tarn, near Brampton, Cumberland.

Some initial experiments were carried out at room temperature, but later experiments were conducted in a constant temperature room at 10° C. ± 1° C. The animals were acclimatized to this temperature for about 1 week before commencing experiments, and they were acclimatized to experimental media for at least 48 hr. before determining influx and loss rates. The media were NaCl solutions made with de-ionized water, and sea water from Cullercoats diluted with Newcastle tap water. Animals were fed with dried leaves of sycamore and elm previously soaked in water, but they were starved for at least 24 hr. before making any determinations.

Sodium influx was measured by the technique described previously (Shaw & Sutcliffe, 1961) using ^{22}Na as the tracer. The influx was continuously recorded over a period of 30–60 min. The sodium concentration of the external solution was measured on an EEL flame photometer.

Sodium loss rates were determined during a period of 60–80 min. by placing groups of about ten animals in 50 ml. de-ionized water. The technique is described by Sutcliffe (1967*a*).

Freezing-point determinations of blood samples and sea-water media were determined by the microcryoscopic method of Ramsay & Brown (1955), accurate to ± 1·5 mM/l. NaCl. Sea-water media are referred to in terms of NaCl solutions with equivalent freezing-point depressions.

RESULTS

The blood concentration and survival in sea-water media

The total concentration of the blood was obtained by measuring the freezing-point depression of blood samples taken from animals acclimatized to sea-water media for 7 days at 12–14° C. The results are shown in Fig. 1. At an external concentration of 0·3 mM/l. NaCl the mean blood concentration in six animals was equivalent to 154 mM/l. (± 3·5 mM/l.) and this is very similar to the value of 144 mM/l. NaCl found in *G. pulex* kept at the same external concentrations (Sutcliffe, 1967*a*). It is also remarkably similar to the value given for *G. lacustris* from the Talzy lakes near Lake Baikal (Basikalova, Birstein & Taliev, 1946). In sea-water media the blood concentration gradually increased and it remained very slightly hyperosmotic to media more concentrated than about 250 mM/l. NaCl. In this respect *G. lacustris* behaves like many other fresh-water animals (Lockwood, 1962; Potts & Parry, 1964). Nevertheless, this species has a surprisingly wide range of tolerance, since animals withstood a sudden transfer from fresh water to 225 mM/l. NaCl (about 40% sea water) with very few deaths at a temperature of 12–14° C. In fact, at this temperature *G. lacustris* remained

healthy in 50 and 60% sea water for more than 8 weeks, and animals were easily acclimatized to 375 mM/l. NaCl (about 70% sea water). At this concentration, however, the animals gradually died off, and attempts to acclimatize them to higher external concentrations were not successful. But it seemed to us that, with time and care, it may prove possible to acclimatize *G. lacustris* to undiluted sea water.

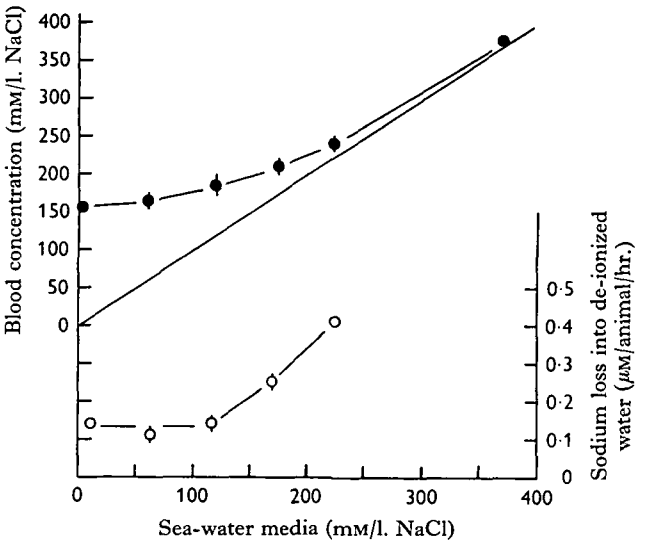

Fig. 1. The total blood concentration (closed circles) and the rate of sodium loss into de-ionized water (open circles) in *G. lacustris* acclimatized to sea-water media at 12 to 14° C. Blood concentrations obtained from freezing-point determinations on individuals; each point is the mean of five to nine individuals, vertical lines indicate standard deviations (±4 to 14 mM/l.). Loss rates are the means of four to twelve groups, with standard deviations (±0·02 mM/l.).

The difference in tolerance between this species and *G. pulex* is very striking. The latter cannot be rapidly acclimatized to concentrations greater than about 225 mM/l. NaCl, and even here there is often a high mortality rate (Beadle & Cragg, 1940; Sutcliffe, 1967*a*), although Sexton (1928) managed to acclimatize *G. pulex* very gradually to undiluted sea water. In fact, the wide tolerance shown by *G. lacustris* resembles that of the trichopteran larva *Limnephilus affinis*, a fresh-water insect which is also adapted for living in brackish water (Sutcliffe, 1961, 1962). *G. lacustris* has not been recorded from brackish-water localities (see Segerstråle, 1954), but apparently it occurs in inland salt lakes in Russia (Basikalova *et al.* 1946). In view of these observations it would be interesting to make a thorough investigation into the osmoregulatory mechanisms (or lack of them) in *G. pulex* and *G. lacustris* along the lines begun by Beadle & Cragg (1940). The only information we have obtained so far is that, like *G. pulex*, *G. lacustris* imbibes sea-water media containing the dye Amaranth, and the sodium loss rate into de-ionized water increases markedly when the animals are acclimatized to external concentrations greater than 115 mM/l. NaCl (Fig. 1). These animals were of the same size and weight as *G. pulex*, and the results are practically identical with those obtained with *G. pulex*. To this extent at least the two species appear to be very similar, and they certainly differ from the brackish-water *G. duebeni*

where the sodium loss rate did not increase until the animals were acclimatized to external concentrations greater than 270 mM/l. NaCl (Sutcliffe, 1967*b*).

Sodium influx and net uptake at low external concentrations

Measurements of sodium influx were made over a range of external concentrations from 0·06 to 2 mM/l. NaCl with animals acclimatized to 0·1 mM/l. NaCl for more than a week at 10° C. The average weight of these animals was 48 mg., so the influx and loss rates may be directly compared with those found in *G. pulex* (Sutcliffe, 1967*a*)

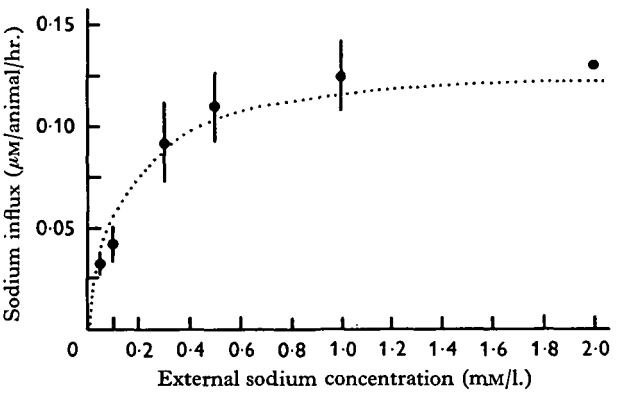

Fig. 2. The relation between the influx and the external sodium concentration in *G. lacustris* acclimatized to 0·1 mM/l. NaCl at 10° C. Each point is the mean influx rate of five to nine groups of animals, except at 2 mM/l. (4 groups). The vertical lines indicate the extent of the standard deviations. The broken line represents: influx = 0·13 [C/(0·14+C)].

Table 1. *The minimum external sodium concentration for balance at 10° C. in* Gammarus lacustris

Group no.	External sodium concentration (mM/l.)
1	0·054
2	0·058
3	0·060
4	0·060
5	0·075
Mean	0·061

The results are shown in Fig. 2 together with a curve derived from the equation: influx = 0·13 [C/(0·14+C)], where C is the external concentration. The curve chosen provides the best fit to the measurements, particularly at the lower external concentrations.

The influx curve is very similar to that found in *G. pulex*. The sodium-transporting system is again fully saturated at an external concentration of about 1mM/l. NaCl, and is half-saturated at an external concentration of about 0·14 mM/l. NaCl. Hence the affinity of the transporting system for sodium ions is almost identical in *G. pulex* and *G. lacustris*, and it is of interest to note that the maximum rate of influx is the same in both species. Furthermore, the minimum external concentration at which

sodium balance can be maintained, 0·06 mM/l. sodium (Table 1), is the same as in *G. pulex*.

When animals acclimatized to 0·1 mM/l. NaCl are in a steady state the rate of sodium influx is balanced by an equivalent loss of sodium, estimated by measuring the loss into de-ionized water (Table 2). When the influx rate in these animals was measured at higher external concentrations it was found that, during the first hour at the new external concentration, the influx rate was increased (Fig. 2). If the influx is a true

Table 2. *Steady-state sodium influx and loss rates in* Gammarus lacustris *at* 10° *C.*

Groups acclimatized to:	Sodium influx (μM/animal/hr.)	*N*	S.D.	Sodium loss (μM/animal/hr.)	*N*	S.D.
0·1 mM/l. NaCl	0·042	5	0·009	0·047	5	0·01
0·3 mM/l. NaCl	0·076	5	0·016	0·070	9	0·017

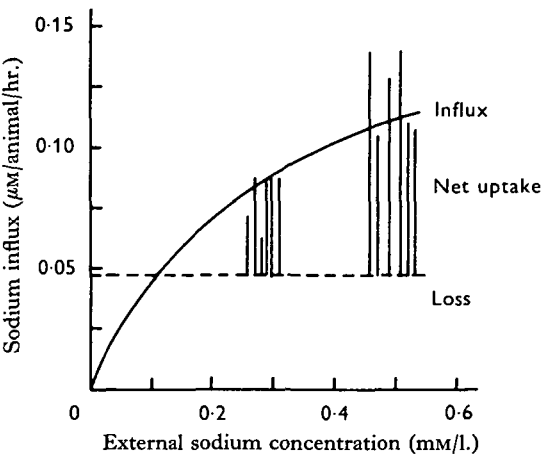

Fig. 3. Net uptake of sodium from 0·3 and 0·5 mM/l. NaCl by *G. lacustris* acclimatized to 0·1 mM/l. NaCl at 10° C. Each vertical line represents the net uptake rate of sodium in a group of animals measured during a 30 min. period. The influx curve is based on Fig. 2. The horizontal broken line represents the mean loss rate into de-ionized water by animals acclimatized to 0·1 mM/l. NaCl (from Table 2).

measure of the rate of movement of unlabelled sodium ions, the increase in influx should be accompanied by a net uptake of sodium, provided that the loss rate of sodium is not suddenly increased. Net uptake of sodium was measured in animals acclimatized to 0·1 mM/l. NaCl. Three groups of fifteen animals were transferred to 20 ml. of 0·3 mM/l. NaCl, and three groups were transferred to 20 ml. of 0·5 mM/l. NaCl. The change in concentration of the external medium was measured at 30 min. intervals during the first hour at the new external concentration. The results of these experiments are shown in Fig. 3.

Now the total uptake rate of sodium will be equal to the sum of the net uptake rate and the passive loss rate of sodium. Assuming that the initial loss rate of sodium at the new external concentration is the same as at 0·1 mM/l. NaCl, i.e. 0·047 μM/hr., it is seen from Fig. 3 that the sum of the loss rate and the net uptake rate agrees very closely

with measurements of the influx rates. It appears, therefore, that in these experiments all of the measured influx can be accounted for by uptake of sodium ions, and there was no exchange diffusion during net uptake. It is also clear that the loss rate was not suddenly increased at the new external concentration. The apparent absence of any exchange component might suggest that the sodium-transporting system in *G. lacustris* is different from that found in *G. pulex*, *G. duebeni* (Shaw & Sutcliffe, 1961), *Astacus pallipes* (Shaw, 1959; Bryan, 1960*a*, *b*) and the mosquito larva *Aedes aegypti* (Stobbart, 1965), all of which exhibit an exchange component linked to the active transport of sodium. However, it will be necessary to make further investigations of sodium influxes in *G. lacustris* before it is certain that there is no exchange component in the sodium influx.

Regulation of sodium influx and loss rates

From a consideration of the influx curve and loss rates in *G. lacustris* acclimatized to 0·1 mM/l. NaCl (Fig. 2, Table 2) it is apparent that if the animals were then transferred to an external concentration of 0·06 mM/l. NaCl the loss rate of 0·047 μM/hr would initially exceed the influx rate of 0·032 μM/hr. and there would be a net loss of sodium. However, a slight adjustment in either the influx rate or the loss rate, or in both, would lead to a balance between the two rates and thus no further net loss of sodium. Both *G. pulex* and *G. duebeni* are able to increase the influx and decrease the loss rate of sodium to maintain balance at very low external concentrations (Shaw & Sutcliffe, 1961), so it is of interest to see if *G. lacustris* can also alter both influx and loss rates.

Sodium loss into de-ionized water was determined in groups of animals acclimatized to a range of external concentrations from 2 to 0·06 mM/l. NaCl. The latter concentration is the lowest at which sodium balance can be maintained over a period of several days. The groups were acclimatized to the external concentrations in a random manner, and the mean loss rates are given in Table 3. At external concentrations from 2 to 0·3 mM/l. NaCl the loss rate remained fairly constant, but at 0·1 mM/l. NaCl the loss rate was reduced by 30–40%, and there was a further reduction in animals acclimatized to 0·06 mM/l. NaCl.

For measurements of changes in the influx rate animals were first acclimatized to 0·1 mM/l. NaCl and the influx was measured at an external concentration of 0·06 mM/l. NaCl. The animals were then acclimatized to 0·06 mM/l. NaCl, and the influx again measured at this concentration. The results are shown in Table 4. The difference between the two influx rates is highly significant ($t = 4{\cdot}59$, $P = 0{\cdot}001$), and represents an increase of 53%. As in *G. pulex*, acclimatization to the lowest possible balance concentration results in an increase in the influx rate by a factor of 1·5, and a reduction in loss rate by a similar amount.

Since the loss rate remained fairly constant at external concentrations from 2 to 0·3 mM/l. it might be expected that, if the animals were in a steady state, the influx rate would also remain constant at these external concentrations. This was tested in the following manner. The influx rates were measured at an external concentration of 0·05 mM/l. NaCl in animals acclimatized to a range of external concentrations from 2 to 0·1 mM/l. NaCl. The same groups of animals were used for all of the influx measurements and groups were acclimatized to a progressively increasing or decreasing range of external concentrations. The measurements were made early on in

Table 3. *The loss rate of sodium into de-ionized water in* Gammarus lacustris *acclimatized to low external concentrations at* 10° *C.*

Groups acclimatized to external concentration (mM/l. NaCl)	Sodium loss rate (μM/animal/hr.)	No. of groups	S.D.
2·0	0·08	6	0·02
1·0	0·08	8	0·017
0·3	0·07	9	0·017
0·1	0·047	5	0·01
0·06	0·038	4	—

Table 4. *The effect of acclimatization to low external concentrations on the sodium influx in* Gammarus lacustris at 10° *C.*

Groups acclimatized to:	Sodium influx from 0·06 mM/l. NaCl (μM/animal/hr.)	No. of groups	S.D.
0·1 mM/l. NaCl	0·032	7	0·007
0·06 mM/l. NaCl	0·049	5	0·006

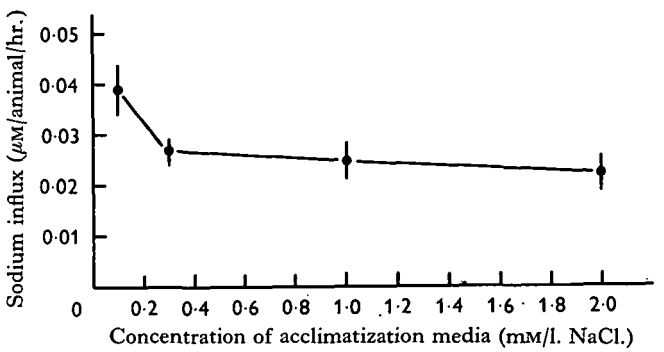

Fig. 4. The rate of sodium influx from 0·05 mM/l. NaCl in *G. lacustris* acclimatized to a range of external concentrations. Each point is the mean influx in five to seven groups, the vertical lines indicate the extent of the standard deviations.

the investigation and were carried out at room temperature, which fluctuated mainly between 14 and 20° C. The results are shown in Fig. 4. It is clear that, like the loss rate, the influx rate remained fairly constant over the range 2–0·3 mM/l. NaCl. Comparing the mean influx in animals over this range, the difference, 0·005 μM/hr. is not significant. But the increase in influx rate by 45% in animals acclimatized to 0·1 mM/l. is highly significant when compared with the influx rate at 0·3 mM/l. NaCl ($t = 6{\cdot}31$, $N = 12$, $P < 0{\cdot}001$).

Finally, we may consider the changes in influx and loss rates when animals acclimatized to 0·1 mM/l. NaCl were transferred to 0·3 mM/l. NaCl and allowed to become acclimatized to the new external concentration. We have already seen that during the initial period at the new concentration there was no change in loss rate and the higher

influx rate (0·09 μM/hr.) resulted in a net uptake of sodium (Fig. 3). However, after 24 hr. acclimatization to 0·3 mM/l. NaCl the influx rate was reduced and the loss rate increased to achieve a new steady state (Table 2).

DISCUSSION

The results show that the overall features of sodium regulation in *G. lacustris* are virtually identical with those found in *G. pulex*. In both species the blood concentration, when in fresh water, is maintained at the low level typical of many fresh-water animals, and in both a balance between sodium uptake and loss at external concentrations between 0·3 and 0·1 mM/l. sodium is achieved by increasing the influx rate and decreasing the loss rate. On the other hand, at concentrations above about 0·3 mM/l. both the influx and loss rates remain approximately constant.

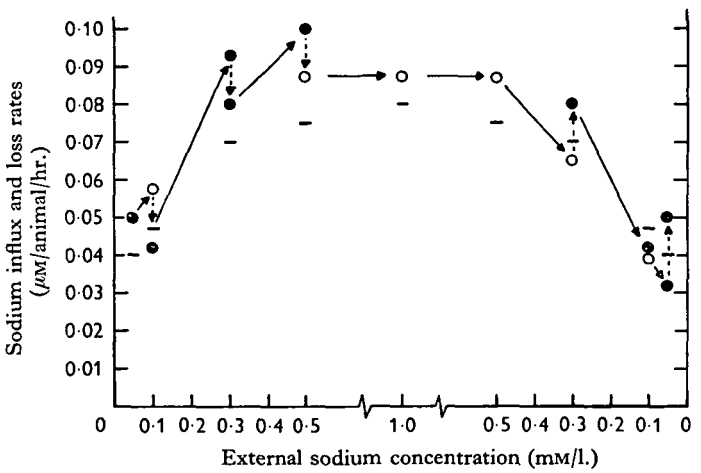

Fig. 5. The relationship between changes in sodium influx and loss rates when *G. lacustris* is placed in a series of increasing (left) and decreasing (right) external sodium concentrations. Solid circles represent measured influx rates, open circles represent influx rates calculated from influx curves. Solid arrows indicate the initial influx rates at the new external concentrations, broken arrows indicate the final influx rates when the animals are fully acclimatized to the new external concentrations. The horizontal bars are loss rates measured with animals fully acclimatized to the external concentrations.

In *G. pulex*, changes in the influx and loss rates are linked to changes in the blood sodium concentration, and it was suggested that a drop in blood sodium activated the sodium-transporting systems at the body surface and in the antennary glands, resulting in the elaboration of a very dilute urine. Part of the reduction in sodium loss rate was also due to a reduction in diffusion across the body surface (Sutcliffe, 1967*a*). It is highly probable that the same mechanism operates in *G. lacustris*, particularly since it is known to occur in other fresh-water crustaceans (Shaw, 1964). Certainly there is a very close relationship between alterations in the rates of sodium influx and loss. This is illustrated in Fig. 5. When animals are moved from 0·1 to 0·3 mM/l. and higher external concentrations the influx rate at the body surface is initially greater, due to the fact that the sodium-transporting system can move a greater amount of sodium at these concentrations where the transporting system is approaching its saturation level.

During this period there is a net uptake of sodium. But the influx rate is then depressed (broken arrows, Fig. 5) and the loss rate is increased to balance the new rate of sodium uptake. In a series of decreasing external concentrations the process is reversed, with an increase in the influx and a reduction in sodium loss as the animals adjust to the new situation.

SUMMARY

1. The sodium balance mechanism of *Gammarus lacustris* in fresh water is virtually identical with that found in *G. pulex*.

2. The sodium transporting system at the body surface has a very high affinity for sodium ions. The system is half-saturated at an external concentration of about 0·14 mM/l. and fully saturated at about 1 mM/l. sodium.

3. The lowest external concentration at which sodium balance was maintained was 0·06 mM/l.

4. Both the total sodium loss rate and the sodium influx rate remained approximately constant in animals acclimatized to the range of external concentrations from 2 to 0·3 mM/l. NaCl. At lower concentrations the loss rate was reduced and the influx increased by a factor of about 1·5.

5. Changes in the sodium influx and loss rates are very closely linked together, and it is shown how these changes are related to the external sodium concentration.

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