THE EFFECT OF EXTERNAL SODIUM CONCENTRATION UPON SODIUM FLUXES IN *CHIRONOMUS DORSALIS* (MEIG.) AND *CAMPTOCHIRONOMUS TENTANS* (FABR.), AND THE EFFECT OF OTHER IONS ON SODIUM INFLUX IN *C. TENTANS*

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SUMMARY

In comparison with other freshwater animals, the sodium uptake mechanism in fourth instar larvae of both *C. tentans* and *C. dorsalis* has a moderate affinity for sodium. In both species half maximum influx (Km) occurs at about 0.57 mM-Na^+ and is unaltered by salt depletion. Maximum influx is achieved in steady-state *C. tentans* at 1.9 mM-Na^+, and in steady-state *C. dorsalis* at 3.0 mM-Na^+. Both of these values increase on depletion. Efflux also appears to be saturable at higher external sodium concentrations.

In *C. tentans*, sodium may be transported independently of chloride, although it seems likely that sodium movement is enhanced by chloride. Sulphate strongly inhibits sodium influx. Nitrate apparently inhibits sodium influx at low concentrations, but this inhibition is progressively overcome at external sodium concentrations approaching 4 mM.

A number of cations interfere with sodium influx in depleted *C. tentans*, notably H^+, Li^+ and, to a lesser extent NH_4^+. It is suggested that these ions compete with sodium for carrier sites. Potassium is apparently transported independently of sodium.

INTRODUCTION

One of the principal factors considered by Shaw (1961) to be of importance for the adaptation of animals to fresh water was the acquisition of a sodium pump with a high affinity for sodium. This aspect of the sodium pump has now been investigated in a large number of freshwater animals (Shaw, 1959*a*, 1961; Shaw & Sutcliffe, 1961; Stobbart, 1965; Alvarado & Dietz, 1970; Greenaway, 1970; Morris & Bull, 1970).

A study of the sodium affinity of the pump in *C. dorsalis* and *C. tentans* has been coupled here with an investigation of the effects of other ions on sodium influx in *C. tentans*. The latter aspect of the work extends an earlier study of *Chironomus* larvae (Koch, Evans & Schicks, 1956) and provides comparison with similar studies on other freshwater animals (Koch & Evans, 1956; Shaw, 1960*a*, *b*; Maetz & Garcia Romeu, 1964; Stobbart, 1965).

MATERIALS AND METHODS

Measurement of sodium flux using ^{22}Na

(a) *Influx.* These were made in the same manner as the short-term measurements described earlier (Wright, 1975 a), which assumed that over the first 2 h or so the departure from linearity of the relationship, time versus rate of influx, would be very small. In this case, it was reasonable to assume that backflux would be negligible. The specific activity of sodium in very dilute solutions was made high enough to enable a measurable amount of ^{22}Na to enter the animals within a reasonably short time. In this way, influx in all solutions was measured for the same length of time.

(b) *Efflux.* Animals were loaded with ^{22}Na for at least 300 h, by which time they had equilibriated with the external medium (Wright, 1975 a). They were then washed and placed in groups of 6–10 larvae in a known volume of unlabelled solution. After a measured length of time, animals were weighed and discarded, and the external solution was evaporated down for counting. 4·5 μm of dextrose was used as a spreader. The more concentrated solutions tended to crystallize on evaporation and these were split to facilitate spreading and minimize self-absorption (which was very small at the concentrations used). Where animals were to be placed in very dilute solutions, the specific activity of the loading medium was high (approximately 500 mCi/g Na). Reasons for this are as previously explained. As with influx, efflux was expressed as m-moles Na/g wet weight/hour.

Chemical analysis

Measurements of sodium were made using a Unicam SP900 flame spectrophotometer. This was also used for checking the concentration of the other metallic ions used, Cs^+, Rb^+, Li^+, K^+, Ca^{2+}, Mg^{2+}, with the appropriate amount of sodium being added to standard solutions to eliminate interference effects. NH_4Cl was checked using an automatic chloride titrator.

All animals used in experiments to test the effect of other ions on sodium influx were depleted (Wright, 1975 a). This was done in order to stimulate fully the sodium pump.

The terms influx and efflux are used as in Wright (1975 a).

RESULTS

Measurements of sodium influx were made using steady-state and depleted specimens of both *C. tentans* and *C. dorsalis*, over the range of external sodium concentration 0·002–6·0 mM. The results are shown in Figs. 1 and 2. The figures also include efflux data, which were obtained from steady-state and depleted *C. tentans* (Fig. 1), and from depleted *C. dorsalis* (Fig. 2). The relationship between flux and external sodium concentration is similar to that which has been established in a number of freshwater invertebrates (Shaw, 1959a; Shaw & Sutcliffe, 1961; Stobbart, 1965; Greenaway, 1970). The relationship can be described approximately by the Michaelis–Menten Equation,
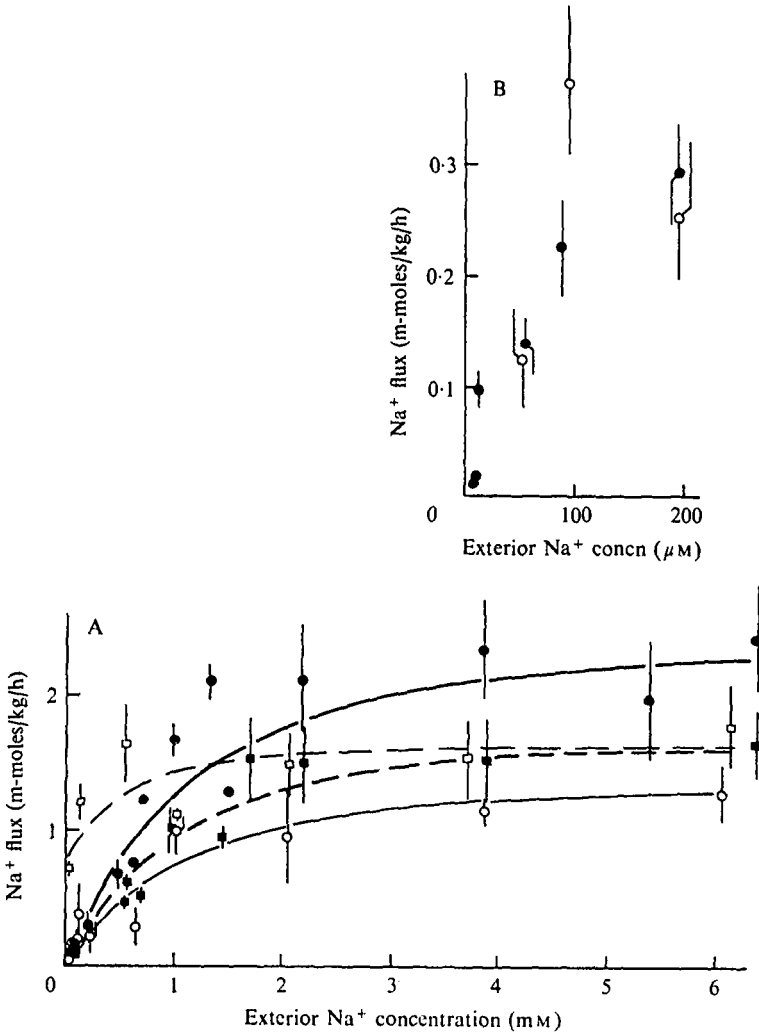
$$\text{Influx} = \frac{K \times C}{Km + C},$$

Fig. 1. The relationship between external sodium concentration and sodium flux in *C. tentans*. – – ■ – –, influx in steady-state larvae (heavy broken line); —●—, influx in depleted larvae (heavy continuous line); – – □ – –, outflux in steady-state larvae (thin broken line); —○—, outflux in depleted larvae (thin continuous line). Each point represents the mean of 4–6 groups of 8 larvae ± S.E. B. Similar to A, but with x-axis in μM.

where K = maximum rate of sodium transport, C = external sodium concentration and Km = external sodium concentration at which half the maximum flux is obtained.

Equations for the lines fitted to the data in Figs. 1 and 2 are given in Table 1. It may be seen that the half saturation of the system in both depleted and steady-state individuals of both species was reached at an external sodium concentration of approximately 0·75 mM. Calculations of net sodium movements from the difference between influx and efflux are in good agreement with values obtained from flame photometry. The initial rate of net sodium uptake from 2 mM-NaCl (Wright, 1975a) was found to be 2·5 m-moles/kg/h for *C. dorsalis* and 1·1 m-moles/kg/h for *C. tentans*. These compare with values of 2·0 and 0·8 m-moles/kg/h respectively, obtained by subtrac-
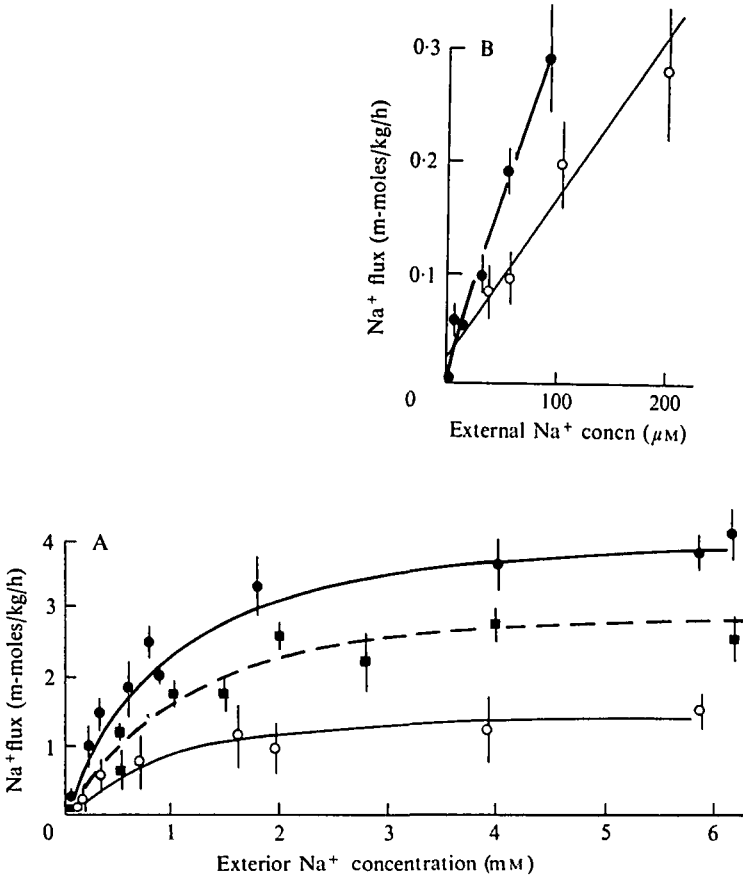
Fig. 2. The relationship between external sodium concentration and sodium flux in *C. dorsalis*. Symbols as in Fig. 1.

tion of component fluxes in Figs. 1 and 2 at an external sodium concentration of 2 mM. It is clear that sodium influx in depleted animals is increased relative to steady-state animals by a factor approaching 2 at higher external sodium concentrations. The relationship between rates of efflux measured in steady-state and depleted animals is difficult to assess. Some indirect evidence has been found which suggests a rise in efflux in depleted *C. tentans* larvae (Wright, 1975*a*, Fig. 10). However, the rise

Table 1. *Equations for the relationship between flux and external sodium concentration*

$\left(\dfrac{K \times C}{Km+C}\right)$, where K = maximum flux rate (m moles kg/h), C = external sodium concentration (mM), and Km = external sodium concentration (mM) at $\frac{1}{2}K$)

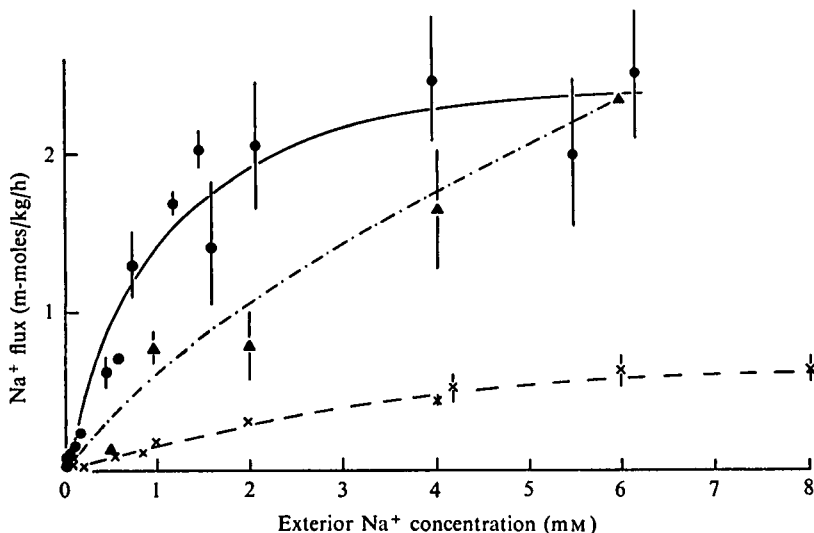| Species	State of animal and flux measured		
	Steady-state influx	Depleted influx	Depleted outflux
C. tenans	$\dfrac{1 \cdot 9 \times C}{1 \cdot 75 + C}$	$\dfrac{2 \cdot 6 \times C}{0 \cdot 75 + C}$	$\dfrac{1 \cdot 5 \times C}{0 \cdot 8 + C}$
C. dorsalis	$\dfrac{3 \cdot 0 \times C}{1 \cdot 75 + C}$	$\dfrac{4 \cdot 2 \times C}{1 \cdot 75 + C}$	$\dfrac{1 \cdot 6 \times C}{0 \cdot 75 + C}$

Fig. 3. The relationship between sodium influx and external sodium concentration shown by depleted *C. tentans* larvae in solutions of various sodium salts. —●—, NaCl; ·—·▲·—·, NaNO₃; ——×——, Na₂SO₄. Each point represents the mean ± S.E. of 4–6 groups of 8 larvae.

appears to be very small, and set against this is a clear indication of reduced urine sodium and integumental permeability in depleted animals (Wright, 1975 a). Data from Fig. 1 seems to suggest an overall lowering of efflux in depleted *C. tentans* larvae, although this is not significant at higher external sodium concentrations. However, a real difference between rates of efflux from steady-state and depleted animals does apparently exist at concentrations lower than 2 mM. Interestingly, this is the acclimatization concentration for steady-state larvae. Taken with influx data from steady-state *C. tentans*, it would appear that a net loss of sodium from steady-state animals occurs at low external concentrations. This contrast with depleted animals, which, at similar external sodium concentrations, maintain a low efflux and increased influx. Of further interest, is the point of intersection of influx and efflux data in depleted *C. dorsalis* (Fig. 2B). This agrees very well with the minimum balance concentration of 0·01 mM determined for this species (Wright, 1975 a). Efflux data for *C. tentans* are too variable for a similar comparison to be made.

The relationship between the sodium influx and external sodium concentrations was also determined for depleted *C. tentans* larvae in NaNO₃ and Na₂SO₄ (Fig 3). The inhibition of sodium influx in Na₂SO₄ is particularly marked, showing for example a reduction of 80% in 1 mM-Na₂SO₄ (= 2 m-equiv. Na) compared with 2 mM-NaCl. This is in contrast to *A. aegypti* (Stobbart, 1965) where, at similar sodium concentrations SO₄²⁻ induces a reduced sodium influx of only 30%. In larval *Ambystoma gracile*, Alvarado & Dietz (1970) noted no marked differences between sodium influx from NaCl and Na₂SO₄.

A more complete investigation into cationic and anionic interference with the sodium pump of *C. tentans* was undertaken using 0·2 mM-NaCl. This was, obviously considerably more dilute than the normal working medium of 2mM-NaCl used in previous work (Wright, 1975a). However, it may be seen from Fig. 1 that variability
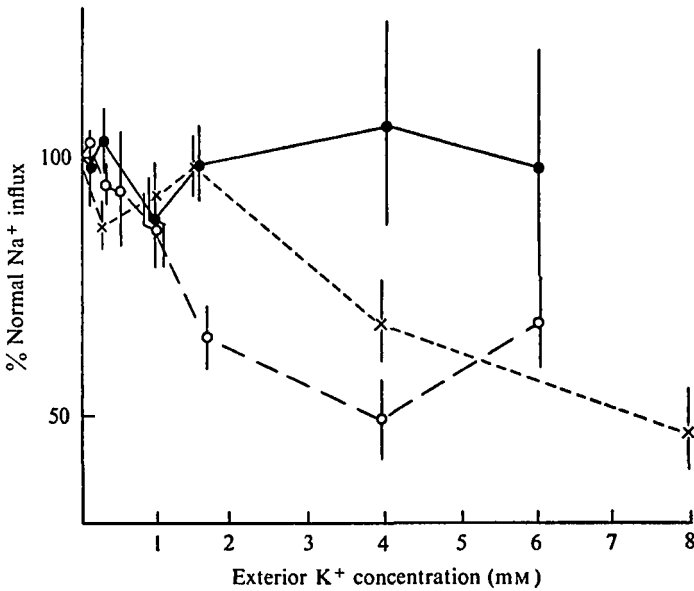
Fig. 4. The effect of different concentrations of potassium salts on sodium influx in depleted *C. tentans*. —●—, KCl added; —○—, KNO₃ added; -- × --, K₂SO₄ added. In all cases (Figs. 4–14 incl.) the sodium concentration = 0·2 mM. Each point represents the mean of 3 or 4 groups of 6–8 larvae ± S.E.
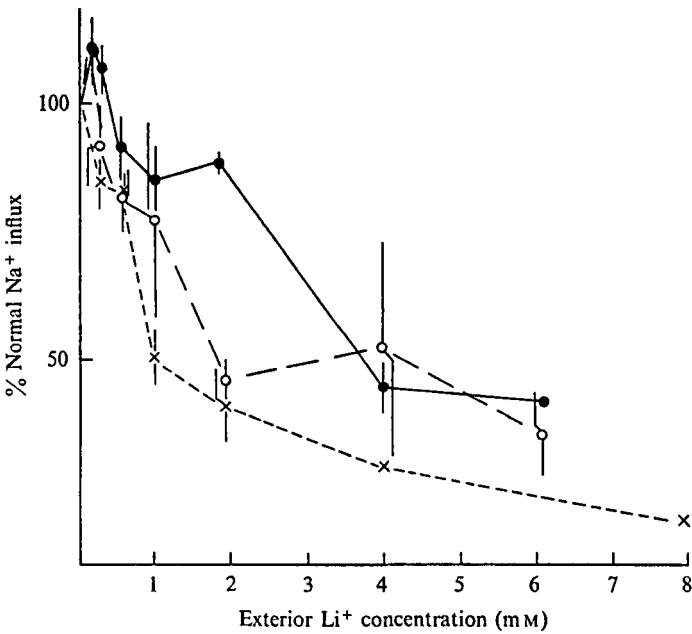

Fig. 5. The effect of different concentrations of lithium salts on sodium influx in depleted *C. tentans*. —●— LiCl added; —○—, LiNO₃ added; -- × --, Li₂SO₄ added. Other conditions as in Fig. 4.
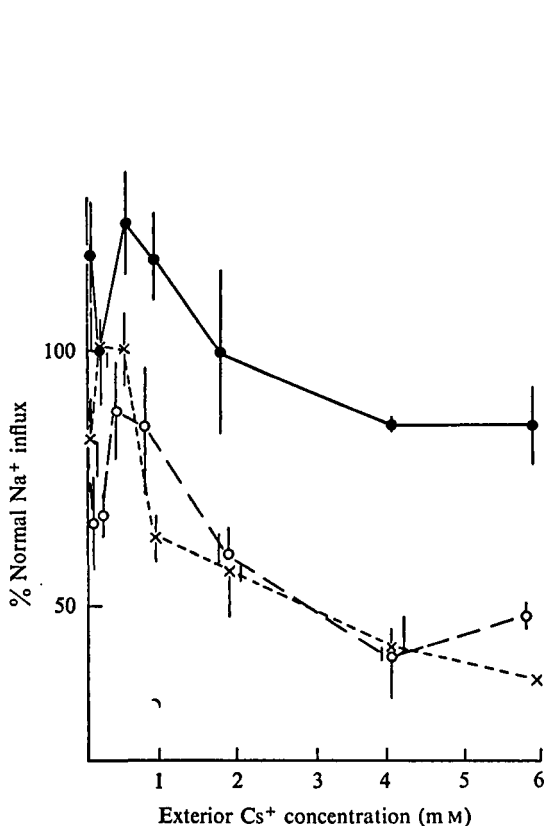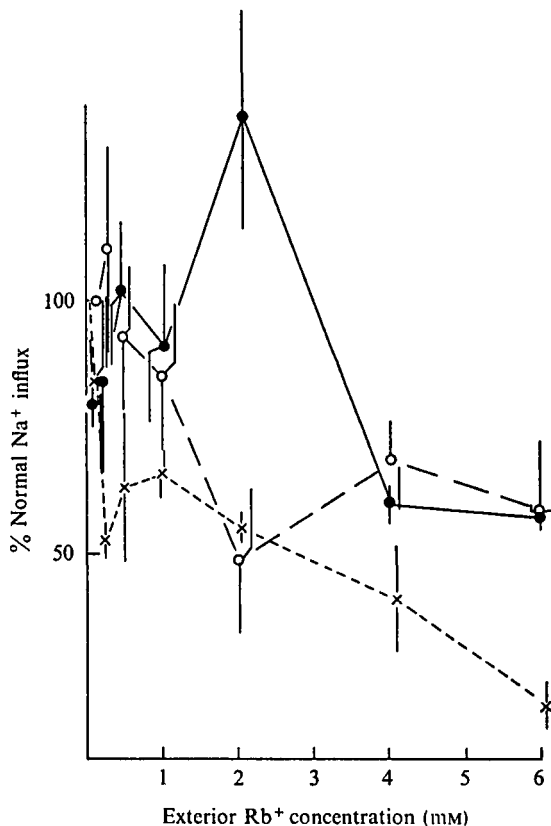
Fig. 6. The effect of different concentrations of caesium salts on sodium influx in depleted *C. tentans*. —●—, CsCl added; —○—, CsNO₃ added; — × —, Cs₂SO₄ added. Other conditions as in Fig. 4.

Fig. 7. The effect of different concentrations of rubidium salts on sodium influx in depleted *C. tentans*. —●—, RbCl added; —○—, RbNO₃ added; — × —, Rb₂SO₄ added. Other conditions as in Fig. 4.

of data increased as the sodium pump reached saturation. In view of this, it was decided that the use of depleted animals, and at an external concentration on the steep upward slope of the graph (Fig. 1) would impart a greater sensitivity to the system.

The cations used were Li^+, Rb^+, Cs^+, K^+, NH_4^+, Mg^{2+} and Ca^{2+}. These were added as either NO_2^-, SO_4^{2-} or Cl^-. The results are expressed in two ways. In Figs. 4–10 each cation is considered individually, and in Figs. 11, 12 and 13, the effects of the anions are summarized. All results are expressed as percentage normal sodium influx (from a 0·2 mM-NaCl solution alone).

The effect of pH on sodium influx is shown in Fig. 14. Two acids, HCl and H_2SO_4, and two alkalis, NH_4OH and $(CH_3)_4NOH$, were used. The $(CH_3)_4NOH$ contained NaCl as an impurity in amounts which were appreciable above a pH of 10. The changes in specific activity that this produced were allowed for in the calculation of influxes at such pH's. A further approximate correction was applied, to account for the increase in influx which the higher sodium concentration would normally produce. Clearly,
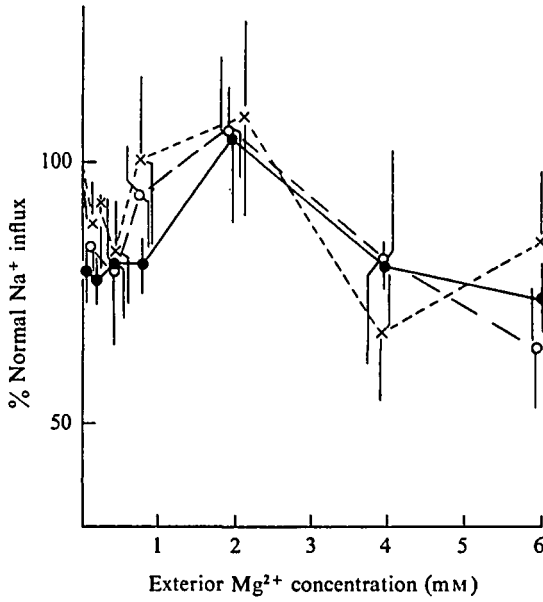
Fig. 8. The effect of different concentrations of magnesium salts on sodium influx in depleted *C. tentans.* —●—, $MgCl_2$ added; —○—, $MgNO_3)_2$ added; — × —, $MgSO_4$ added. Other conditions as in Fig. 4.

Fig. 9. The effect of different concentrations of calcium salts on sodium influx in depleted *C. tentans.* —●—, $CaCl_2$ added; —○—, $Ca(NO_3)_2$ added; — × —, $CaSO_4$ added. Other conditions as in Fig. 4.
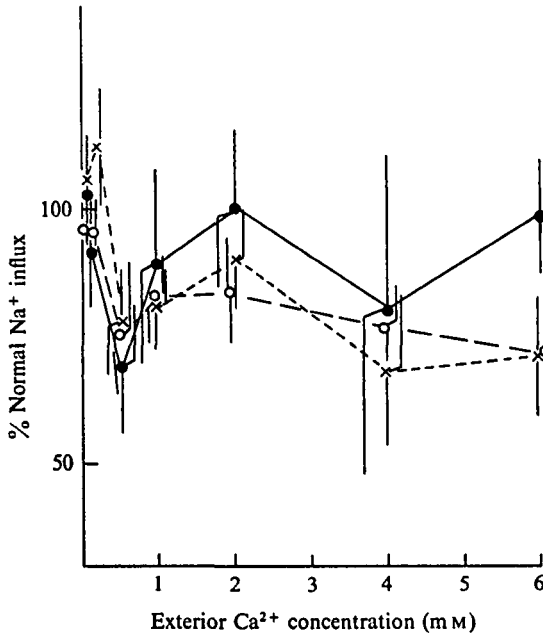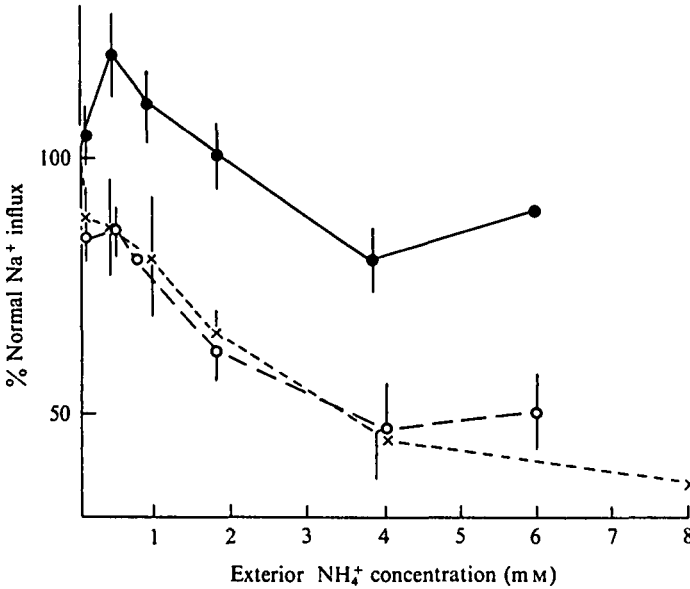
Fig. 10. The effect of different concentrations of ammonium salts on sodium influx in depleted *C. tentans*.—●—, NH₄Cl added; —O—, NH₄NO₃ added; − −×− −, (NH₄)₂SO₄ added. Other conditions as in Fig. 4.
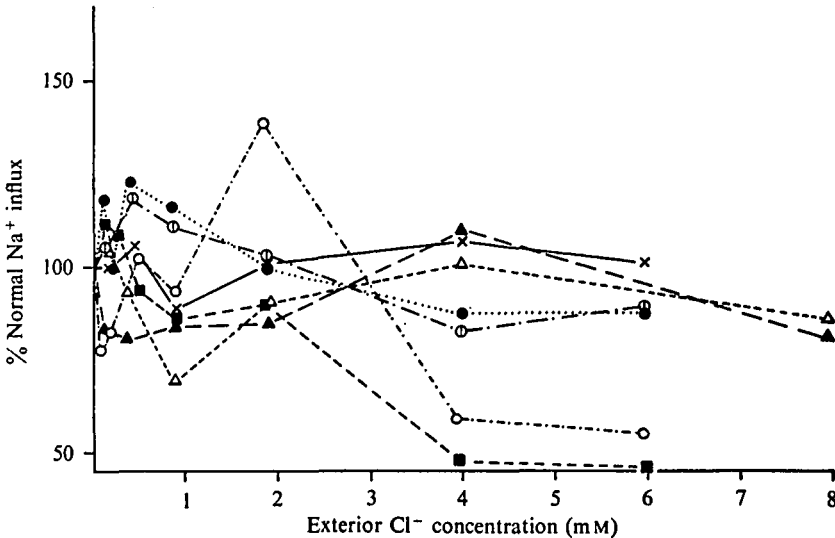

Fig. 11. The effects of various chlorides on sodium influx in depleted *C. tentans*. Sodium concentration = 0·2 mM. Cations: K⁺, —×—; Cs⁺, ···●···; Rb⁺, −·−O−·−; Ca²⁺, − −△− −; Mg²⁺, − − −▲− − −; NH₄, ·—⊕—·; Li, − −■− −.

a low pH greatly inhibits sodium influx. In this respect *C. tentans* is similar to *A. aegypti* larvae (Stobbart, 1967) and *Astacus* (Shaw, 1960b). However, a stimulation of influx with increased pH is not as well marked as in *A. aegypti* (Stobbart, 1967). In so far as sodium influx on 0·2 mM-NaCl (pH approx. 5·6) rarely reaches 0·2 m-mole/ kg/h, some enhancement of influx could be said to have taken place at pH 6 and 9. However, the inhibition of sodium influx in *C. tentans* at pH 7 and 8, giving the
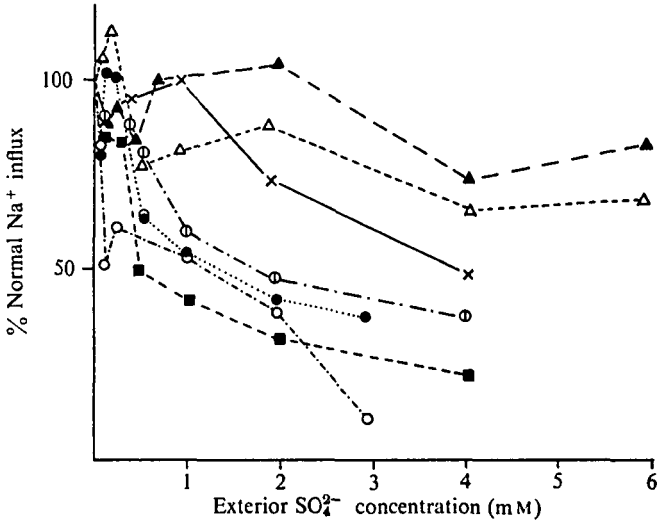
Fig. 12. The effects of various sulphates on sodium influx in depleted *C. tentans*.
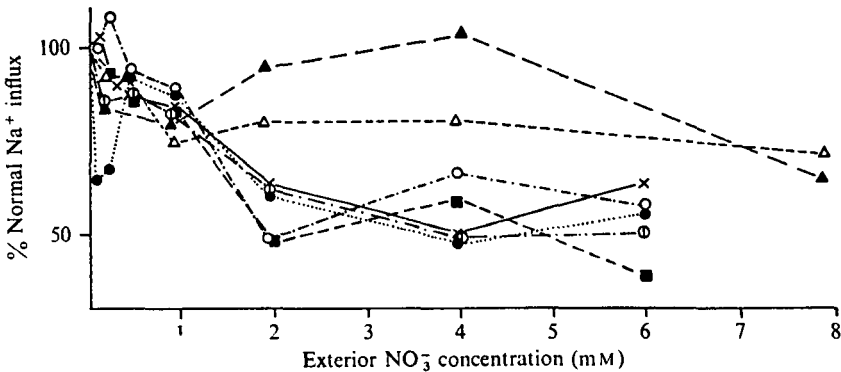Symbols as in Fig. 11.

Fig. 13. The effects of various nitrates on sodium influx on depleted *C. tentans*.
Symbols as in Fig. 11.

graph a bimodal shape, is quite unlike the condition observed in *A. aegypti*. This may be due to a difference in the reaction of the sodium pump of these two species to NH_4^+ and related ions, and is discussed later.

DISCUSSION

Km values for *C. dorsalis* and *C. tentans* are compared with other freshwater animals in Table 2. Clearly the affinity of the pump for sodium is low in chironomid larvae compared with some other freshwater animals, and it would appear that, in considering their freshwater adaptation, this feature is of secondary importance to their relatively low permeability. *Km* values for depleted and steady-state *C. tentans* larvae are very similar, indicating that the affinity of the sodium pump is not altered by the depletion process. This has been found to be the case with a number of other freshwater animals (Shaw, 1959a, b; Horne, 1967; Stobbart, 1967 (reference to unpublished data); Alvarado & Dietz, 1970; Greenaway, 1970), and it seems likely
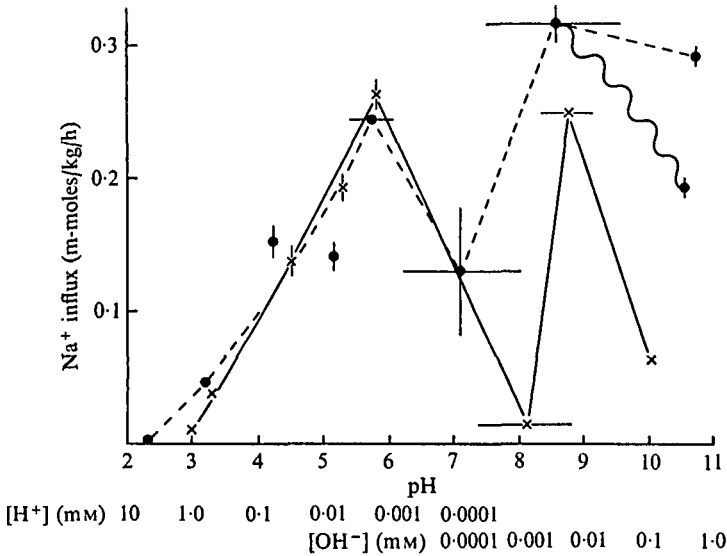
Fig. 14. The effect of pH upon the influx of sodium from 0·2 mM NaCl in depleted *C. tentans*. In animals — × — the solution was acidified with HCl and made alkaline with NH₄OH. In animals – – ● – – the solution was acidified with H₂SO₄ and made alkaline with (CH₃)₄NOH. ∿∿● represents the corrected figure, taking into account contamination of (CH₃)₄NOH by sodium (see text). Horizontal lines represent pH drift during experiment. Other details as in Figs. 4–13.

Table 2. Km *values for some freshwater and brackish-water animals*

Species	Km (mM-Na⁺)	Reference
Gammarus pulex	0·10–0·15	Sutcliffe (1967a)
G. duebeni	1·5–2·5	Sutcliffe (1967b)
G. lacustris	0·10–0·15	Sutcliffe & Shaw (1967)
Astacus pallipes	0·2–0·3	Shaw (1959a)
Potamon niloticus	0·05	Shaw (1959b)
Eriocheir sinensis	1·0	Shaw (1961)
Limnaea stagnalis	0·25	Greenaway (1970)
Aëdes aegypti	0·55	Stobbart (1965)
Camptochironomus tentans	0·75	Present study
Chironomus dorsalis	0·75	Present study
Platichthys spp.	0·8	Maetz (1971)
Salmo gairdneri	0·45	Kerstetter *et al.* (1970)
Lampetra planeri ammocoete larva	0·26 (tapwater acclimated)	Morris & Bull (1970)
Lampetra planeri ammocoete larva	0·13 (depleted)	Morris & Bull (1970)
Ambystoma gracile larvae	0·3–0·55	Alvardo & Dietz (1970)

that the increased operation of the pump is a result of the elaboration of more carrier molecules. With the ammocoete larvae of *Lampetra planeri*, however, it is interesting to note that salt depletion results in a change in both K and Km, indicating a change in sodium affinity of the pump (Morris & Bull, 1970).

Shaw (1959a) has produced evidence which suggests that in *Astacus*, increase in efflux at higher external sodium concentrations, such as is found for *C. tentans* and *C. dorsalis* (Figs. 1 and 2), is due to the introduction of an exchange component (Ussing, 1947) that is probably linked to a carrier involved in active sodium transport.

This presupposes a direct link between influx and efflux at carrier level, and there is conclusive evidence that such a mechanism exists in *A. aegypti* (Stobbart, 1959, 1974). In some freshwater animals the relationship between sodium efflux and the external sodium concentration has also been explained in terms of back transport by an unsaturated carrier system (Kirschner, 1955). As yet there is insufficient evidence to differentiate between the exchange mechanism and back transport in *C. tentans*.

In considering the effects of other ions in sodium influx it is assumed throughout this discussion that cationic interference effects are due to the progressive occupation of the sodium carrier molecule. Such a carrier must be assumed as, otherwise, sodium would diffuse through the anal papillae down the electrochemical gradient. When estimating the degree of inhibition of sodium influx by other ions, difficulties obviously arise from the superposition of a cation and an anion effect. Such problems can be clarified a little by a process of comparison and elimination. Reference to Figs. 11, 12 and 13 suggests, for example, that SO_4^{2-} is more inhibitory than NO_3^-, and that both are more inhibitory than Cl^-. Fig. 3 indicates that this is a reasonable assumption. However, it may be seen that for any one particular cation the general shape of the graph is similar for the three anions concerned (see particularly Figs. 6, 8, 9 and 10). Thus, a cation effect is discernable against a background anion effect.

It can be said that Cl^- is stimulatory in effect only in the sense that the chlorides are the only salts which can, in some cases, 'break free' from or, to some extent, counter the general pattern of inhibition. This is in contrast to *A. aegypti* (Stobbart, 1965, 1967), where Cl^- is much more obviously stimulatory in its effect on the sodium pump. A quantitative estimate on the effects of different ions on the pump is very difficult.

From Fig. 3 it is clear that when sodium is accompanied by SO_4^{2-} *only*, the sodium influx is much smaller (generally < 20%) than influx from NaCl. In the interference experiments (Figs 4–13), such a degree of inhibition by the sulphate salt is rarely found. However it must be remembered that in these experiments an amount of Cl^- equal to the Na^+ (0·2 mM) is always present, albeit swamped by SO_4^{2-} and NO_3^- at higher concentrations. It is suggested then, that it is the permanent presence of Cl^- which maintains the XSO_4 inhibited influx (Figs. 4–10, X = interfering cation) above the Na_2SO_4 inhibited influx (Fig. 3). It is unlikely that the increased SO_4^{2-}:Na^+ ratio (Figs. 4–10) would act in this way. The role of NO_3^- in these experiments is confusing as in some cases the nitrate salts hardly differ from sulphate salts with respect to their effect on sodium influx. It would appear that their effect on sodium influx in the presence of a little chloride is similar to that of sulphate, although Fig. 3 shows that, on its own, NO_3^- appears inhibitory at low concentrations and stimulatory at high concentrations. The latter case clearly indicates movement of sodium that is independent of chloride transport. This has been demonstrated in a number of freshwater animals (Krogh, 1939; Shaw, 1960b; Maetz & Garcia Romeu, 1964; Kerstetter, Kirschner & Rafuse, 1970; Stobbart, 1971). However, there is a marked differential effect on the sodium pump by the three anions used in this study. The degree of anion/cation interaction varies considerably amongst freshwater invertebrates. In *Astacus*, for example, the nature of the anion makes little difference to sodium movement (Shaw, 1960a). *Aëdes* does differentiate between sodium salts, although to a lesser extent than *C. tentans*. A situation similar to that in *C. tentans* is seen in *Eriocheir sinensis*, where sodium influx is greatly lessened in Na_2SO_4, and in fact there is a total

cessation of net sodium movement in Na_2SO_4 and $NaNO_3$ (Koch, 1965). Both influx and net sodium movement are restored to high levels in this animal by the addition of NH_4Cl. Koch (1965) assumes on this evidence that sodium movement in *E. sinensis* is passive and relies on an active chloride pump. Stobbart (1967) discusses the effect in terms of a mutual enhancement of the sodium and chloride mechanisms, and it is likely that a similar mechanism operates in *C. tentans*. The enhancement is considered to be an indirect one, based on a mutual dependence on the dissociation $H_2CO_3 \rightleftharpoons HCO_3^- + H^+$ in facultative Na^+/H^+ and Cl^-/HCO_3^- exchanges. It may be seen, then, that an anion may have an effect on sodium movement through the mediation of the chloride pump, despite having no direct effect on the sodium pump. It is also possible however, that the pumps operate entirely electrogenically. Arguments against such a mechanism, in normal operation, are put by Stobbart (1967). However, it is possible that the sodium pump, at least, is capable of both electrogenic and electroneutral transport, and that the mode of operation is dependent upon prevailing conditions. This is further investigated in a later paper (Wright, 1975b).

Because of the degree of anionic interference, there are problems in attempting to gauge the interference exerted on the sodium pump by the various cations. As a first exercise, it is probably useful to look at the chloride salt. From this, it is clear that Li^+ is generally inhibitory, and so are Rb^+, NH_4^+ and Mg^{2+} at higher concentrations, although in the latter case difficulties arise through there being no significant difference between the effect of any of the salts. In contrast with *A. aegypti*, the K^+ ion has apparently no effect on sodium influx. By implication it appears that Na^+ and K^+ are transported independently in *C. tentans*. This is further considered elsewhere (Wright, 1975b). It is perhaps rather surprising that Rb^+ appeared to be inhibitory in view of its ability to mimic the action of potassium (and caesium) in many tissues (Taylor, Paton & Daniel, 1971). The results reported here for potassium and lithium agree with work by Koch *et al.* (1956) on *Chironomous plumosus*, where '...it was observed that while increasing concentrations of K^+ did not interfere with the uptake of Na^+, Li^+ had a marked effect on the simultaneous uptake of Na^+'. The isolated gill of *Eriocheir sinensis* behaves similarly with respect to these three cations (Koch & Evans, 1956). These authors conclude that the lithium effect could be due to either direct inhibition of the pump, or competition with sodium for the pump. Evidence to be presented later (Wright, 1975b) suggests the latter.

A further point of contrast with *A. aegypti* is the inhibitory effect of NH_4^+ on the sodium pump of *C. tentans*. As in other cases, it is difficult to separate the anion effect from the cation effect. Moreover, synergistic effects cannot be eliminated. Nevertheless, significant inhibition of sodium flux occurs at 4 mM-NH_4Cl. Support for this would appear to come from Fig. 14, where between pH 7 and 8 it is tempting to postulate interference by NH_4^+ (and $(CH_3)_4N^+$), with what otherwise appears to be a mildly stimulatory effect on sodium influx by OH^-. A sodium pump capable of transporting NH_4^+ ions has been described in a number of other freshwater animals (Shaw, 1960b; Dietz, Kirschner & Porter, 1967; Maetz, 1972, 1973). Maetz (1971, 1973) favours the view that Na^+/NH_4^+ exchange may be supplemented by a Na^+/H^+ exchange when sodium absorption is increased. Kerstetter *et al.* (1970) cite Na^+/H^+ as the norm, with NH_3 excretion occurring by diffusion. Stobbart (1971) has clearly shown Na^+/H^+ exchange in *A. aegypti* larvae, despite being unable to demonstrate

any influence by NH_4^+ on the sodium pump. Na^+/H^+ in *C. tentans* is strongly impli‐
cated in Fig. 14, where sodium influx is greatly inhibited at low pH's, although an
indirect disruptive effect on tissues at high H^+ concentrations cannot be ruled out.
Regarding $NH_4^+(H^+)/Na^+$ exchange, it seems unnecessary to apply a rigid rule, at
least in *C. tentans*. It appears that in this animal both H^+ and, to a lesser extent,
NH_4^+ ions are capable of attaching to the sodium pump. Under natural conditions it
may be that both compete as exchange cations for Na^+. It is interesting to note that
NH_4^+/Na^+ exchange has been demonstrated in the hindgut of the Dipteran larva,
Sarcophaga bullata (Prusch, 1972).

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