SOME EFFECTS OF TEMPERATURE AND CONCENTRATION OF THE MEDIUM ON THE IONIC REGULATION OF THE ISOPOD *ASELLUS AQUATICUS* (L.)

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INTRODUCTION

The temperature of small bodies of fresh water may undergo considerable changes within a short period. Such changes in temperature are likely to affect the rates of all biological processes including the mechanisms responsible for the active uptake of ions from the medium and the production of a hypotonic urine. Although the osmotic uptake of water and any passive loss of ions from the body may have high temperature coefficients it is unlikely that these will be the same as the temperature coefficients of the active mechanisms. Since ions have to be replaced at the same rate as they are lost from the body if the haemolymph concentration is to be maintained at a constant level, it is of interest to investigate the means by which fresh-water poikilotherms are able to withstand the potential hazards of change in the temperature of their medium.

Wikgren (1953) has discussed the previous work in this field in relation to his own findings on *Potamobius*, *Petromyzon* and various teleosts. He has shown that the ionic regulation of *Petromyzon* is affected in two ways by a reduction in the temperature of its medium to 1–2° C: first, there is a decrease in the urine volume. This is accompanied by a decrease in salt readsorption so that the Cl concentration of the urine remains similar to that at higher temperatures. Second, the rate of ion uptake from the medium is markedly reduced. As a result there is an initial net loss of ions from the body at low temperatures.

Evidence suggesting the presence of an effective and sensitive process for the active uptake of sodium by *Asellus* has already been described (Lockwood, 1959*a*). The present report is mainly concerned with investigation into the effect of different temperatures and different concentrations of the medium on the rate of loss of ions from the body, on the ionic concentration of the haemolymph and on the rate of uptake of ions from the medium. Some data are also given of the sodium fluxes.

DEFINITIONS AND THEORETICAL CONSIDERATIONS

In this paper the term sodium 'flux' is used to indicate the total rate of sodium movement as determined using labelled sodium. 'Influx' incorporates both active transport of sodium into the animal and also exchange diffusion. Conversely,

'efflux' is made up of loss of sodium from the body by diffusion and in the urine plus exchange diffusion. Since, by definition, the component of exchange diffusion is the same in both directions at any one time, the sodium content of the body of the animal remains constant only when the active uptake of sodium balances the diffusion and urine loss.

The term 'fully loaded animal' is applied to one which has been allowed to exchange its sodium with a medium containing tracer for a time sufficient to ensure that the specific activity is the same in both animal and medium.

The following symbols have been used in this paper:

$O.P._l$ haemolymph osmotic pressure (expressed as mM/l. NaCl)

Na_l haemolymph sodium concentration

Cl_l haemolymph chloride concentration

Na_o sodium concentration of the medium

If in the study of the exchange of sodium between an animal and its medium it is permissible to regard the internal sodium as present in a single phase, the flux may be calculated from the equation

$$R = \frac{Na_l}{t} \ln \left(\frac{{}^*Na_\infty}{{}^*Na_\infty - {}^*Na_t} \right) \quad (1)$$

where R is the flux in m-equiv./l. haemolymph/hr.,

Na_l is the sodium concentration of the haemolymph in the steady state,

$^*Na_t$ is the tracer activity in a sample of haemolymph at time t, and

$^*Na_\infty$ is the tracer activity in a similar sample of haemolymph when exchange of tracer with the medium is complete.

Replacing Na_l by 100 gives the flux in terms of % haemolymph sodium exchanged per hour.

The use of the above equation, which treats the whole sodium content of the animal as being in a single phase, is justified if the exchange of sodium between the haemolymph and any other compartment is either very rapid or very slow compared with the exchange between the haemolymph and medium. It will be shown that the exchange of sodium between haemolymph and non-haemolymph compartments in *Asellus* is slow compared with the exchange between haemolymph and medium. The above equation can therefore be used to calculate the sodium flux between the haemolymph and medium provided that its use is restricted to the first few hours of an experiment. During this time exchange between haemolymph and tissues will be small.

Prior to some of the experiments to be described in this paper each animal was fully loaded in a solution containing ^{22}Na. Depletion of the sodium content of the animal was brought about by exposing it to a current of deionized water, and the sodium-depleted animal was allowed to replace the lost sodium from the original loading solution. Since no exchange diffusion is possible if the medium contains no sodium and since there is no net effect of exchange diffusion when the specific

activity of ^{22}Na is the same in the medium and in the animal, it follows that in these circumstances any change in the count rate of the animal represents a net change in its sodium content.

MATERIALS AND METHODS

Asellus aquaticus was obtained from Coe Fen, Cambridge, and in later experiments from a number of fresh-water sites around Edinburgh.

Osmotic pressures were measured by the method of Ramsay & Brown (1955), sodium by flame photometry and chloride by the first method of Ramsay, Brown & Croghan (1955). Details of the procedure and the method of haemolymph collection have already been described (Lockwood, 1959*a*). Haemolymph samples for the measurement of isotope activity were collected and stored below liquid paraffin as in the case of samples for osmotic pressure determinations. Aliquots of haemolymph were pipetted on to planchettes or polythene disks and were evaporated to dryness before counting. Sufficient haemolymph could be obtained from a single large animal to allow duplicate samples to be taken. Samples of medium were pipetted directly on to planchettes, evaporated and counted. Whole animals were counted in one of the Perspex washing-out trays previously described (Lockwood, 1959*b*).

In most cases sufficient counts were recorded to give a statistical accuracy of $\pm 2\%$. The usual corrections for background and count rate were applied to the raw data and in addition, during long-term experiments, a ^{22}Na standard was counted before and after each ^{22}Na sample in order that corrections might be made for variations in the counting efficiency of the apparatus.

Animals were not fed during the course of experiments.

RESULTS

The sodium flux

Two methods have been used in the study of the sodium flux. In the first, animals were transferred from a medium to which they had been acclimatized (1·1 mM./l. NaCl) to a measured weight of a similar medium containing ^{22}Na. Aliquots of the medium were taken at intervals, dried down on to polythene disks and counted. After counting, each disk was rinsed in the medium to remove the sample and the medium was brought up to its initial weight by the addition of deionized water. Aliquots of the medium were counted several times a day for 6 days and then again after 30 days to obtain the value after complete exchange. The temperature was $20 \pm 1{\cdot}5°$ C. (Fig. 1*a*).

A semi-logarithmic plot of $M_t - M_\infty$ (where M_t is medium count at time t and M_∞ is the steady-state count) against time would be linear if a single rate constant were governing the sodium exchange. Fig. 1*b* indicates that this is not the case in *Asellus*. Two rate constants have been derived graphically from the results of this plot, the slower giving a half-time of exchange of 114 hr. and the more rapid one of 19 hr. Direct extrapolation to obtain the proportion of the total sodium

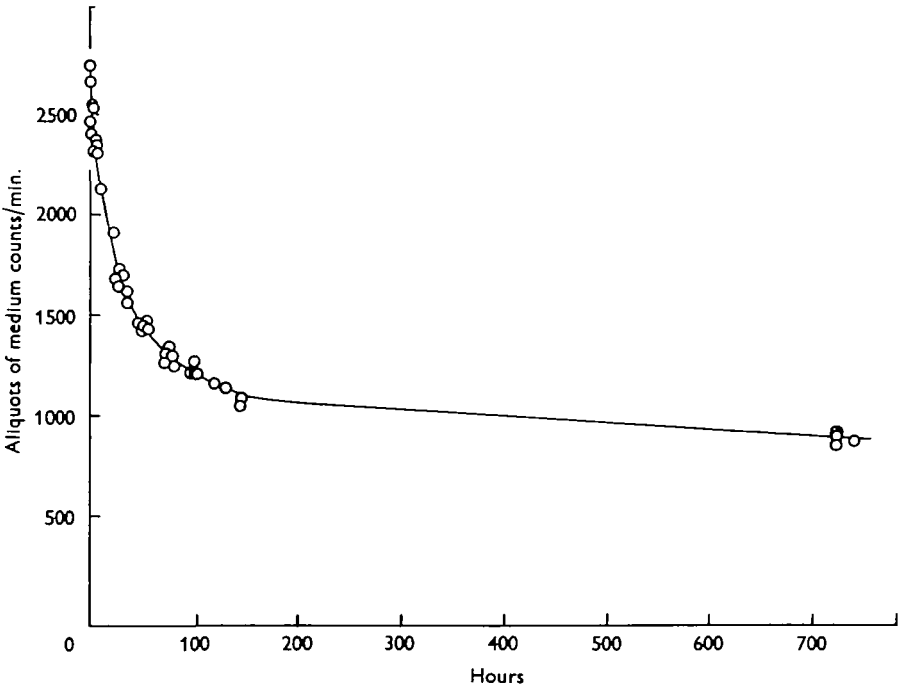

Fig. 1 *a*. Falling count of aliquots of medium during uptake and exchange of ^{22}Na by five animals.

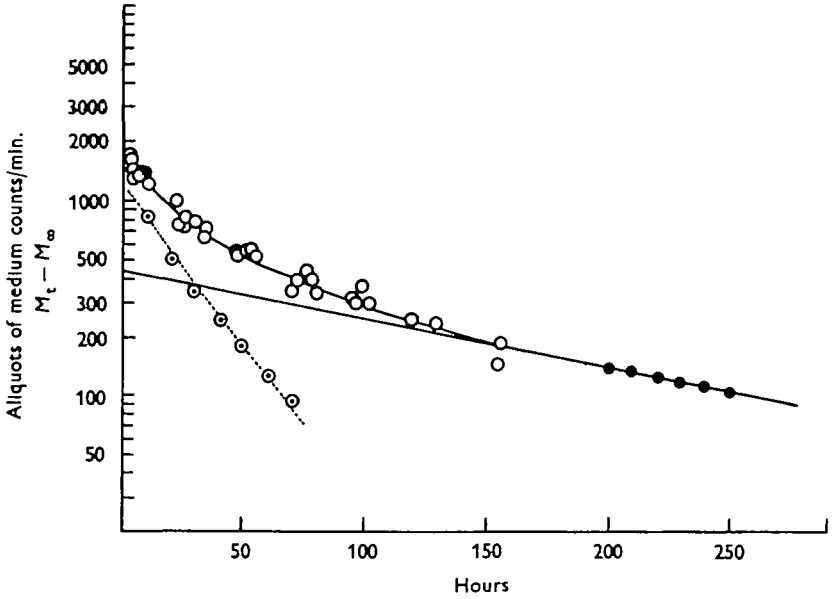

Fig. 1 *b*. Semi-logarithmic plot of data from Fig. 1 *a* to show the two compartments of Na aliquot counts ($M_t - M_\infty$) where M_t is the observed count and M_∞ the count when the exchange of ^{22}Na is complete. ——, back extrapolation for slow component of flux. · · · ·, fast component of flux.

accounted for by the slow rate is liable to error, but the value obtained of between 20 and 30% of the total sodium is similar to that found for the proportion of extrahaemolymph sodium in *Asellus* (Lockwood, 1959*c*) and it is presumed that the slow exchange corresponds to the exchange of the sodium in Zenker's organ.

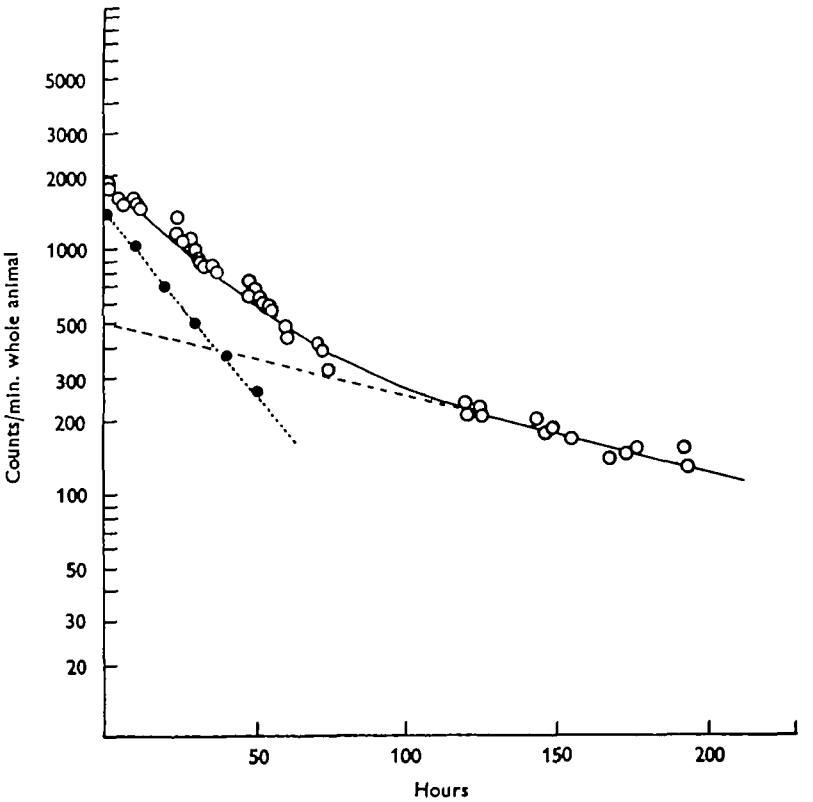

Fig. 2. Loss of ^{22}Na by a fully loaded animal washed with a non-tracer medium. ○, counts of whole animal: – – –, back extrapolation of slow component of flux: ●, fast component of flux.

In the second method the sodium flux was examined by following the decrease of activity of a fully loaded animal exposed to a current of tracer-free medium. The loss of tracer was again defined by two rate constants (Fig. 2), the half-times for exchange being 20 and 103 hr. respectively.

In these experiments, and in others involving exchange of sodium, care was taken to select animals of closely similar size since preliminary experiments had shown the existence of a relationship between the size of animal and the rate of exchange of sodium per unit weight (Fig. 3).

The effects of temperature on ion regulation

(*a*) *The response of haemolymph concentration to temperature*

Small groups of animals were kept in 1·7 mM./l. NaCl at different temperatures. After 2 weeks the haemolymph was sampled and the $O.P._i$ and Na_i of the haemo-

lymph were compared in animals from different temperatures. The results of three similar experiments (Table 1) provide a general confirmation for the finding of Heuts (1943) that the $O.P._i$ and Cl_i of *Asellus* decrease with temperature to a minimum value at about 4° C. and then rise again at temperatures below this. The rise in $O.P._i$ and Na_i below 5° C. is not associated with an increase in the total sodium

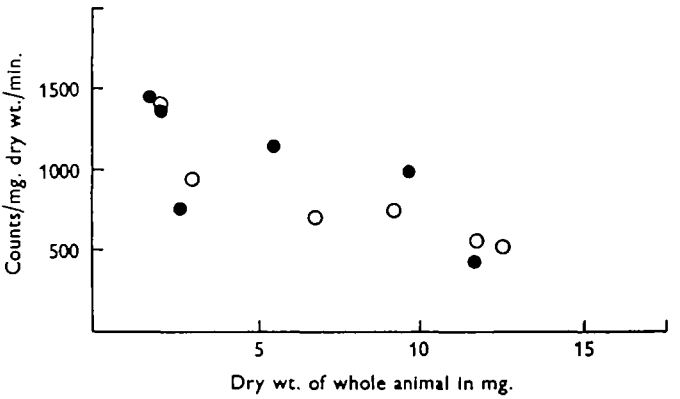

Fig. 3. Rates of exchange of sodium per unit body weight of animal. ●, ○, results derived from two similar experiments.

Table 1. *The effect of temperature difference on* $O.P._i$ *and* Na_i

Experiment 1

Temperature (° C.)	$O.P._i$ mM./l. NaCl	Na_i mM./l.
1	156	—
1	165	152
1	164	145
11	142	126
11	137	126
11	142	128
24	150	137
24	142	135

Experiment 2
Conc. of medium
2·4 mM./l. NaCl

	3·5° C.	12° C.	18° C.	22° C.
Na_i mM./l.	127 ± 2·7	123 ± 1·8	126 ± 0·9	129 ± 1·4
	n = 11	n = 11	n = 10	n = 10

Experiment 3
Conc. of medium
57 μM./l. NaCl

	5° C.	8° C.	15° C.	20° C.	24·5° C.
Na_i mM./l.	110 ± 2·6	96 ± 4·2	103 ± 2·5	106 and 109	110 ± 1·5
	n = 11	n = 3	n = 10	n = 2	n = 10

Mean ± standard error. n = number of observations.

content of the body. Animals fully loaded at room temperature and then transferred to a temperature of 1° C. in the same loading medium all showed a net loss of sodium (Table 2). When the temperature was raised once more the animals showed a net uptake of sodium from the medium.

It is possible that the rise in the ion concentration of the haemolymph at low

temperatures results from the accumulation of metabolites in the cells and a consequent water shift to the cells. A similar water shift to the cells has been postulated to explain the maintenance of a relatively high haemolymph concentration when *Asellus* is washed with deionized water (Lockwood, 1959*a*).

Animals kept in a very dilute medium (57 μM./l. NaCl) at different temperatures showed a relationship between Na_i and temperature (Fig. 4) similar to that found at the higher concentration of NaCl. At all temperatures however Na_i was considerably below the 'normal' level.

Table 2. *The loss of sodium from animals fully loaded at room temperature and transferred to* 1° *C in the same medium*

	% initial sodium remaining in body. Period at 1° C.			
Animal	1 day	2 days	3 days	4 days
1	94	—	—	—
2	92	90	84	—
3	—	95	96	—
4	—	91	87	—
5	—	98	80	71
6	—	—	—	79

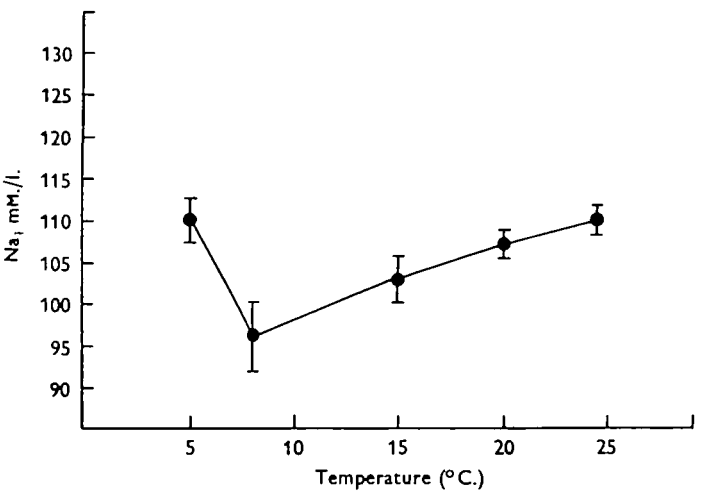

Fig. 4. The influence of temperature on the steady state Na_i of animals in very dilute NaCl (57 μM./l.). ●, means: I, range of standard error.

The experimental finding on the short-term relationship between temperature and Na_i and $O.P._i$ may be compared with the observed changes in the $O.P._i$ of *Asellus* during the year. In Fig. 5 are shown the means and standard errors for $O.P._i$ of freshly caught individuals in different months. There is a small rise in the $O.P._i$ as the water temperature falls in winter. During the remainder of the year, however, the $O.P._i$ is remarkably constant and the differences from month to month are not significant.

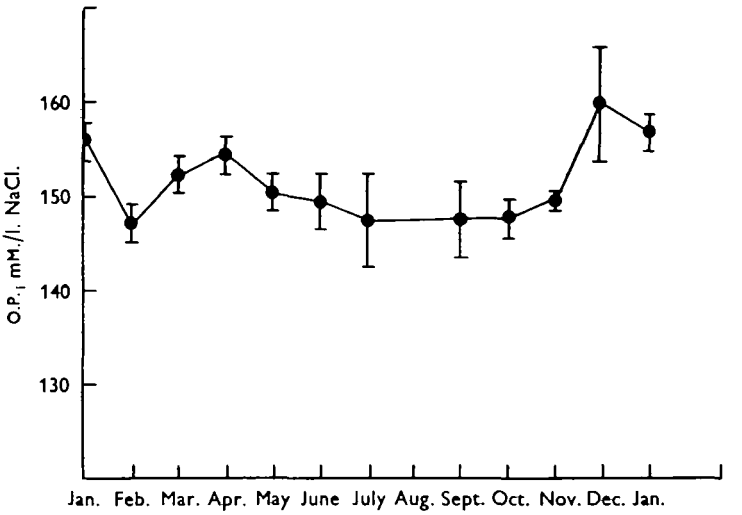

Fig. 5. Range and standard deviation of O.P.$_i$ month by month.

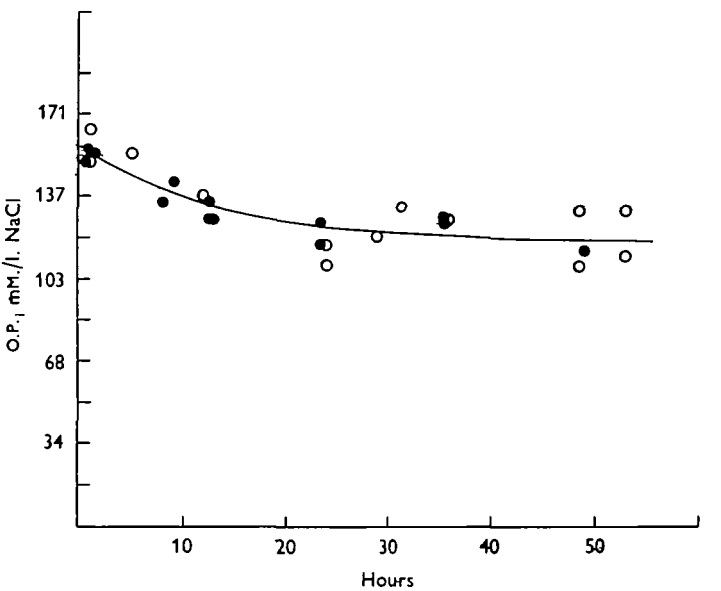

Fig. 6. Comparison of the rate of fall in O.P.$_i$ of animals washed with deionized water at different temperatures. ●, O.P.$_i$ of animals washed out at 24° C.: ○, O.P.$_i$ of animals washed out at 1°C.

(*b*) *The rate of sodium loss at different temperatures*

Two batches of animals were acclimatized for 36 hr. to temperatures of 1 and 24° C. and each batch was then exposed to a slow stream of deionized water at the temperature to which it had been acclimatized. Haemolymph samples were taken at intervals and the rates of fall of O.P.$_i$ at the two temperatures were compared. No significant difference was detectable between the rates (Fig. 6). Since Na_i

forms a constant proportion of O.P.$_1$ under a wide range of conditions (Lockwood, 1959*a*), this result suggests that the rate of ion loss is not affected by temperature. This conclusion was checked by washing out single fully loaded individuals successively at different temperatures and Fig. 7 illustrates the results of one such experiment. After loading, this animal was washed out at 16 ± 1° C. until its count had fallen to about 70% of the initial value. It was then allowed to take up sodium from the original loading medium and was finally washed out at 2 ± 1° C. The rates of loss of sodium were closely similar after the first few hours at the two

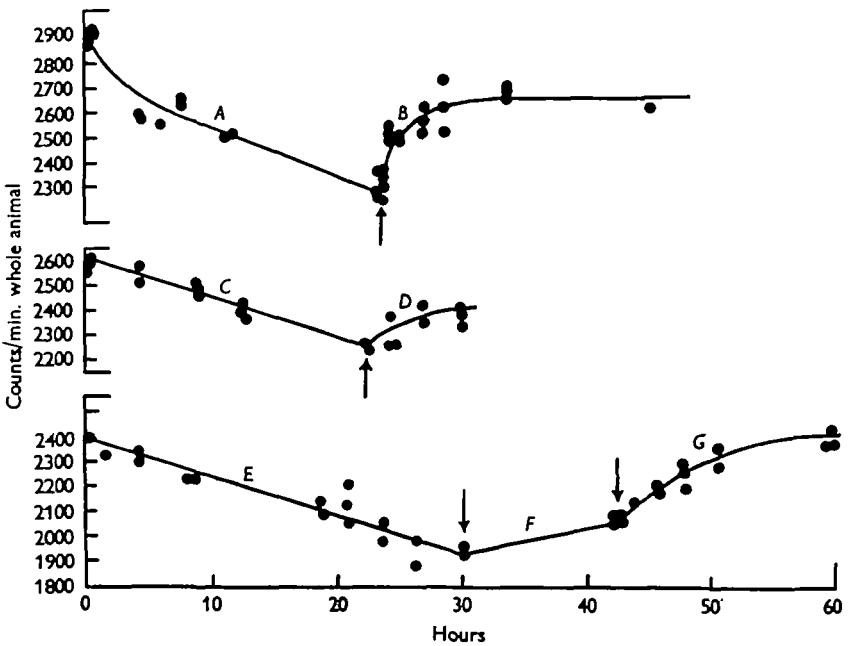

Fig. 7. The effect of temperature on the rate of loss and uptake of sodium. *A*, loss of sodium in deionized water at 16 ± 1° C. *B*, uptake from original loading medium at 16 ± 1° C. *C*, loss of sodium in deionized water at 2 ± 1° C. *D*, uptake from original loading medium at 2 ± 1° C. *E*, loss of sodium in deionized water at 2 ± 1° C. *F*, uptake from original loading medium at 2 ± 1° C. *G*, uptake from original loading medium when temperature was raised to 16 ± 1° C.

temperatures. Similar experiments conducted over other temperature ranges indicate that the rate of loss of sodium is effectively independent of temperature (Fig. 8).

It is assumed that the values obtained for the initial rate of loss of sodium from the bodies of animals in deionized water correspond to the normal diffusion and urine loss. As might be expected there is some variation in the rate of loss. The mean rate found for the first 24 hr. of washing out fully loaded animals was 1·00 ± 0·36% of total body sodium per hour (n = 20). As some 20–30% of the total sodium is not in the haemolymph the mean rate of loss from the haemolymph would be expected to be of the order of 1·2–1·5%/hr. This agrees reasonably well with the initial rate of fall of the O.P.$_1$ of animals in distilled water of 1·6%/hr.

(Lockwood, 1959*b*) considering that the initial rate of loss of sodium is somewhat more rapid than the mean rate over 24 hr.

The half-time of net loss of haemolymph sodium, taking the rate of loss as being 1·6%/hr., would be 43 hr., whereas the half-time found for exchange of haemolymph sodium in the flux experiments was less than half this value (19–20 hr). The difference is presumed to be accounted for by the exchange diffusion component of the flux.

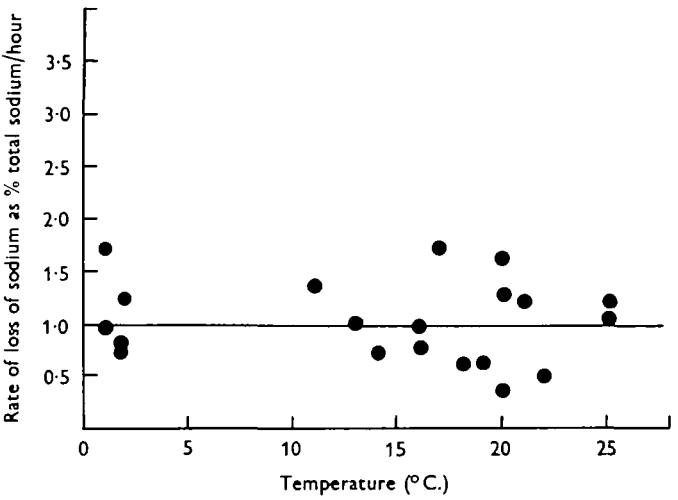

Fig. 8. Rate of loss of sodium (as % of total sodium/hr.) at various temperatures, during the first 24 hr. of washing out in deionized water.

(*c*) *The effect of temperature on the rate of active uptake of sodium by animals with a reduced haemolymph concentration*

Individual fully loaded animals were washed with deionized water at 1° C. until their count had fallen to a convenient level (70–50% of the initial count). They were then replaced in the loading medium (1·1 mM/l. NaCl) at 1° C. and allowed to take up sodium. After some hours the temperature of the medium was raised to 11° C. and the rate of uptake of sodium markedly increased. The animals were then washed out again at 11° C. to the same level as before and were finally replaced in the loading medium at 11° C. The course of one such experiment is shown in Fig. 9 and a comparison is made of the net rates of uptake at 1 and 11° C. in Fig. 10. In the experiment shown in Fig. 9 the initial net uptake at 11° C. is 2·0% of the initial total body sodium per hour or some five times as rapid as the net uptake at 1° C. which is only 0·4%/hr. However, in determining the temperature coefficient of the uptake mechanism account must also be taken of the sodium loss from the body during the period of uptake. In this case the rate of loss during the initial washing out phase was 1·3% of total body sodium/hr. The gross uptake of sodium at 11° C. is thus about twice that at 1° C., 2·0+1·3 = 3·3%/hr. as against 0·4+1·3 = 1·7%/hr.

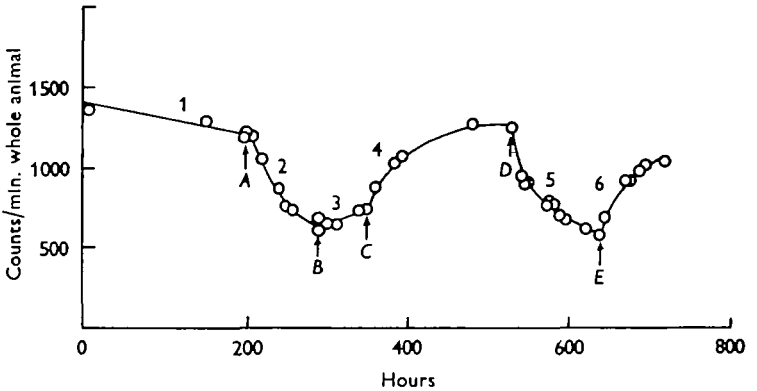

Fig. 9. The effect of temperature on the loss of sodium in deionized water and on the rate of uptake of sodium from a tracer medium. An animal was fully loaded with ^{22}Na at room temperature and at zero time the temperature was lowered to 1° C. The count rate of the whole animal fell while it was kept in the original loading medium at 1° C. (1). At *A* the animal was exposed to deionized water at 1° C. and suffered loss of sodium (2). At *B* it was replaced in the original loading medium at 1° C. and a slow uptake of sodium occurred (3). The temperature of the medium was raised to 11° C. at *C* and the rate of uptake increased (4), but declined again as the total sodium in the animal approached the normal level. At *D* the animal was transferred to deionized water at 11° C. and the loss of sodium (5) was similar to that during period 2. Finally, at *E* it was replaced in the tracer medium at 11° C. and uptake (6) was similar to that during period 4.

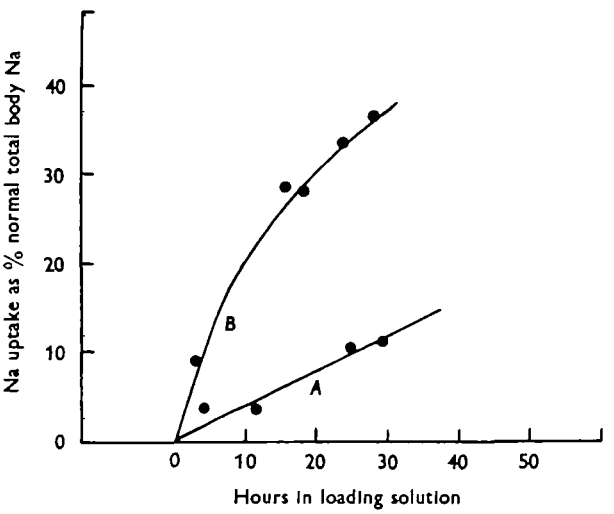

Fig. 10. The effect of temperature on the net rate of sodium uptake. A comparison of the rates of uptake at 1 and 11° C. by a single individual. *A* = uptake at 1° C., *B* = uptake at 11° C.

The maintenance of the haemolymph concentration at low concentrations of medium

Shaw (1959*a*) has shown that the rate of uptake of sodium by the crayfish is dependent on the concentration of the medium only when this is less than 1 mM./l. NaCl. At levels more concentrated than the critical level of 1 mM./l. NaCl the

rate of uptake does not vary with the concentration of the medium. The critical level at which the concentration of the medium ceases to be a rate-limiting process is clearly of importance to fresh-water animals since any fall below this level must necessarily result in a drop in the steady-state haemolymph concentration.

A study has been made of the critical level in *Asellus*, but as this animal is too small for repetitive haemolymph sampling an indirect approach has been used. It is reasonable to assume that the concentration of the medium affects the carrier complex directly or indirectly in a manner independent of the mechanism which regulates the rate of uptake in response to changes in the Na_i. If the two processes are distinct then the critical concentration is the minimum concentration of the medium at which an animal can maintain its normal Na_i. The minimum Na_o

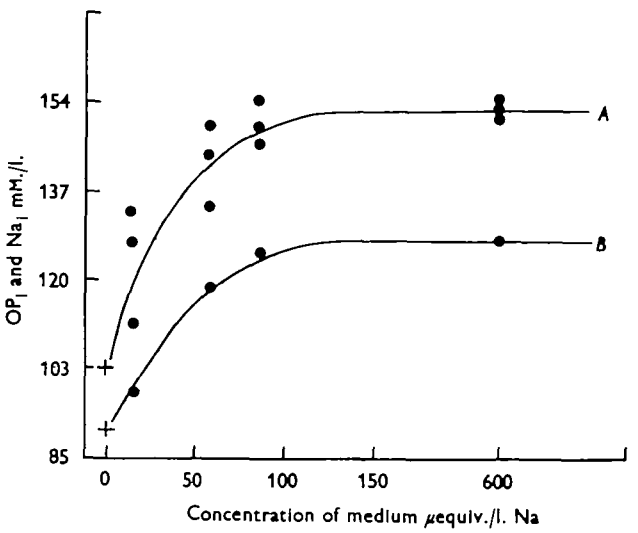

Fig. 11. Steady states of O.P.$_i$ and Na_i of animals in an external medium of low NaCl concentration. *A*, O.P.$_i$: *B*, Na_i: +, mean levels of O.P.$_i$ and Na_i of controls after the initial period of washing out in deionized water (see text).

at which *Asellus* can maintain its 'normal' Na_i was determined as follows. A number of animals were washed with deionized water until the O.P.$_i$ and Na_i of controls had fallen to about 100 mM./l. and 90 mM./l. respectively. Groups were then placed in various dilute solutions of NaCl in the concentration range 20–600 μM./l. After 7 days O.P.$_i$, Na_i and Na_o were determined. The two groups of animals in media less concentrated than 90 μM./l. had O.P.$_i$ and Na_i intermediate between the levels found in controls after the initial washing-out phase and the corresponding levels in normal untreated controls (Fig. 11). The critical concentration for the saturation of the carrier complex of *Asellus* is therefore of the order of 90 μM./l. at 19° C.

From this experiment it may also be observed that the animal is able to maintain a steady-state Na_i at levels below the normal Na_i. Steady states are maintained only when loss of sodium is balanced by the active uptake of sodium. If the concentra-

tion of the medium falls below the critical level, uptake will show a corresponding decline. This decline is balanced, however, by the increased rate of uptake resulting from a fall in Na_i and a steady state is regained at a lower Na_i level than 'normal.' Interpretation of the results of the experiment in this manner involves the assumption that *Asellus* displays an increased rate of uptake when the Na_i falls below 'normal.' Such an effect has already been shown on a variety of species (Krogh, 1937; Shaw, 1959; Heuts, 1943), and has also been demonstrated on *Asellus*. This was done by washing fully loaded animals with deionized water and then replacing them in the medium in which they were originally loaded. The rate of uptake declines as the total sodium in the body approaches the 'normal' level. See, for examples, Figs. 7 and 9.

DISCUSSION

Asellus aquaticus has been shown to have only some 70–80 % of its total sodium in the haemolymph, much of the remaining 20–30 % being associated with uric acid in the cells of Zenker's organ (Lockwood, 1959*c*). The sodium content of these cells might be expected to have only a slow rate of exchange with the haemolymph, and it is therefore not surprising to find that the sodium flux in this animal is governed by two rate constants. It is presumed that the component of the flux responsible for 20–30 % of the total exchange and having the slow half-time of over 100 hr. at 19° C. is that of the sodium in Zenker's organ. The half-time of exchange of the other component of the flux has a value of about 20 hr. Rather few determinations have as yet been made on the sodium fluxes of arthropods but this latter value is intermediate between the values for exchange between haemolymph and external medium in the saline water crustacean *Artemia* (Croghan, 1958) and in the larva of the fresh-water mosquito *Aëdes* (Treherne, 1954).

The Na_i is maintained in a steady state when the active uptake of sodium is equal to the diffusion and urine losses. The rate of loss is slow (mean value 1·00 % total sodium/hr. or about 1·2–1·5 % Na_i/hr.) and fairly constant over the temperature range studied (1–24° C.). A transient increase in the rate of loss has sometimes been observed if the temperature is raised rapidly. The rate of loss of animals acclimatized to a given temperature is equivalent to a half-time of exchange of Na_i of 58–46 hr., that is, of the order of twice the value obtained in the corresponding flux experiments. The sodium influx from medium to haemolymph must therefore be separated into two components, exchange diffusion and active uptake. A similar exchange diffusion component of the sodium flux of arthropods has been shown by Croghan (1958) on *Artemia* and Stobbart (1959) on *Aëdes*.

If Na_i is lowered below the 'normal' value by washing an animal with deionized water the rate of active uptake of sodium is increased when the animal is returned to a normal medium. It is assumed that this increase in the rate of uptake is brought about by the mobilization of extra transporting sites.

Changing the temperature of the medium might be expected to result in a change in the rates of reactions involved in active transport and hence in the rate of operation of individual sites. The rate of active uptake of sodium by animals with

a reduced Na_i displays such a temperature dependence, the uptake at 11° C. being about twice that at 1° C. Since the loss and uptake of sodium are not affected to the same extent by an alteration of the temperature of the medium the Na_i will also change. In fact, however, the change in the mean steady-state value of Na_i over the temperature range 8–24·5° C. is small, being only a few mM./l.

The small extent of this change in Na_i indicates the presence of a sensitive homeostatic mechanism and the following account describes a negative feed-back system which might act as the primary means by which the relative constancy of the Na_i is maintained under the influence of temperature change.

Lowering the temperature of the medium has the effect of decreasing the rate of active uptake of sodium. Since the rate of loss of sodium is fairly constant over a wide range of temperature such a fall in temperature has the effect of lowering the Na_i below the original steady-state level. But because the rate of active uptake increases as Na_i decreases, this fall below the 'normal' Na_i results in an increase in the rate of uptake of sodium. After a certain fall has occurred in the Na_i the rate of uptake is raised to a level at which it is again equivalent to the loss, and the Na_i will then again be in a steady state. The overall effect is thus a fall in the steady-state level of Na_i with decreasing temperature. Such a decrease in the concentration of the haemolymph as the temperature is lowered has been observed in *Gammarus pulex* as well as in *Asellus* (Heuts, 1943). Conversely, if the temperature is raised the steady state is regained at a higher concentration. It is clear that with such a mechanism regulating the Na_i under the influence of temperature the steeper the slope of the curve relating rate of uptake of sodium to the Na_i the smaller will be the change in the steady-state Na_i for any given temperature change. It is interesting therefore that Shaw (1959*a*) found that in the crayfish the rate of active uptake rises to a maximum when Na_i has fallen by only approximately 5–10%. In consequence this animal may be expected to be extremely well protected against the effects of temperature change.

In both *Asellus* and *Gammarus pulex* there is a marked rise in the haemolymph concentration at temperatures below about 4–5° C. (Heuts, 1943) and this appears to be incompatible with the suggested homeostatic mechanism outlined above. The experimental finding that the total sodium in the body is in fact reduced still further at low temperatures suggests that this effect is an artifact resulting from an accumulation of metabolites in the cells accompanied by a water shift from the haemolymph. However, despite this initial loss of sodium at low temperatures, a net uptake has been observed even at 1° C. in an animal with a low Na_i.

Shaw (1959*a*) has shown that in the crayfish the rate of active uptake of sodium is related to the concentration of the medium when this latter is less than about 1 mM./l. Above this critical value the rate of active uptake of sodium is independent of the concentration of the medium. In *Asellus* the corresponding critical value, obtained by an indirect method, is about 90 μM./l. NaCl—a level some eleven times less than that of the crayfish. A low critical value for the saturation of the carrier would seem to be of prime importance to a fresh-water animal in regions where the ion content of the water is low, and this may be one of the principal

reasons for the well-known occurrence of *Asellus* in soft-water regions where the crayfish is not found. In Grasmere *Asellus* has been reported (Moon, 1957) from water only some 60 μM./l. Na above the experimentally determined critical value.

The high temperature coefficient of active uptake relative to that of loss found in *Asellus*, if more generally applicable, may have a wider physio-ecological significance. According to von Martens (1858) tropical and subtropical estuaries and fresh waters contain more quasi-marine species than do the corresponding temperate waters. In addition, a number of temperate brackish-water species have apparently given rise to very similar forms which live in fresh water near the warmer southern regions of the range of the parent species. Such forms include *Jaera balearica* (Margalef, 1952) and *Palaemonetes antennarius*. Various suggestions have been made in an attempt to explain the possible influence of high temperatures on the invasion of fresh water by brackish-water animals. These include the relative stability of temperatures in tropical fresh waters (von Martens, 1858) and a suggestion by Pannikar (1940) that the optimum osmotic pressure of the haemolymph may be lower at high temperatures. The present results suggest a further possibility, though caution must naturally be exercised in the application of conclusions derived from results based on a single fresh-water species to more general considerations. If the range of a brackish-water species is limited by the minimum Na_i and Cl_i at which it is sufficiently physiologically normal to compete effectively in its environment, then it can achieve these concentrations in a dilute medium more readily if the temperature is high than if it is low. A corollary of this is that for a given limiting haemolymph concentration penetration will be further towards fresh water the warmer the medium. Some support for this contention is provided by the fact that in very dilute media *Asellus* maintains a higher Na_i at high temperatures than it does at low temperatures, and by Broekhuysen's (1936) finding that the eggs of *Carcinus maenas* would develop at a salinity of 20‰ at 16·3° C., but could not tolerate less than 26‰ when the temperature was lowered to 10° C. Similarly, Broekema (1941) found than *Crangon crangon* survived low salinities better when the temperature was high than when it was low, and he discusses this in relation to the seaward migration of this animal at the beginning of winter. Shaw (1959*b*) has shown that the fresh-water crab *Potamon niloticus* loses sodium to the medium when the temperature is lowered and he points out that this species is not found in the colder upper reaches of the East African rivers.

However, high temperatures do not assist all brackish water species to move towards more dilute waters. *Gammarus duebeni* may be cited as an example of a species in which the temperature coefficients of loss and uptake of sodium bear an inverse relationship to those found in *Asellus* (unpublished observations). This is also supported by recent work by Kinne (1959) on the survival of this animal at different temperatures and salinities. Animals in this category might be expected to be more likely to invade fresh water in cold regions.

SUMMARY

1. Some effects of temperature and concentration of the medium on the sodium metabolism of the isopod *Asellus aquaticus* have been studied.

2. The steady-state haemolymph concentration rises with the temperature in the range 5–24° C. The change is, however, only a few mM./l. over this temperature range. The osmotic pressure ($O.P._l$) and sodium concentration of the haemolymph (Na_l) also rise at temperatures below about 4–5° C.

3. The rate of sodium loss from the body is unaffected by temperature.

4. The rate of active uptake of sodium is increased as the temperature is raised.

5. The rate of active uptake of sodium is related to the haemolymph concentration, rising as the Na_l falls.

6. The Na_l is maintained at the normal level in media containing more than 90 μM./l. NaCl. Steady states can also be maintained at reduced haemolymph concentrations in media less concentrated than 90 μM./l.

7. It is suggested that the homeostatic mechanism regulating the Na_l under the influence of temperature operates as follows. As the temperature falls there is a greater decrease in the rate of uptake than in the rate of loss and hence the Na_l falls. A fall in the Na_l, however, results in a rise in the rate of uptake. Therefore after a certain fall in Na_l the loss is again balanced by uptake and a new but lower steady state is reached.

8. It is tentatively suggested that high temperatures may assist some brackish-water species in the invasion of more dilute media.

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