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SODIUM BALANCE IN THE FRESHWATER PRAWN, *PALAEMONETES ANTENNARIUS*

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INTRODUCTION

Palaemonid prawns are found in a wide range of environments from fresh water to sea water. The species *Palaemonetes varians* which occurs in Britain can live in any salinity between slightly concentrated sea water and very dilute brackish water, but cannot survive in salinities lower than about 0·5% sea water. Its flexible osmoregulatory requirements are reflected in its widespread distribution. The closely related *P. antennarius* is found in southern Europe in completely fresh water, but while it is common in some localities it is restricted in its distribution.

P. varians in sea water maintains a blood concentration equivalent to about 70% sea water, and in dilute brackish water one equivalent to about 50% sea water (Potts & Parry, 1964). In all experimental media between 5% and 100% sea water the urine is almost isosmotic with the blood (Parry, 1957). Sodium and chloride balance in *P. varians* has been described by Potts & Parry (1964). In waters more concentrated than the blood, sodium ions are actively extruded, the resulting negative potential maintaining the chloride balance. In solutions less concentrated than the blood, chloride ions are actively taken up and the resulting potential, again negative, helps to maintain sodium balance although there is also a sodium pump taking up sodium ions, which is essential in the lower concentrations. This system contrasts with that of the freshwater crayfish or of the frog where active sodium uptake is the primary means of osmotic regulation in fresh water, the resulting positive potential helping to maintain chloride balance.

P. antennarius maintains a blood concentration equivalent to about 40% sea water (Δ = 0·75° C.) and the urine is almost as concentrated as the blood (Δ = 0·67° C.). The rate of urine production averages 2·2% body weight/hr. (Parry, 1957). The more restricted range of habitat in completely fresh water of *P. antennarius* suggests an osmoregulatory mechanism specialized for salt uptake from low concentrations but unable to acccommodate to different environments. In contrast to *P. varians*, *P. antennarius* does not alter the urine flow in response to salinity change, nor can water fluxes be controlled (as shown by weight changes) although the prawns show some tolerance to changes of the medium. Not only are the prawns very permeable to water, but also to salts (Parry, 1957, 1961). The discontinuous distribution of *P. antennarius* in southern Europe has been attributed to the inability of the prawns to take up salts below a particular threshold concentration (Parry, 1961).

The experiments reported in this paper were undertaken to clarify the hypotheses as to salt balance in this freshwater prawn.

MATERIALS AND METHODS

P. antennarius was collected from two localities near Verona in northern Italy; at Peschiera where the river Mincio flows out of Lake of Garda, and at Caldiero, a warm spring in the Po valley. Peschiera water contains 1·12 mM-Ca/l. and 0·18 mM-Na/l. (Marchesoni, 1952); Caldiero water contains *ca.* 2 mM-Ca/l., 1·22 mM/l. sodium + potassium, and some magnesium and sulphate (Frederici, 1948). Caldiero water rises at 28° C. and prawns are found in the hottest part of the basin.

Flux measurements were made with ^{24}Na-labelled sodium chloride using a GM end-window counter. Rates of influx and efflux were determined by methods previously described (Potts & Parry, 1964). These experiments were made in Italy, with freshly collected animals.

A number of animals were brought back to England for chemical analysis and potential measurements. The animals are very easily maintained in aquaria and it is unlikely that these animals differed physiologically from those used locally.

Sodium and potassium concentrations were measured with an EEL flame photometer. Total body sodium and potassium were measured after dissolving the dried animal in a small quantity of concentrated nitric acid and diluting appropriately. Sodium concentrations in the blood were measured after diluting weighed quantities of blood with deionized water. Blood samples were obtained from the heart or dorsal abdominal vessel with fine silica glass capillaries filled by surface tension. Animals were dried overnight at 105° C. for estimation of water content.

Potential measurements were made with the aid of fine glass electrodes filled with 3 M-KCl, a cathode follower and pen recorder. Animals were restrained in a Perspex-and-wax vessel by threads across the thorax and abdomen. The water level could be adjusted so that the dorsal surface of the animal projected above the water. As the surface is hydrofuge, electrodes could then be inserted behind the posterior edge of the thorax or between the first two abdominal segments without short circuiting. Animals were equilibrated in each solution for 3 min., then four measurements were taken at minute intervals before the water was changed. The readings in any solution rarely varied more than 5 mV. from the mean. The order in which the solutions were applied was randomized. This rather hasty procedure was necessary because the experiments were often prematurely terminated by violent movements of the animals and because the stock of animals in England was very limited.

P. antennarius is found in water containing about 0·2 mM-Na/l., but in aquaria the animals did not survive well in solutions containing 0·25 mM-Na/l. or less. The animals were found to survive for several days in a solution containing 150 mM-Na/l. but not in one containing 250 mM-Na/l. Experiments were therefore made with animals equilibrated for at least 24 hr. in solutions containing 0·5, 10 and 150 mM-Na/l. Efflux measurements were also made on animals transferred to distilled water after adaptation to 0·5 mM-Na/l. and influxes were measured in animals transferred for a short period to solutions containing 0·05, 0·125 and 0·25 mM-Na/l. All experiments were carried out at $16 \pm 2°$ C.

RESULTS

Sodium content of Palaemonetes antennarius

The sodium content of the blood and whole body of animals adapted to a solution containing 0·5 mM-Na/l. and of the blood of animals adapted to solutions containing 10 and 150 mM-Na/l. are shown in Table 1. Sufficient animals were not available for measurements of the total body sodium in the higher concentrations. The figures in square brackets are calculated from blood concentrations on the assumption that the total body sodium is proportional to the blood concentration.

In the medium with 0·5 mM-Na/l., the water content of the animal was 83·6%, and potassium was 81 mM/kg. body water. In *P. varians* in 2% sea water, the water content was 78%, and potassium was 90 mM/kg. body water (Potts & Parry, 1964).

Table 1. *Sodium content of the medium and the blood of* Palaemonetes antennarius

Medium (m-equiv. Na/l.)	0·5	10	50
Blood (m-equiv. Na/kg.)	177 ± 10 (7)	197 ± 10 (7)	271 ± 40 (4)
Whole animal (m-equiv. Na/kg. water)	75 ± 6 (5)	[83]	[115]
Whole animal (m-equiv. Na/kg. body)	63	[70]	[96]

Mean ± standard error (no. of observations).

Table 2. *Electrical potential between blood and medium in* Palaemonetes antennarius

Blood negative to medium. For details see text.

	Potential (mV.)						
Medium (m-equiv. Na/l.)	Salt-deficient animals			Normal animals			Salt-loaded animal
	(1)	(2)	(3)	(4)	(5)	(6)	(7)
0·5	44	28	25	27	28	44	23
10	28	34	23	—	22	—	35
150	41	54	26	31	—	—	19

Potential across the body wall

All potential measurements showed that the animals' body fluids were negative with respect to the media. This implies that the chloride uptake is more powerful than the sodium uptake. *P. varians* is similarly negative with respect to the medium, but in the frog the sodium pump is more powerful (if indeed a chloride pump exists in the frog) and the body fluids are positive with respect to the medium. In *Astacus*, the sodium pump is the primary factor in controlling the blood concentration but the chloride pump is sufficiently powerful to maintain the animal almost electrically neutral (Shaw, 1960).

No systematic variation of potential with the concentration of the medium is apparent in Table 2. This is probably because the experiments were so short that adaptation did not take place. Again no systematic differences are observable between normal, salt-loaded and salt-deficient animals. This does not necessarily mean that the activity is the same in all animals. The potential will depend on the balance of the activity of the sodium and chloride pumps. In the dilute medium both pumps

must be active. In the absence of a sodium pump a potential of 150 mV would be required to maintain the blood sodium at 177 mM/l. in a medium containing 0·5 mM-Na/l.

Effluxes

The effluxes of sodium from *P. antennarius* in three solutions covering most of the viable range of the animals, and in distilled water, are given in Table 3. It has been assumed in calculating the effluxes that the exchange of sodium between tissues and blood is rapid compared with the exchange across the body surface, so that the animal may be treated as a one compartment system. Any error in the assumption will be small, as the rate constants of exchange between blood and tissues are usually much larger than the rate constants for the effluxes (Table 3). The data available allows the calculation of the effluxes in terms of mM/kg. blood/hr. in all three salinities, but in terms of mM/kg. animal/hr. only in the normal salinity of 0·5 mM-Na/l. For the purpose of general comparison, the effluxes from animals in the solutions containing 10 and 150 mM-Na/l. have been estimated from the computed total body sodium on the assumption that the water content of the body is the same in all three solutions. The error in this assumption will be small.

Table 3. *Efflux of sodium from* Palaemonetes antennarius *in different media*

	Distilled water	0·5 (m-equiv. Na/l.)	10 (m-equiv. Na/l.)	150 (m-equiv. Na/l.)
Rate constant of efflux hr.$^{-1}$	0·063 ± 0·08 (8)	0·053 ± 0·010 (8)	0·081 ± 0·007 (14)	0·081 ± 0·010 (8)
Efflux (m-equiv./kg. blood/hr.)	11·1 ± 2·1	9·4 ± 1·8	16 ± 1·6	22 ± 4·2
Efflux (m-equiv./kg. animal/hr.)	3·9	3·3	4·8	6·5
Urine loss (m-equiv./kg. animal/hr.)	3·5	3·5	4·3	3·7

Mean ± standard error (no. of observations).

Table 4. *Influxes of sodium in* Palaemonetes antennarius *in different media*

(m-equiv. blood/hr.)

Medium in which influxes were measured (m-equiv. Na/l.)	Previous medium			
	Distilled water 3 hr.	0·5 m-equiv. Na/l. 24 hr.	10 m-equiv. Na/l. 24 hr.	150 m-equiv. Na/l. 24 hr.
0·05	3·2 ± 0·6 (10)	—	—	—
0·125	5·4 ± 1·0 (10)	5·1 ± 0·8 (8)	—	1·32 ± 0·26 (6)
0·25	10·3 ± 1·7 (5)	—	—	—
0·5	14·1 ± 0·9 (13)	9·7 ± 0·8 (16)	—	10·3 ± 1·0 (4)
10	68 ± 8 (10)	—	20 ± 3·7 (8)	13·5 ± 2·9 (4)
150	65 ± 9 (17)	—	—	31 ± 4·5 (13)

Mean ± standard error (no. of observations).

Influxes

Influxes were measured in three groups of animals: animals in equilibrium with their media, animals salt-loaded by being kept in a solution containing 150 mM-Na/l. for 24 hr., and animals rendered salt-deficient by being kept for 3 hr. in a considerable volume of distilled water, changed at hourly intervals, having previously been adapted

to the solution containing 0·5 mM-Na/l. Influxes were measured not only in the three solutions in which the animals could survive indefinitely, but also in very dilute solutions containing 0·25, 0·125 and 0·05 mM/l. (Table 4). Influxes into two small groups of animals from Caldiero were also measured (not included in Table 4). After transfer from Caldiero water to a medium containing 0·5 mM-Na/l. the average rate of influx into these Caldiero animals was 12·3 mM/kg. blood/hr., and after transfer to a solution containing 0·125 mM-Na/l. the influx into four animals averaged 6·0 mM/kg. blood/hr. From these results it seems unlikely that the Caldiero animals differ significantly from those from Peschiera.

DISCUSSION

Agreement of influx and efflux

The agreement between the measured influxes and effluxes in animals equilibrated to solutions containing 0·5, 10 and 150 mM-Na/l. is fairly good (Tables 3 and 4); in only one case is the difference greater than the sum of the standard errors.

The initial sodium loss from animals transferred from the solution containing 0·5 mM-Na/l. to deionized water is 3·9 mM-Na/Kg. animal/hr. but the efflux would decline as the total body sodium declines. Two independent estimates of the rate of loss of salt from *P. antennarius* are available (Parry, 1957). The first estimate was based on the increase in the conductivity of distilled water containing the animal on the assumption that the increase in the conductivity was due to lost sodium chloride alone. The estimated chloride loss by this method was 34 mM/kg. animal/hr. The second estimate was based on the rate of decline of the blood concentration, measured by the freezing-point depression, after transfer to distilled water. The rate of decline of the total osmotic concentration averaged 3%/hr. over 6 hr., corresponding to a rate constant of 0·03 hr.$^{-1}$. However, sodium and chloride ions account for only about 50% of the osmotically active substances in the body. If the total body osmotic pressure declines by 3%/hr. but virtually all this loss is of sodium chloride alone, then the rate of loss of these ions would be as high as 7·5%/hr., and the blood volume would decline rapidly. The real rate of loss of ions doubtless lies between 3 and 7·5%, in agreement with tracer measurements. The estimate based on conductivity measurements is too high, and the observed increase in conductivity might well be due to the accumulation of bicarbonate and other ions.

Effluxes

The efflux will consist of two components, loss through the body wall and loss in the urine. Urine loss can be estimated approximately from the data of Parry (1957). The rate of urine production in fresh water averaged 2·2% of the body weight/hr. If it is assumed that the relative contribution of sodium to the total osmotic pressure is similar to that in the blood, then sodium loss in the urine will be 3·5 mM-Na/kg. animal/hr., when the animal is in a medium containing 0·5 mM-Na/l., and 4·3 mM/kg. in a medium containing 10 mM-Na/l. In a medium containing 150 mM-Na/l. the osmotic gradient between the blood and medium will be reduced and the rate of urine production might be smaller in proportion, but the urine would be more concentrated. In these conditions the urine loss might be about 3·7 mM-Na/kg. animal/hr.

In the medium containing 0·5 mM-Na/l. the computed urine loss exceeds the observed total loss. The calculated loss may well be overestimated since sodium may be less concentrated in the urine than in the blood, but it is clear that extrarenal loss must be very low. In higher salinities extra-renal loss becomes significant. This might indicate exchange diffusion, or an increase in blood concentration combined with a decline in the potential across the body wall, allowing a greater escape of sodium. In distilled water the efflux appears to be slightly greater than in fresh water. If this increase is real it is unlikely to be due to an increase in urine production (Parry, 1961) and cannot be due to exchange diffusion. It could again indicate a decline in the potential across the body wall following the decline in active uptake.

Influxes

The rate of influx depends on the salt load of the animal; salt-deficient animals have a much higher rate of uptake from any solution than have equilibrated animals, while salt-loaded animals have a lower rate of uptake. A similar phenomenon has been observed in *Astacus* (Shaw, 1959; Bryan, 1960*a*, *b*, *c*).

The influx in any solution must contain two components, one due to active uptake, the other to passive inward diffusion. However, when the external concentration is 0·5 mM-Na/l. or less, the passive component of sodium influx must be negligible even in the presence of a potential attracting positive ions into the animal.

The relation between uptake and external concentration clearly follows a curve of the kind described by Shaw (1959) for *Astacus* uptake. At low external concentrations the influx is roughly proportional to external concentration but at higher concentrations the influx begins to level off, presumably as a sodium carrier becomes saturated. The influx into animals transferred to a solution containing 0·125 mM-Na/l., from one containing 0·5 mM-Na/l., is only slightly less than the influx into animals made salt-deficient by washing in distilled water (Table 4). This is probably because the animals living in the 0·5 mM/l. solution are near their viable limit and are already a little salt-deficient. Salt-loaded animals from 150 mM/l. solution have a much lower rate of uptake.

It is noteworthy that the rate of influx declines rapidly when the external salinity falls below 0·5 mM/l. while the efflux remains almost constant. This may indicate a threshold effect and could be an important factor in determining the distribution of *P. antennarius*. The water of Lake Garda and the River Mincio contains about 0·2 mM-Na/l. as well as a high concentration of calcium, but the animals are not found in other lakes draining into the Po Valley. The water of the other lakes, has lower concentrations of ions; for instance, Lake Maggiore contains 0·5 mM-Ca/l. and only 0·03 mM-Cl/l. (Parry, 1961).

In concentrated solutions the influxes reach very high levels. It is difficult to determine how much of this influx is due to passive diffusion aided by the electrical potential, how much to active uptake and how much to exchange diffusion. A potential difference of as much as 40 mV, will only double the passive influx and halve the passive efflux (Potts & Parry, 1964). In dilute media, with a blood concentration of 177 mM-Na/l., passive influx is very low (< 1 mM/kg. animal/hr. or < 3 mM/kg. blood/hr.) so that passive influx from a solution containing 150 mM-Na/l. is still unlikely to exceed 10 mM/kg. animal/hr. One might postulate, therefore, that most

of the influx is active, and that the levelling of the influx at higher concentrations in salt-deficient animals is due to saturation of the uptake mechanism. In equilibrated animals in the 150 mM/l. medium influx is less than in the salt-deficient animals but active uptake is still rapid. As a result, the animals become hyperosmotic in media initially isosmotic with their blood, and will eventually die. In contrast, *P. varians* in these conditions ceases active uptake.

SUMMARY

1. The sodium content of the freshwater prawn *Palaemonetes antennarius* and the rates of influx and efflux of sodium in fresh water and a variety of other media are described.

2. The greater part of the influxes in all media is due to active uptake. The relationship between active uptake and the external and internal concentrations is described and it is concluded that the animal is living close to its viable limits of dilution.

3. The greater part of the efflux in fresh water is due to loss in the urine.

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