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STUDIES ON FRESHWATER OSMOREGULATION IN THE AMMOCOETE LARVA OF *LAMPETRA PLANERI* (BLOCH)

II. THE EFFECT OF DE-IONIZED WATER AND TEMPERATURE ON SODIUM BALANCE

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INTRODUCTION

The sites and extent of ion loss and uptake have been investigated in ammocoetes (Hardisty, 1956) and in adult river lampreys (Morris, 1956, 1960; Bentley, 1962; Bentley & Follett, 1962) and it has been shown that the mechanism of ion balance is in many respects similar to that of a freshwater teleost. In the river lamprey the urinary loss of ions is small in comparison with the loss from the skin and the branchial epithelium (Wikgren, 1953; Morris, 1960; Bentley & Follett, 1962) and this is balanced by an active transport mechanism involving sodium, potassium and chloride (Morris, 1960).

Many investigators have pre-treated animals in distilled or de-ionized water in order to promote ion loss and to produce a negative ion balance prior to measuring the rates of ion uptake. In some cases (Wikgren, 1953; Morris, 1960) a combination of low temperature and de-ionized water has been found to be more effective. We have used this technique in experiments which will be reported later, and we decided to try to analyse the factors which were involved in promoting negative ion balance in this way, not only because the pre-treatment can affect the validity of subsequent experiments, but also because this gave an opportunity of studying the effects of temperature on sodium uptake and sodium loss.

MATERIALS AND METHODS

Ammocoete larvae (3–5 g.), in the year previous to metamorphosis, were used in these investigations. Details of the sources of animals, the methods of collection and identification, together with the methods of chemical analysis employed, are described in a previous publication (Bull & Morris, 1968). A Unicam SP. 90 atomic absorption spectrophotometer was used for calcium determinations.

Net flux of sodium was measured on individual animals which were allowed to swim freely in 100 ml. of de-ionized water contained in darkened conical flasks which had been coated internally with silicone. The water was aerated slowly by filtered compressed air delivered from clean glass tubes. Two ml. samples were withdrawn for analysis by flame-photometry. When changes of water were necessary, the experi-

mental solutions were carefully siphoned off, both flask and animal were washed with de-ionized water, and a fresh 100 ml. introduced.

Experiments to determine *influx and outflux rates of sodium* in animals were also conducted on individuals which were placed in smaller volumes of solution (25–30 ml.) in order to obtain reliable measurements of the change of radioactivity. Containers were constructed from Pyrex boiling tubes (150 × 25 mm.) by sealing an open side-arm (30 × 8 mm.) 10 mm. from the bottom. The animal and the radioactive solution were held within the horizontal tube by means of a neoprene bung covered by plastic sheet and the vertical side arm served as a means of introducing a glass tube for aeration as well as allowing pipettes to be introduced for taking samples. The tubes were painted black to minimize disturbance to the animals during experiments. Experimental solutions were made up from diluted, sodium chloride-free Ringer solution diluted 1/100 with de-ionized water. Sufficient ^{24}Na in isotonic saline was then added to give a count rate of 3000–8000 counts/min. after which a calculated amount of sodium chloride was added to give the required total external sodium level. Animals were allowed at least 15 min. to settle in the containers before withdrawing the initial samples of 1 ml. for chemical analysis by flame photometry and 0·2 ml. for the determination of count rates. The latter samples were transferred to planchets previously treated with dilute detergent. They were dried under infra-red lamps and counted under an end-window Geiger Müller tube connected through a quench unit to a decatron scaler and counter. Sufficient counts were made to keep the counting error below $\pm 1{\cdot}5\%$ of the total. Count rates were corrected to zero time to allow for the decay of ^{24}Na and sodium influx was then calculated from plots of log count rate against time, using the formulae derived by Jørgensen, Levi & Ussing (1946). The formulae assume that there is no marked decrease of radioactivity in the external solution and no significant loss of radioactivity from the animal, and checks were made to see that both these conditions were fulfilled during the experiments. Assessments of net flux were obtained from sodium analyses, whilst outflux was derived from the measurements of net flux and influx.

SODIUM BALANCE IN DE-IONIZED WATER AT 10° C.

Krogh (1937) was the first to use distilled water as a method of depleting animals of ions in order to measure ion loss and to prepare the animals for subsequent measurements of ion uptake.

Figure 1 illustrates the net exchange of sodium which takes place when an ammocoete is subjected to five successive changes of de-ionized water (100 ml.) maintained at 10° C. This example is typical of six similar experiments which were performed on individual ammocoetes and shows that ion loss takes place rapidly during the first period of immersion. In later periods (2–5), the initial ion loss is balanced by sodium uptake, so that the animal maintains an equilibrium with its environment at a particular concentration of sodium. This concentration appears to stabilize as the experiments continue and eventually reaches a minimum value (*the minimum equilibrium concentration*) after the 4th or 5th change of de-ionized water in each of the six experiments. The minimum equilibrium concentration, which varies between 0·005 and 0·03 mM-Na/l. for ammocoetes, is a measure of the efficiency of the ion balance mechanism in

fresh water and indicates that the sodium transport mechanism is able to operate in very low concentrations of sodium, lower than that of the crayfish (0·009–0·02 mM Na/l.; Shaw, 1959) or of the roach (0·02 mM-NaCl/l.; Krogh, 1937).

Shaw (1957) calculated the normal rate of sodium loss of the crayfish from the sodium level attained after a period of immersion of 3 hr., since at this stage the sodium level of the environment is well below the minimum equilibrium concentration and

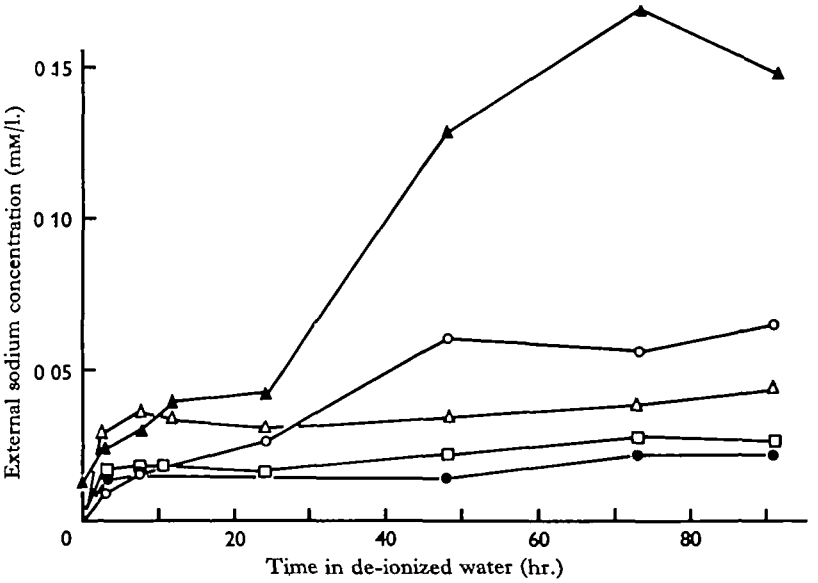

Fig. 1. The attainment of a minimum external concentration for a typical individual: weight 2·4 g. ▲, ○, △, □ and ● represent 1st, 2nd, 3rd, 4th and 5th 90 hr. periods respectively.

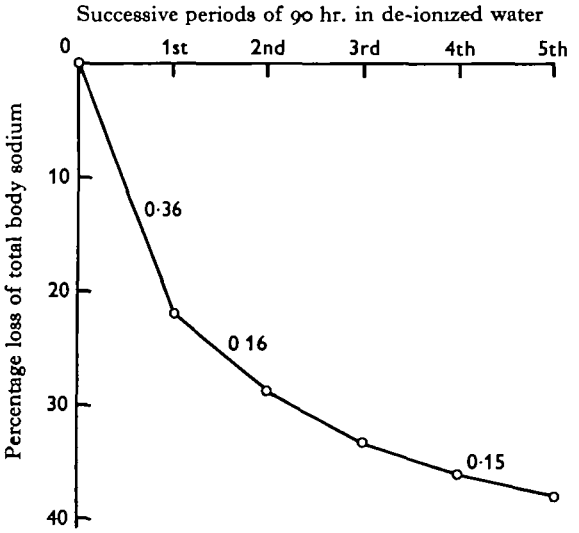

Fig. 2. The percentage loss of total body sodium lost by ammocoetes during successive periods in de-ionized water at 10° C. Drawn from mean values calculated from experiments on six individuals (mean weight 3·5 g.) Figures refer to mean loss rates of sodium during the first 3 hr. after immersion and are expressed in μM-Na/g./hr.

presumably the uptake mechanism is unable to operate. In ammocoetes the initial sodium loss varies during succeeding periods of immersion, and calculations show that animals lose about 50% of the sodium lost throughout the experiments during the first immersion period, whilst they manage to reduce the loss to between 1 and 5% of the total output by the third and fourth immersion.

Figure 2 illustrates the way in which sodium loss affects the sodium content of the whole animal and has been calculated using the value of 26·04 μM-Na/g. animal obtained by Bull & Morris (1968). The results indicate that the high rate of sodium loss during the first period (mean value of $0{\cdot}36 \pm 0{\cdot}006$ μM-Na/g./hr. for six animals) is likely to be an abnormal situation arising from factors which will be discussed later. The rate of sodium loss for succeeding periods ranges from 0·04 to 0·33 μM-Na/g./hr. and the mean value of $0{\cdot}16 \pm 0{\cdot}016$ μM-Na/g./hr. is within the range of isotopically measured loss rates (Table 1) and may be taken as approaching the normal rate of sodium loss.

The only comparable experiments are those of Hardisty (1956), who obtained value of 1·8 μM-Cl/g./day from ammocoetes kept in distilled water. Assuming that these animals followed a similar response pattern to ours, they should have reached their equilibrium concentration in about 10 hr., from which we calculated a loss rate of 0·18 μM-Cl/g./hr., which is in reasonable agreement with our assessment of sodium loss.

SODIUM BALANCE IN DE-IONIZED WATER AT 1° C

Temperature and net flux

In animals like the ammocoete, where the rate of ion loss in de-ionized water is relatively low, prolonged treatment is required before the animal gives the maximum rate of sodium uptake (Fig. 1). Wikgren (1953) was the first to realize that this time period could be shortened by maintaining animals in distilled water at low temperature (1° C).

Figure 3 gives examples of typical individual responses taken from a group of five similar experiments. At low temperature the high initial rate of sodium loss is reduced after a period of 7 hr., and in some cases (lower curve) there is little or no net loss of sodium after this time. During the 48 hr. of low-temperature treatment, the animals lose 8–38% of their total body sodium, which is almost the same range as that obtained for the first immersion period at 10° C. (11–33%). On raising the temperature to 10° C., a net uptake of sodium occurs which reduces the sodium content of the solution to the minimum equilibrium concentration within 50–72 hr.

Temperature and flux rates

Wikgren (1953) believed that the temperature responses illustrated above (Fig. 3) were caused by a variety of factors. The initial ion loss was attributed to metabolic inhibition of the mechanism responsible for ion uptake, whilst the attainment of a plateau was thought to be brought about by a decrease in permeability caused by low temperature, and to a lesser extent by a 'reactivation' of the uptake mechanism retarded by low temperature.

The experiments described below were designed to test these possibilities further, and involved measuring the sodium uptake and loss rates of four individual animals

at 10° C. by means of ^{24}Na prior to transferring them to de-ionized water at 1° C. for 44 hr. No isotopic measurements were made at this stage because of experimental difficulties associated with lack of 'carrier' ions in de-ionized water, but net fluxes were calculated from conventional analyses. Because the concentration of sodium in

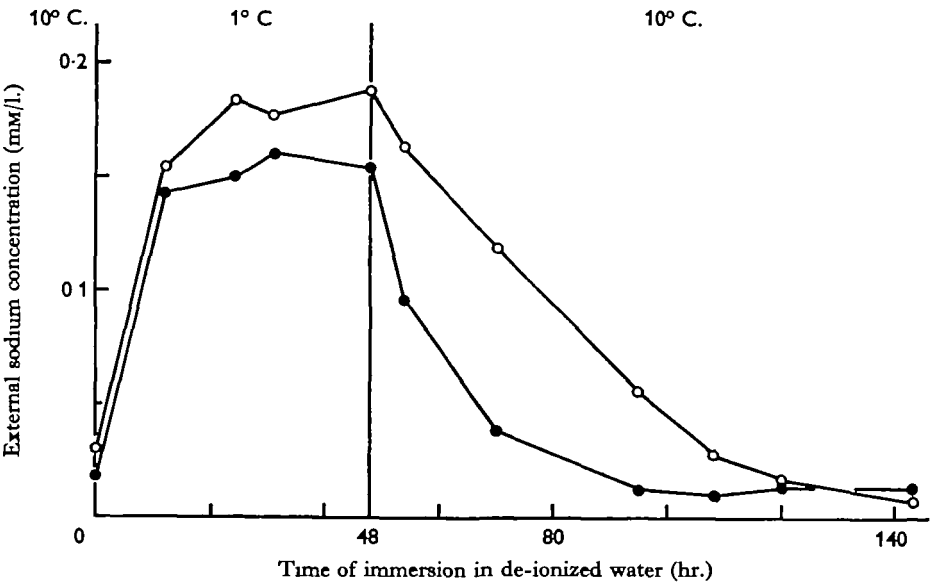

Fig. 3. The effect of low temperature on the sodium balance of two ammocoetes; ○, 2·2 g.; ●, 1·96 g.

Table 1. *The effect of temperature and de-ionized water on sodium flux rates in ammocoetes*

(The values given at 1° C., 7 hr. have been calculated from net flux rates; whilst those at 1° C., 44 hr. are derived from net flux measurements and the loss rates at 10° C. in the following column, using a Q_{10} of 2·55. For further explanation, see text.)

Treatment ...	*R*/100 10° C., 2 hr.			De-ionized water 1° C., 7 hr.		
Animal	Concn. mM/l.	Influx μM/g./hr.	Outflux	Concn. mM/l.	Influx μM/gm./hr.	Outflux
J	0·182	0·162	0·162	0	0	0·318
K	0·194	0·099	0·0465	0	0	0·289
L	0·216	0·264	0·093	0	0	0·295
M	0·172	0·199	0·161	0	0	0·214
Mean values		0·181	0·091	0	0	0·279

Treatment ...	*R*/100 1° C., 44 hr.			De-ionized water 10° C., 47 hr.		
	Concn. mM/l.	Influx μM/gm./hr.	Outflux	Concn. mM/l.	Influx μM/gm./hr.	Outflux
J	0·150	0·082	0·135	0·185	0·180	0·180
K	0·120	0·189	0·205	0·194	0·168	0·274
L	0·200	0·041	0·140	0·651	0·382	0·182
M	0·200	0	0·242	0·317	0·039	0·227
Mean value		0·078	0·180		0·142	0·216

the environment can affect sodium flux rates (Bull & Morris, unpublished data), and the rate of uptake is also dependent on the presence of other ions in solution (Fig. 6), the measurements at 10° C. were made in matched solutions, prepared by adding labelled sodium solutions to sodium-free Ringer solution which had been diluted 1/100 with de-ionized water (*R*-Na)/100.

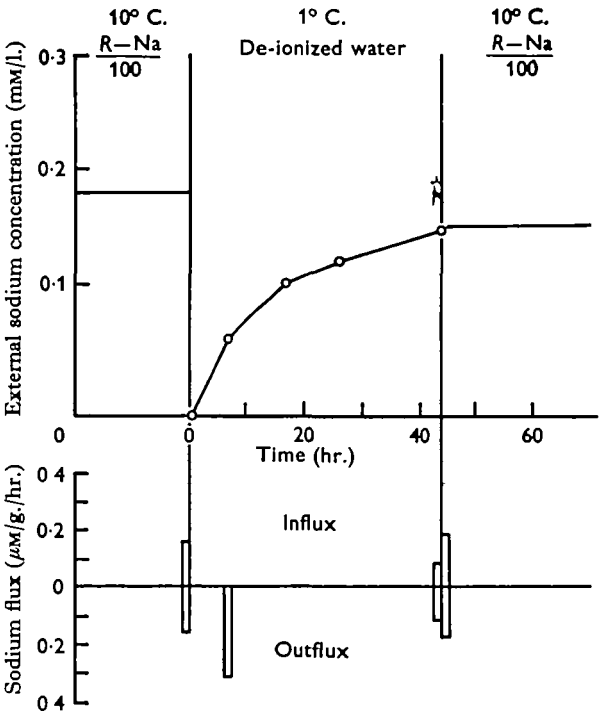

Fig. 4. The effect of temperature on sodium flux in an ammocoete larva; weight 2·9 g. (For further explanation see text.)

The results calculated from four experiments of this type are given in Table 1 and a typical example is illustrated in Fig. 4.

There are two main variables which affect the *sodium loss rates* in these experiments.

The first of these is the effect of transferring the animals to de-ionized water. Table 1 (cf. periods 1 and 2) shows that the mean loss rate increases from 0·091 μM-Na/g./hr. at 10° C. to 0·279 when ammocoetes are immersed in de-ionized water at 1° C. The same effect is evident from previous experiments performed at 10° C. (Fig. 2) where animals lose 0·36 μM-Na/g./hr. when they are first immersed, compared with the assessment of 0·16 μM-Na/g./hr. for acclimatized animals. There seems little doubt that the increased loss rate can be attributed to the specific effect of de-ionized water rather than a more general handling effect, because much lower outflux rates were recorded at 10° C. in *R*/100 in the initial and final stages of these experiments (Table 1, periods 1 and 4) where the degree of handling was much the same.

The second variable is *temperature*. A comparison of the mean sodium loss rates resulting from initial immersion experiments (Table 2) shows that ion loss is reduced by a factor of 0·75 at 1° C. relative to 10° C. This gives a Q_{10} of 2·55 for sodium perme-

ability, which is in close agreement with the value of 2·6 for water permeability obtained by Wikgren (1953) for the river lamprey.

Adaptation to de-ionized water. Figures 1 and 2 show that there is a gradual reduction in initial sodium loss rate with each successive immersion in de-ionized water at 10° C. There is also the possibility of a similar adaptation taking place during the period of immersion in de-ionized water at 1° C. No measurements of flux rates were made towards the end of this period (44 hr.), but it is possible to calculate the sodium outflux at this time from the outflux measured at 10° C. in the period immediately following, if one assumes a Q_{10} of 2·55 (see above). The results of these calculations are shown in Table 1, (1° C.:44 hr.) where they have also been used in conjunction with net flux to evaluate influx. The results suggest that there is a reduction in the rate of sodium loss with time and hence that there is adaptation to de-ionized water.

Table 2. *Sodium loss rates of ammocoetes kept under various conditions in de-ionized water*

(Mean sodium loss rates are followed by standard errors, whilst the figures in parentheses give the number of individuals on which the estimate is based.)

Conditions	Temp. (° C.)	Sodium loss rate (μM/g./hr.)	Source
Initial immersion	10	0·360 ± 0·006 (6)	Fig. 2, period 1.
Acclimatized	10	0·160 ± 0·016 (6)	Fig. 2, periods 2–5.
Initial immersion + Ca (1 mM/l.)	10	0·045 ± 0·008 (6)	Fig. 5.
Initial immersion	1	0·279 ± 0·026 (4)	Table 4, 1° C., 7 hr.
Acclimatized	1	0·180 ± 0·030 (4)	Table 4, 1° C., 44 hr. (calculated value)
Initial immersion + Ca (1 mM/l.)	1	0·079 ± 0·009 (6)	Fig. 5.

Turning now to the *sodium influx rates*, the main effect of de-ionized water is to inhibit sodium uptake until the external concentration of sodium is high enough to allow transport to recommence (Figs. 1, 4; Table 1). The effect of temperature on influx is seen in Table 1 and Fig. 4. Judging from the calculations of sodium influx at 1° C. (44 hr.), though there is some individual variation, the trend is a reduction in influx with decreased temperature (cf. periods 1 and 3) followed by an increased influx of sodium when animals are returned to 10° C. This reduction in sodium influx at low temperature is also apparent when one considers the net flux measurements. Animals lose sodium faster at 1° C. (mean loss of 23·5% of the total body sodium in 44 hr.) than at 10° C. (mean loss of 23·5% of total body sodium in 90 hr.). Since the rate of sodium outflux has been shown to be lower at 1° C., it follows that the rate of uptake must also be lower at this temperature.

Thus the main conclusions reached in this analysis are that de-ionized water not only prevents sodium uptake until the ions reach the minimum equilibrium concentration, but also increases sodium loss. There is evidence that animals adapt by decreasing the sodium loss. Low-temperature treatment decreases sodium loss, but, since this is accompanied by partial inhibition of sodium uptake, there is a faster net loss of sodium than at higher temperatures.

THE EFFECT OF CALCIUM ON SODIUM LOSS IN DE-IONIZED WATER

One possible reason why sodium loss increases when ammocoetes are immersed in de-ionized water is that the animals lose calcium to the environment, and it is well known that the level of calcium at the cells is known to affect both water and ion permeability (Potts & Parry, 1964). The effects of calcium were studied in two different types of experiment.

Table 3. *The rate of calcium loss from ammocoetes to de-ionized water*

(The figures for each temperature are mean values derived from six individual experiments and these are followed by the standard error of the mean. Differences between means are not statistically significant.)

Temp. (° C.)	Ca loss, μM/g. animal	
	18 hr.	48 hr.
10	0·063 ± 0·02 (5)	0·024 ± 0·01 (5)
1	0·129 ± 0·04 (6)	0·155 ± 0·03 (6)

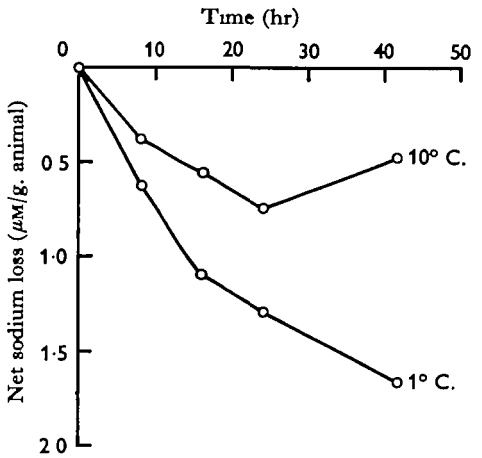

Fig. 5. The effect of calcium (1 mM/l.) on sodium loss in de-ionized water at 1° and 10° C. Values are means from six individual experiments. Differences between treatments are significant at 0·001 level of *P*.

The first group of experiments was concerned with the measurement of calcium loss to de-ionized water. Two groups of six individuals were immersed in 100 ml. of de-ionized water; one group of animals was kept at 10° C. the other at 1° C. The results of these experiments are recorded in Table 3.

The second type of experiment was designed to show the effect of added calcium on the rate of sodium loss in de-ionized water. Two groups of six individuals were immersed in de-ionized water containing 1 mM-$CaCl_2$/l. The two series of experiments, one performed at 1° C. and the other at 10° C., are illustrated in Fig. 5, whilst the mean loss rates are compared with those obtained from previous experiments without added calcium in Table 2.

The first series of experiments show that at 10° C. animals initially lose calcium slowly to the environment, but they are able to take up the ion later and thus maintain

a mean minimum equilibrium concentration of 0·87 ± 0·6 (6) μM-Ca/l. At low temperature the rate of calcium loss is greater than at 10° C. (Table 3) and animals continue to lose calcium during the immersion period presumably because low temperature inhibits the calcium uptake mechanism. It is thus the loss of calcium which is responsible for the high rate of sodium loss to de-ionized water, and adaptation, such as that shown during successive immersions in Fig. 1, is probably the result of a gradual lowering of the minimum equilibrium concentration for calcium so that the animals can then bring about a lowering of the minimum equilibrium concentration for sodium. The fact that high sodium loss rates can be abolished by adding calcium (Fig. 5) strengthens this argument, and animals at 10° C. are able to reach a minimum equilibrium concentration of 3·1 ± 0·14 (6) μM-Na/l. during the first immersion period. Higher sodium loss rates at 1° C. may be the result of a simultaneous inhibition of calcium and sodium uptake, thereby increasing sodium loss and decreasing sodium uptake.

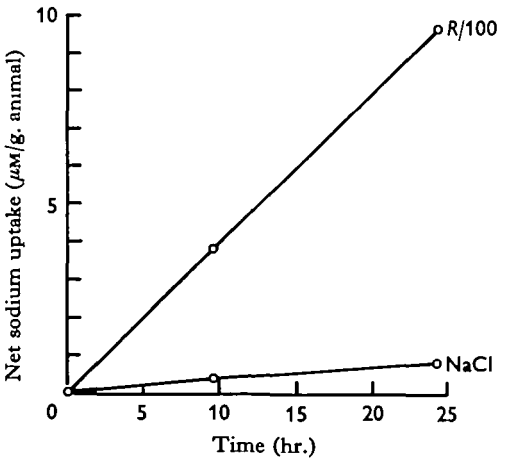

Fig. 6. The effect of additional ions (R/100: Ringer solution diluted 1/100) on sodium uptake compared with sodium chloride, both solutions containing 0·33 mM-Na/l. Ammocoetes were pre-treated in de-ionized water for 48 hr. at 1° C. Values are mean values from five individual experiments and the differences between treatments are significant at the 0·01 level of P.

THE EFFECT OF OTHER IONS ON SODIUM UPTAKE FOLLOWING IONIC DEPLETION AT 1° C.

Since calcium appears to play an important part in determining ion loss, it seemed equally likely to affect the net gain of sodium which occurs when depleted animals are allowed to take up sodium at 10° C. In experiments like those illustrated in Fig. 3, where the animal is kept in the same solution, the presence of calcium is ensured from the calcium loss which takes place from the animal. Figure 6 shows the effect of transferring depleted animals to sodium chloride solutions and to Ringer solution diluted 1/100 with distilled water, i.e. a calcium-containing solution.

These results, taken in conjunction with previous findings, suggest that the apparent inability of animals to take up sodium from pure sodium chloride solutions may be due to an increase in sodium loss rate resulting from lack of calcium. When calcium is present (Fig. 6, R/100) a net uptake of sodium occurs.

IONIC DISTRIBUTION IN TISSUES AFTER TREATMENT IN DE-IONIZED WATER AND LOW TEMPERATURE

In order to discover how de-ionized water and low-temperature treatment affected the ionic distribution within the tissues of whole animals, analyses were made on plasma and muscle samples taken from groups of five individuals at various stages using the type of experimental sequence illustrated in Fig. 4. The analyses involved measuring sodium, potassium and chloride in both types of tissue, together with determinations of the plasma freezing-point depression and the water content of muscle. Figure 7 summarizes the mean values of the series of analyses, and since

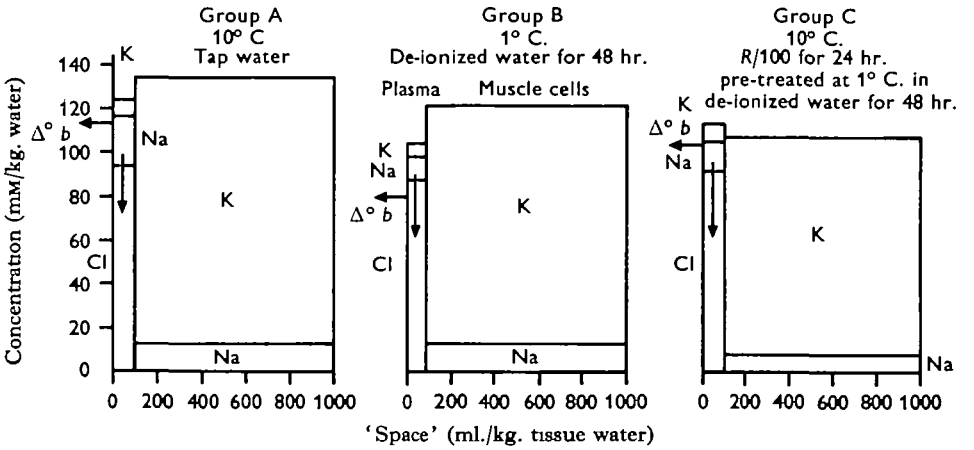

Fig. 7. The effect of low temperature and de-ionized water on the ion compartments of dorsal muscle taken from whole ammonocoetes.

Table 4. *Table of probabilities from Student 't' tests performed on the ionic compartments of dorsal muscle taken from whole animals.*

(Treatments A, B and C refer to those described in Fig. 7 and in the text, and absence of values indicates that there was no significant difference.)

	Treatment	A–B	B–C	C–A
Plasma	Na	0·001	0 05	0 01
	K	—	0·001	0·05
	Cl	0·05	—	—
		0·001	0·01	0·01
Muscle cells	Na	—	—	—
	K	—	—	0·05

chloride in dorsal muscle has been shown to be confined to the extracellular space, it has been possible to calculate the ionic distribution within the extra-cellular and intra-cellular compartments (Bull & Morris, 1968). The first group of animals (A) were taken from tap water maintained at 10° C. and thus represent the ionic distribution for normal animals in November. The second (B) and third (C) groups were transferred to de-ionized water at 1° C. for 48 hr., after which group B were killed and samples taken.

Group C were transferred to 10° C. and left to take up ions for 24 hr. before they were killed. The significance of the difference of the means between groups has been assessed by applying Students '*t*' test and the probability values are given in Table 4.

The differences in total ionic content between the extracellular and intracellular spaces in each group shown in Fig. 7 are not statistically significant. The fat content of the tissues of individual ammocoetes is very variable in November, the time at which these experiments were performed (Bull & Morris, 1968), and the presence of fat can give rise to variation in the levels of ions within a group since the calculations of ionic distribution are based on an assessment of water content taken from the fresh weight of the animals.

The loss of sodium and chloride from the extracellular space is significant, however, when groups A and B are compared, and since animals lose more sodium in proportion to chloride during treatment, part of the sodium loss must be accompanied by an undetermined anion. Within the muscle cells the sodium level remains constant, presumably because this is maintained by the concentration gradient from the extracellular space. There is a significant loss of potassium from the cells, which confirms earlier observations on the river lamprey (Morris, 1960) which showed that an adult lamprey lost considerable amounts of potassium (105 μM) during experiments of this type, much more than the total potassium content of the blood (44 μM).

There is a net uptake of both sodium and chloride into the blood on raising the temperature (Groups B and C, Fig. 7) and the level of potassium also rises significantly, presumably as a result of an active uptake mechanism for potassium which has already been demonstrated in the river lamprey (Morris, 1960). There is no significant difference between the potassium level of muscle after this treatment, which may mean that there is a considerable lag before the muscles regain their lost potassium. Whole muscles show an insignificant amount of variation throughout the experiments.

Thus the effect of de-ionized water at low temperature shows its main effect on sodium loss and to a lesser extent on chloride loss from the blood. The potassium level of muscle suffers most and there is evidence that on raising the temperature the animals recover all three ions.

DISCUSSION AND CONCLUSIONS

These studies indicate that de-ionized water affects ion balance in ammocoete larvae in two ways. De-ionized water promotes calcium loss and increases the permeability of the external surfaces of the animal. As a result of this, ammocoetes show an abnormally high rate of ion loss during the initial stages of immersion in de-ionized water. Attempts to measure ion loss by this method (Krogh, 1937; Hardisty, 1956; Shaw, 1959; Morris, 1960) could therefore give rise to much higher assessments of ion loss than the animal experiences in its natural environment, and in the present experiments, ion loss increased by a factor of 2 or 3. Animals are able to reduce their permeability during successive immersions in de-ionized water and this may be an indication that they are able to reduce the minimum equilibrium level for calcium; and at this time their sodium loss rates are similar to those measured by isotopic methods under more normal circumstances.

De-ionized water also prevents sodium influx until sodium loss from the animal

raises the environmental sodium to a level where uptake can commence. This level reaches a minimum value after successive immersions in de-ionized water because, as in the crayfish (Shaw, 1959), sodium influx increases as the internal sodium content of the ammocoete decreases (Bull & Morris, unpublished data). The progressive reduction of the minimum equilibrium concentration is thus the result of two adaptive processes; one of these is the decrease in ion loss brought about by adaptation of the calcium uptake mechanism, whilst the other involves an increase in the rate of sodium uptake. Low temperature appears to raise the minimum equlibrium concentration for sodium when animals are immersed in de-ionized water and there is no doubt that the main effect is brought about by metabolic inhibition of the sodium uptake mechanism. What evidence there is suggests that sodium loss decreases in response to lowered temperature in the absence of calcium (Table 2) whilst added calcium greatly reduces the rate of sodium loss.

Krogh (1936) drew attention to the necessity of including a range of physiologically important ions in the environment when attempting to demonstrate net uptake of ions by teleosts following ionic depletion in distilled water. Our experiments on ammocoetes show that they need the same conditions and indicate that the demonstration of net ion uptake may depend upon the presence of calcium in the environment since it is this which enables the animal to reduce simultaneous outflux to normal proportions. Little is known about the effect of calcium on sodium influx. Shaw (1960) was unable to obtain any effect in sodium-depleted *Astacus* at concentrations below 1 mM/l., though he recorded a reduction in influx at concentrations above this.

SUMMARY

1. An investigation has been made of the factors which cause sodium loss from ammocoetes when they are immersed in de-ionized water at 1° and 10° C.

2. Sodium influx ceases when animals are first immersed in de-ionized water, but can recommence when the animal loses sufficient sodium to the environment. The concentration of sodium required for influx to take place decreases with succeeding periods of immersion in de-ionized water at 10° C. and reaches minimum equilibrium concentrations as low as 0·005 mM-Na/l.

3. Low temperature inhibits sodium influx and thus promotes net loss of sodium to de-ionized water.

4. Low temperature also decreases the initial loss of sodium to de-ionized water and probably lowers the permeability of the external surfaces of the animal to ions. This effect is small compared with the inhibition of ion uptake so that the combined result is to increase the net loss of sodium from the animal.

5. Since animals lose calcium to de-ionized water and show a decreased rate of sodium loss when calcium salts are added, it is believed that the high rates of sodium loss in de-ionized water are attributable to the effect of calcium on permeability.

6. Lack of calcium may also explain why animals which have been depleted of sodium by low-temperature treatment take up sodium much faster at higher temperatures from dilute Ringer solutions than from pure sodium chloride solutions.

7. When animals lose ions to de-ionized water at low temperature, sodium and chloride are lost from the extracellular space, whilst the muscle cells lose potassium.

These ions are recovered into the extracellular space when animals are allowed to take up ions at 10° C. from diluted Ringer solution later.

Some of this work forms part of a thesis submitted by J. M. Bull to the University of Nottingham for the degree of Ph.D. Our thanks are due to Professor E. J. W. Barrington, F.R.S., for his encouragement and interest, and also to the Science Research Council for financial support.

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