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THE RELATIONSHIP BETWEEN TRANSEPITHELIAL SODIUM MOVEMENT AND POTENTIAL DIFFERENCE IN THE LARVA OF *CAMPTOCHIRONOMUS TENTANS* (FABR.) AND SOME OBSERVATIONS ON THE ACCUMULATION OF OTHER IONS

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SUMMARY

In fourth instar larvae of *Camptochironomus tentans*, net sodium uptake from 2 mM-NaCl has an electrogenic component. During net uptake the transepithelial potential (TEP) alters from a value of ~ -40 mV (sign refers to haemolymph), in depleted animals, to ~ 0 mV. The TEP in depleted larvae is dependent upon external sodium concentration above about 1 mM-Na^+, becoming increasingly electropositive (haemolymph relative to medium) at high sodium concentrations. This effect is exaggerated in Na_2SO_4 compared with NaCl. At an external concentration of 2 mM-NaCl, chloride is carried by an electroneutral mechanism, probably a closely coupled Cl^-/anion exchange. However, it is possible that chloride transport could become somewhat electrogenic at higher concentrations.

Lithium competes with sodium for the electrogenic pump.

Observed TEPs differ greatly from those required to maintain passively the haemolymph concentrations of sodium and chloride.

INTRODUCTION

There is an increasing body of information about potential differences across epithelia that are concerned with ion transport. The work has largely concerned freshwater vertebrates, and has yielded valuable information regarding the nature of the ionic pumps, notably the sodium pump.

On one hand, considerable use has been made of isolated epithelial preparations, such as the amphibian bladder (Brodsky & Schilb, 1966; Herrera, 1968; Solinger *et al.* 1968) and skin (Linderholm, 1951; Koefoed-Johnsen & Ussing, 1957; Aceves, Erlig & Edwards, 1968). On the other hand, the potential difference across the body wall of whole animals has been the subject of much recent interest (Brown, 1962; Maetz & Campanini, 1966; Dietz, Kirschner & Porter, 1967; Alvarado & Stiffler, 1970; Kirschner, 1970).

Investigations of transepithelial potentials (hereafter referred to as TEPs) in invertebrates can also be divided into studies of intact animals (Smith, 1969*a*, *b*; Bryan, 1960; Stobbart, 1974) and isolated epithelial preparations (Croghan, Curra & Lockwood,

1965). A comparison of Bryan's (1960) data on *Astacus*, with that of Croghan *et al.* (1965) shows that there may be considerable differences between TEP measurements in whole animals and isolated epithelial preparations.

The large haemolymph compartment in chironomid larvae and their vermiform shape afforded a very good opportunity to study TEPs in intact animals, especially in view of the localized and extreme posterior position of the ion transporting membrane. Investigations were confined to the large fourth instar larvae of *Camptochironomus tentans* and were prompted by various points emerging in a previous paper (Wright, 1975*b*).

Data concerning the movement of Na^+, K^+ and Cl^- during net ionic uptake into the haemolymph and the body as a whole extended existing information on body compartmentation of these ions (Wright, 1975*a*), and attention was paid to this.

MATERIALS AND METHODS

Collection and maintenance of experimental animals was as described earlier (Wright, 1975*a*), as was the method of salt depletion. Study was confined to fairly large specimens of fourth instar *C. tentans*, generally 20–30 mg in weight.

Measurement of potential difference (TEP)

Animals were mildly anaesthetized with ether vapour, and laid ventral side uppermost on a bed of beeswax resin (Krogh & Weis-Fogh, 1951). A little of the surrounding resin was melted with a warm needle to hold the larva. This quickly cooled and hardened without damage to the animal. Control tests showed that salt-depleted animals treated in this way were able to take up sodium normally, if the posterior end was covered by a drop of 2 mM-NaCl solution. A high resistance electrode was inserted into the thorax of the larva. The weakest point of the cuticle was at the prothoracic legs, and it was found that, if the electrode was applied at this point, the larva could be pulled onto it like a glove, using fine forceps. The low resistance electrode was held in contact with a small pool (0·3 ml) of external medium which bathed the posterior end of the animal, including the anal papillae. The size of this pool did not appear to affect the TEP over the period measured. The experimental set-up used, is represented semi-diagramatically in Fig. 1. The electrodes were of the mercury-calomel-saturated KCl agar type, having a tip potential of less the 5 mV. This was checked regularly. Leakage of KCl from the electrodes was small (< 1 mμ-mole/min), and the addition of unnaturally high amounts of KCl to a 2 mM-NaCl external medium did not appreciably alter the TEP. Measurements were made with a Vibron Electrometer model 33B-2, with a high input impedance.

Once the thorax of the animal was pierced, drying and shrinkage occurred after a few minutes. This limited time available for TEP measurement, and it was decided to record the potential difference after a period of 2 min. In some cases a degree of drift in potential was noted, which varied in magnitude and sign in different specimens. In measuring the TEP in such cases, it was decided that recording the potential after a set time was preferable to integration of the curve produced on the trace, particularly as the large individual variation necessitated the use of large numbers of animals.

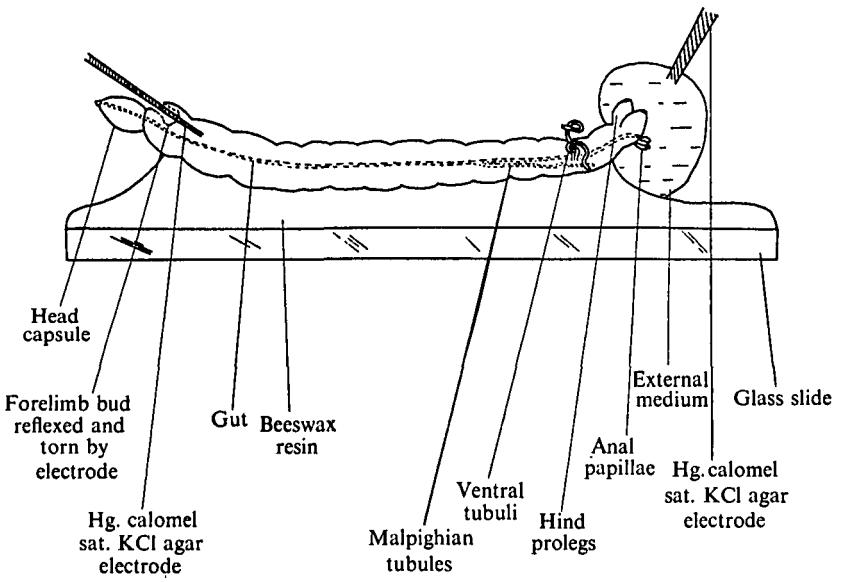

Fig. 1. Semi-diagrammatic representation of preparation used to measure transepithelial potentials (TEPs) in larvae of *C. tentans*.

The sign convention adopted in describing TEPs is that the potential of the inner solution (haemolymph) is measured with respect to the outer solution (medium).

Chemical measurements of sodium, potassium and chloride

These determinations were made as described earlier (Wright, 1975*a*).

Penetration of sulphate into larvae

Sulphate penetration was measured in animals with and without their mouths blocked by beeswax resin (Krogh & Weis-Fogh, 1951). A 1 mM-Na_2SO_4 solution labelled with ^{35}S was used. The ^{35}S was supplied as a $H_2{}^{35}SO_4$ solution by the Radiochemical Centre, Amersham. At the specific activity used, the excess H^+ ions in the experimental solution were negligible. Groups of eight animals were labelled for 2 h. For counting of radioactivity, animals were macerated with 0·2 ml of distilled water, using fine grinding sand and a glass rod. This continued until, when viewed under a microscope, no recognizably intact tissue was seen. A further 0·3 ml of distilled water was added, before spreading, drying, and counting as described earlier (Wright, 1975*a*).

As a control 0·2 ml of a labelled $Na_2{}^{35}SO_4$ solution of known specific activity was used during maceration In this way, it was established that a self-shielding factor reduced the count by 15·8 ± 2·5% ($N = 4$). A suitable correction was applied.

RESULTS

Table 1 gives values for the TEP in steady-state and depleted *C. tentans* larvae, and illustrates the variability encountered throughout this investigation. Despite considerable individual variation, a highly significant ($P < 0{\cdot}001$) increase in TEP was found on depletion, the haemolymph becoming increasingly electronegative with

Table 1. *Some values for the transepithelial potential difference in steady-state and depleted* C. tentans *larvae, in* 2 mM-*NaCl*

P.D. mV (sign refers to haemolymph)

Steady state		Depleted		
−3·0	+7·0	−33·0	−31·5	−35·0
+27·5	−37·0	−32·0	−28·5	−55·0
−16·0	+21·0	−16·5	−10·0	−49·0
+4·0	−10·5	−41·0	−23·5	−36·0
−19·5	+36·0	+7·0	−38·0	−47·0
−33·0	−30·0	−65·0	−22·0	+39·0
+32·0	−30·0	−65·0	−22·0	+35·0
−35·0	+16·0	−43·0	−12·0	−40·0
+40·0	−16·5	−35·5	−29·5	−58·0
−8·5	−24·5	−36·9	−44·0	−27·0
−17·5	+27·5	−43·3	−28·0	−60·0
+56·0	+14·5	−37·2	+5·0	−25·0
+6·0	−54·0	−32·4	−31·0	+30·0
+9·0	−26·0	−35·0	−51·0	−24·5
+16·0	+2·5	−18·5	−71·0	−57·0
—	—	−49·0	−39·0	—
mean = −0·53 ± S.E. 4·80		Mean = −32·29 ± S.E. 3·23		
N = 30		N = 47		

Table 2. *Transepithelial potential differences in freshwater animals*

Species	TEP mV sign refers to internal medium	Concentration of ext. NaCl (mM)	Comments	References
C. tentans	−10	1·0	*In vivo*, steady state	Present study
C. tentans	−46	1·0	*In vivo*, depleted	Present study
Anguilla anguilla	−18	0·5	*In vivo*	Maetz & Campanini (1966)
Blennius pholis	−3	*ca.* 45	*In vivo*	House (1963)
Salmo gairdneri	−10	1·0	*In vivo*	Kirschner (1970)
Rana pipiens	+60	1·0	*In vivo*, depleted	Kirschner (1970)
R. pipiens	+28	1·0	*In vitro*	Brown (1962)
R. pipiens	+13	1·0	*In vivo*	Brown (1962)
Ambystoma tigrinum	+18	1·0	*In vivo*, depleted	Dietz, Kirschner & Porter (1967)
A. gracile	+20	1·0	*In vivo*	Alvarado & Stiffler (1970)
Astacus fluviatilis (= *pallipes*)	−4·1–−6·6	2·0	*In vivo*	Bryan (1960)
Austropotamobius pallipes	−60	2·0	*In vitro*	Croghan *et al.* (1965)
Aëdes aegypti	−47	2·0	*In vivo*, depleted	Stobbart (1974)
A. aegypti	+37	2·0	*In vivo*, steady state	Stobbart (1974)

respect to the external medium. Table 2 compares these TEPs with those measured in other freshwater animals. In this comparison the external sodium concentration was standardized as far as possible. It may be seen that *C. tentans* is similar to *Aëdes*, *Astacus* and the teleosts, in having the body fluid electronegative to the medium at low external salt concentrations.

The time course for net sodium and chloride uptake from 2 mM-NaCl is followed in Fig. 2. The concomitant change in TEP is shown in Fig. 3. This experiment was repeated using 1 mM-Na_2SO_4 (Figs. 4 and 5) and 2 mM-KCl (Figs. 6 and 7). In all cases

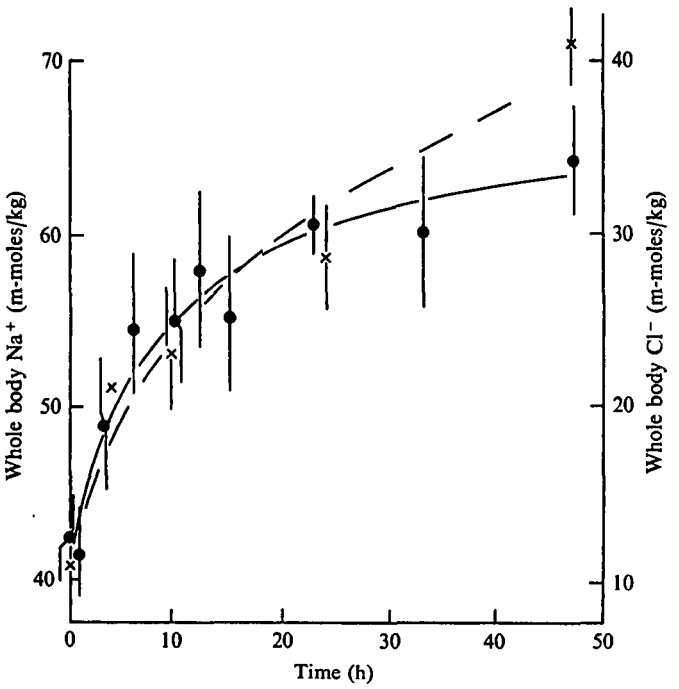

Fig. 2. Net uptake of sodium and chloride from 2 mM-NaCl by *C. tentans* associated with TEP measurements recorded in Fig. 3. —●—, whole body sodium. Each point represents the mean of 4 groups of 6–8 larvae ± S.E. —×—, whole body chloride. Each point represents the mean of 2 or 3 groups of 4–6 larvae ± S.E.

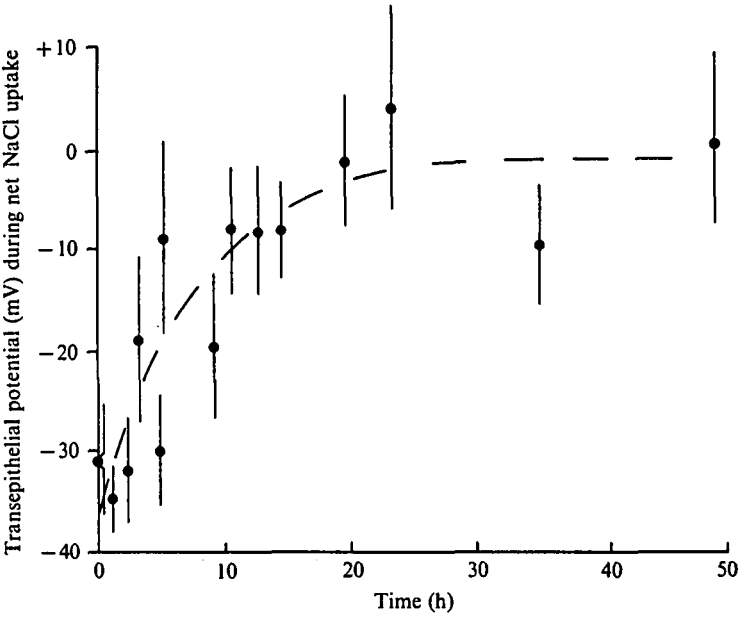

Fig. 3. Transepithelial potential difference measurements during the net NaCl uptake from 2 mM-NaCl by *C. tentans* shown in Fig. 2. Each point represents the mean ± S.E. of groups of 10–23 individuals, except at $t = 0$ where $N = 55$ animals. Line fitted by eye. Sign of TEPs refers to haemolymph with respect to external medium.

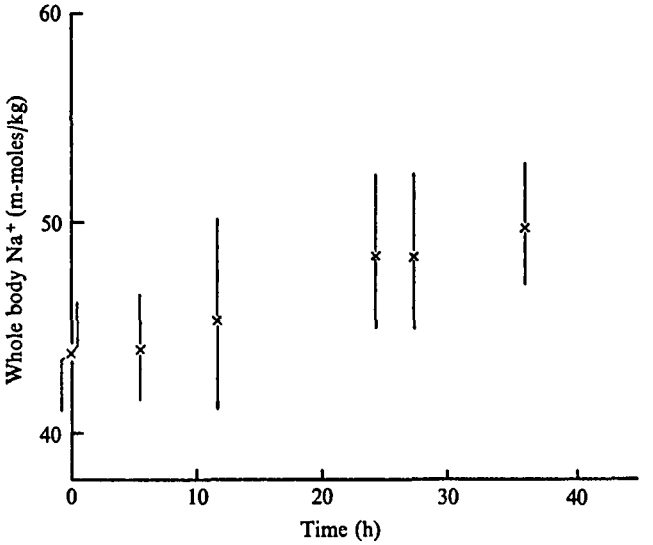

Fig. 4. Net sodium uptake from 1 mM-Na_2SO_4 by *C. tentans* associated with the TEP measurements recorded in Fig. 5. Each point represents the mean of 3 groups of 8 larvae ± S.E.

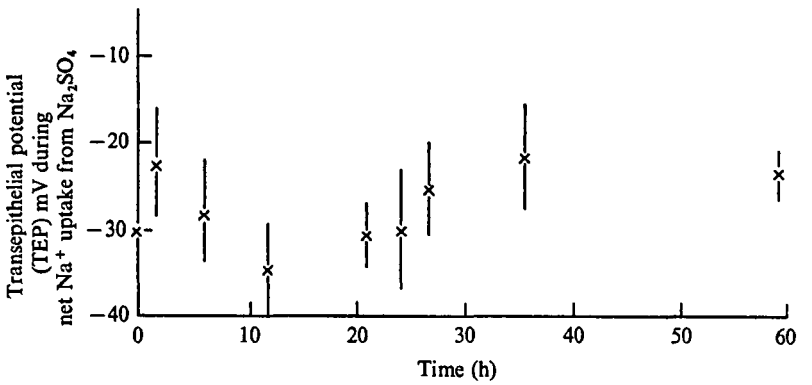

Fig. 5. Transepithelial potential difference measurements during the net sodium uptake from 1 mM-Na_2SO_4 by *C. tentans* shown in Fig. 4. Body sodium determinations not made at 2, 21 and 60 h. Sign of TEPs refers to haemolymph with respect to external medium.

animals used for measurement of TEP were the same as those used for measurement of body ions. A smaller number of animals were set aside from each experimental batch for measurement of haemolymph Na^+ during net uptake from 2 mM-NaCl haemolymph K^+ during net uptake from 2 mM-KCl, and haemolymph Cl^- during net uptake from 2 mM-NaCl and 2 mM-KCl (Fig. 8). Net NaCl uptake from 2 mM-NaCl was accompanied by a drift in TEP from approximately −36 mV to about 0 mV. This change in TEP coincided reasonably well with the time course of net sodium uptake. By contrast, during net sodium uptake from 1 mM-Na_2SO_4 no clearly defined drift in TEP could be seen, and despite some scatter of the data, the TEP after 60 h (−23·45 mV) had altered little from the value of −29·77 mV at time 0 h. This stability of TEP was associated with a very much reduced net sodium uptake. Similarly, no significant alteration in TEP was seen during net KCl uptake from a

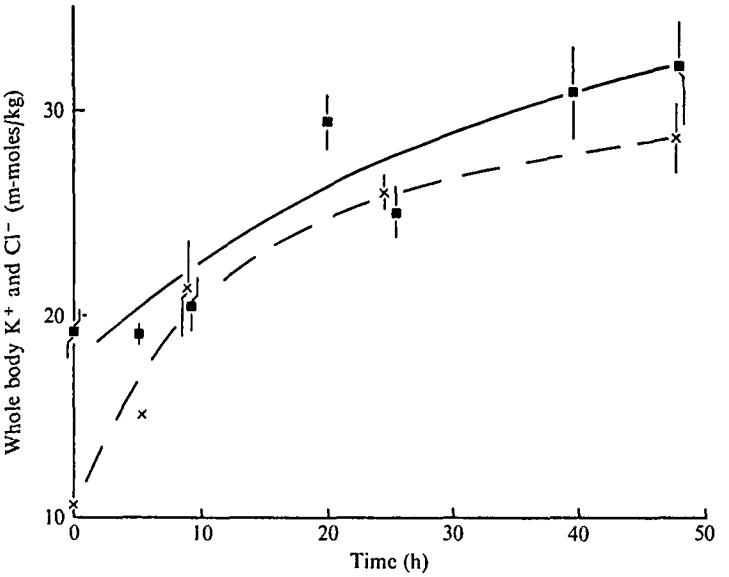

Fig. 6. Net potassium and chloride uptake from 2 mM-KCl by *C. tentans*, associated with TEP measurements recorded in Fig. 7. —■—, whole body potassium. Each point represents the mean ± S.E. of 3 or 4 groups of 6 larvae. – – × – –, whole body chloride. Each point represents the mean ± S.E. of 2 or 3 groups of 5 larvae.

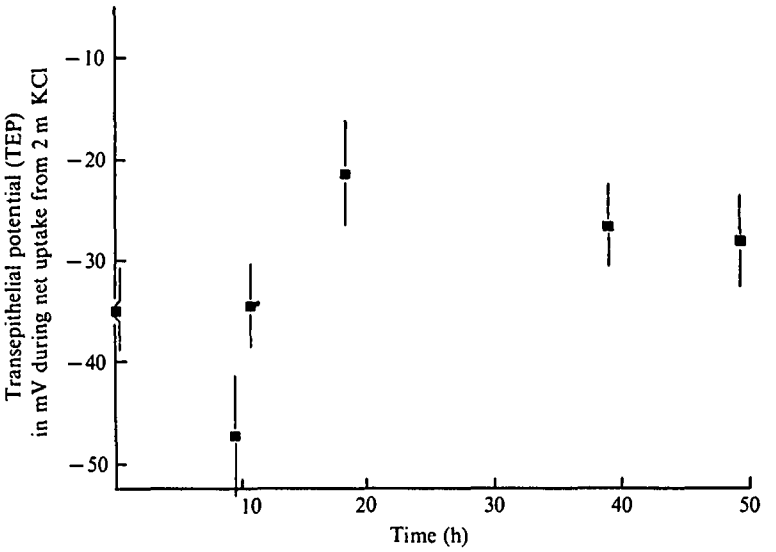

Fig. 7. Transepithelial potential difference measurements during the net KCl uptake from 2 mM-KCl by *C. tentans* shown in Fig. 6. Each point represents the mean ± S.E. from 7 to 12 individuals. Sign of TEPs refers to haemolymph with respect to external medium.

2 mM-KCl solution. TEP values at 0 h and 49 h were −34·74 mV and −29·13 mV respectively.

The inhibition of net sodium uptake from 1 mM-Na_2SO_4 (Fig. 4) emphasizes the strong inhibitory influence of SO_4^{2-} on sodium movement noted earlier (Wright, 1975*b*) and reveals a further similarity, in this respect, with *E. sinensis* (Koch, 1965;

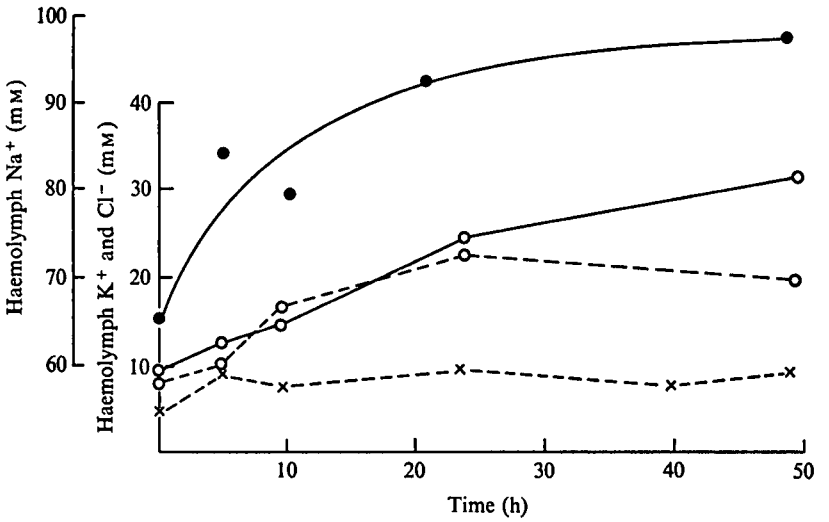

Fig. 8. Haemolymph ion levels during net NaCl and KCl uptake by *C. tentans* (Figs. 2 and 6). —●—, haemolymph sodium during net uptake from 2 mM-NaCl; —○—, haemolymph chloride during net uptake from 2 mM-NaCl; - -○- -, haemolymph chloride during net uptake from 2 mM-KCl; - -×- -, haemolymph potassium during net uptake from 2 mM-KCl. Each point represents the average from 2 samples of pooled haemolymph from 6 to 8 larvae.

Table 3. *Influx of labelled* SO_4^{2-} *into* C. tentans *from* 1 *mM-*$Na_2S^{35}O_4$ *solution (m-moles/kg/h)*

Depleted animals	Depleted animals with mouth blocked
0·0158	0·0040
0·0186	0·0042
0·0169	0·0034
Mean 0·0171 ± 0·0007 (S.E.)	0·00387 ± 0·0002

Each figure represents group of 6–8 larvae.

Wright, 1975*b*). Linear regression analysis of this data does indicate a significant uptake ($P < 0.05$) but this is obviously very slight. Penetration of SO_4^{2-} into the larvae from a 1 mM-$Na_2^{35}SO_4$ solution was followed (Table 3). There is some ingestion of SO_4^{2-} into the gut. However, if gut-blocked animals are considered, the influx of SO_4^{2-} is clearly very small.

The effect of external sodium concentration on TEP was studied in depleted animals placed in NaCl and Na_2SO_4, and in steady-state animals placed in NaCl (Fig. 9). The results obtained from depleted larvae are very similar to those obtained from salt-depleted specimens of the amphibians *Ambystoma tigrinum* (Dietz *et al.* 1967) and *Rana pipiens* (Kirschner, 1970). In both steady-state and depleted *C. tentans* the TEP remains stable at external NaCl concentrations below 1 mM, although that of depleted animals is displaced 30–40 mV negative in comparison with steady-state larvae. From 1 to 5 mM-NaCl in the external medium, there is a significant correlation ($P < 0.01$) between TEP and external NaCl concentration in steady-state animals. In depleted animals, the tendency towards a less negative TEP above an external sodium concentration of 1 mM is curtailed at higher NaCl concentrations. However, when SO_4^{2-}

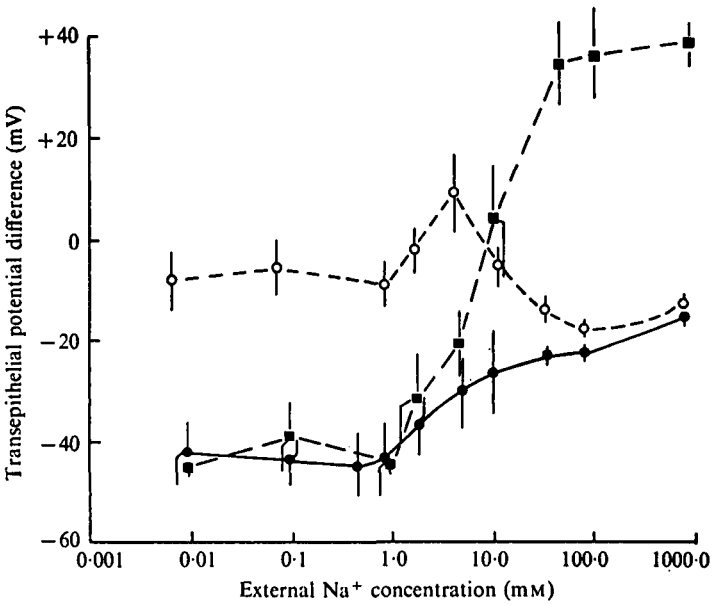

Fig. 9. The relationship betweeen TEP and the external sodium concentration in steady-state and depleted *C. tentans* larvae in NaCl, and depleted *C. tentans* larvae in Na_2SO_4. --■--, depleted larvae in Na_2SO_4;—●—, depleted larvae in NaCl; --○--, steady-state larvae in NaCl; Each point represents the mean ± S.E. of 8 to 30 individuals. Sign of TEP refers to haemolymph with respect to external medium.

replaces Cl^-, the TEP continues the positive trend, reaching about +40 mV at higher external Na_2SO_4 concentrations.

Assuming haemolymph sodium and chloride concentrations to be 93 mM and 30 mM respectively (from Fig. 8), in steady-state animals the equilibrium potential (E) at any external NaCl concentration can be calculated using the Nernst equation, which defines E for ion i:

$$E_i = \frac{RT}{Z_i F} \ln \frac{C_i 1}{C_i 2}, \qquad (1)$$

where R, T, F and Z_i have their usual meanings, and $C_i 1$ and $C_i 2$ are internal and external concentrations respectively. The equilibrium potential as defined here is, in effect, the potential required if observed concentration differences of the ion are to be maintained passively. It is equal but of opposite sign to the potential which would be given by the ion diffusing passively through the anal papillae between compartments of the observed concentrations. Equilibrium potentials for sodium and chloride at different external NaCl concentrations are compared in Table 4 with measured TEPs. Both ions are clearly far from equilibrium, and, for maintenance of steady-state, both ions must be actively secreted into the haemolymph.

Smith (1969*b*) shows how an asymptotic ionic flux-concentration curve may be obtained without necessarily postulating the influence of a carrier (Wright, 1975*b*). This situation results from passive movement of an ion, caused by TEP changes following alteration in concentration of the external medium, and therefore applies to an ion distributed, more or less, according to its electrochemical equilibrium. He concedes, however, that where large concentration differences occur between haemo-

Table 4. *Relationship between equilibrium transepithelial potentials (TEPs) for sodium and chloride, and observed TEPs in different concentrations of NaCl*

Ion species	State	Internal (haemolymph) concentration (mM)	External (medium) concentration (mM)	Equilibrium TEP* (mV). Sign refers to haemolymph	Observed TEP (mV) sign refers to haemolymph
Na^+	Steady	93	0·5	−131·6	*ca.* −9·0
Cl^-	state	30	0·5	+103·1	
Na^+	Depleted	66	0·5	−123·0	−46·8
Cl^-		10	0·5	+75·4	
Na^+	Steady	93	1·0	−114·2	−9·8
Cl^-	state	30	1·0	+85·7	
Na^+	Depleted	66	1·0	−105·5	−45·4
Cl^-		10	1·0	+58·0	
Na^+	Steady	93	2·0	−96·7	−0·52
Cl^-	state	30	2·0	+68·2	
Na^+	Depleted	66	2·0	−88·1	−36·8
Cl^-		10	2·0	+40·5	
Na^+	Steady	93	5·0	−73·6	+10·0
Cl^-	state	30	5·0	+45·1	
Na^+	Depleted	66	5·0	−65·0	−28·6
Cl^-		10	5·0	+17·4	
Na^+	Steady	93	10·0	−56·2	−5·0
Cl^-	state	30	10·0	+27·7	
Na^+	Depleted	66	10·0	−47·5	−24·7
Cl^-		10	10·0	0·0	
Na^+	Steady	93	100·0	+1·8	−17·4
Cl^-	state	30	100·0	−32·1	
Na^+	Depleted	66	100·0	+10·5	−21·5
Cl^-		10	100	−58·0	

* Potential required if observed concentration differences of the ion are to be maintained passively; equal to but of opposite sign to the potential which would be given by the ion diffusing passively through the membrane compartments of observed concentrations.

lymph and external medium, such a curve would arise from a carrier mediated relationship, and so could be described by the Michaelis–Menten equation. Although, regarding sodium, the latter condition clearly exists in *C. tentans*, it was considered of interest to compare the actual flux-concentration curve observed in this species (taken from Wright, 1975*b*) with that expected to result from passive movement caused by TEP changes resulting from alterations in concentration of the external medium. The object of this was to provide a quantitative estimate of the degree of disparity between the two curves.

To obtain values for expected effluxes, use was made of the modified Goldman equation (Goldman, 1943; Smith, 1969*b*), which describes net flux of ionic species i from phase 1 to phase 2, assuming that the constant field theory holds;

$$\mathcal{J}_i = P_i(Z_i FV_{12}/RT).\frac{C_i 2 - C_i 1 \exp(Z_i FV_{12}/RT)}{1 - \exp(Z_i FV_{12}/RT)}, \qquad (2)$$

where $\mathcal{J}_i$ = net flux of $_i$ in moles/cm/s, P_i = permeability coefficient of $_i$ in cm/s, V_{12} = electrical potential of phase 1 with respect to phase 2, $C_i 1$ and $C_i 2$ = concentrations of $_i$ in phase 1 and 2 respectively, Z_i, F, R and T have their usual meanings.

Table 5. *Comparison of expected efflux in depleted* C. tentans, *assuming passive sodium movement, with observed efflux* (*Wright*, 1975b; *Fig.* 1)

External sodium concentration (mM)	*Expected efflux (Moles cm^{-2} sec^{-1})	*Expected efflux as m-moles/kg/h	Observed efflux m-moles/kg/h
0·01	$4{\cdot}41 \times 10^{-12}$	0·00561	0·017
0·1	$4{\cdot}00 \times 10^{-12}$	0·00513	0·375
0·5	$4{\cdot}25 \times 10^{-12}$	0·00545	0·521
1·0	$4{\cdot}41 \times 10^{-12}$	0·00561	1·008
2·0	$5{\cdot}05 \times 10^{-12}$	0·00641	0·997
5·0	$5{\cdot}93 \times 10^{-12}$	0·00761	1·130

* Predicted from equation (3) using TEP measurements from Fig. 9.

Before the equation is workable, an estimate must be obtained of the surface area of the anal papillae, in order to place the ionic flux in appropriate units. To a reasonable approximation, the anal papillae of a 20 mg larva of *C. tentans* can be thought of as four cylinders of dimensions 0·5 mm length × 0·25 mm diameter, thus having a total surface area of 0·0176 cm^2. P_i also has to be defined. Equation (2) assumes that active transport does not occur, and, in order to evaluate P_i, a net flux must be found which is essentially passive. It was decided that efflux into a very dilute external medium would approximate to a passive loss. As the flux concentration data for depleted larvae was to be used for comparison, it was thought appropriate to take the lowest observed efflux of 0·125 m-moles/kg/h at 50 μM-NaCl (Wright, 1975*b*, Fig 1*b*). This represented a flux of $3{\cdot}93 \times 10^{-10}$ moles/cm/sec. Substitution in equation (2) gives a value for P_i of $1{\cdot}64 \times 10^{-7}$ cm/sec. This can only be taken as an approximate value, in view of the variability of efflux data already noted. It must be remembered too that permeability is probably higher in steady-state *C. tentans*. The equation for unidirectional efflux measured using a tracer is

$$\text{Efflux} = \frac{P_i Z_i F V_{12}}{RT} \frac{C_i 1 \exp(Z_i F V_{12}/RT)}{\exp(Z_i F V_{12}/RT)^{-1}}. \qquad (3)$$

The value for P_i derived from equation (2) was substituted in equation (3). This gave the efflux value which would be expected on purely passive grounds at a given TEP. Effluxes derived in this way have been compared with observed effluxes at six different external sodium concentrations between 0·01 mM and 5·0 mM (Wright, 1975*b*, Fig. 1). The data are shown in Table 5, with units for efflux given as both m-moles/kg/h and moles/cm^2/sec. The data are also presented in Fig. 10, where the great disparity between expected passive efflux and observed efflux may be clearly seen.

An experiment was designed to investigate further the inhibitory role of lithium on the sodium pump, as indicated in an earlier paper (Wright, 1975*b*). Net sodium uptake from a solution containing 2 mM-NaCl + 10 mM-LiCl was followed, along with concomitant TEP changes (Figs. 11 and 12). A $Li^+ : Na^+$ ratio of 5 is known to be sufficient to inhibit sodium uptake almost completely. Control animals were placed in 2 mM-NaCl only. As mentioned earlier, the same animals were used for both total body sodium and TEP measurement. Control animals gave similar results to those described in Fig. 3. It is interesting to note, however, that while no net sodium

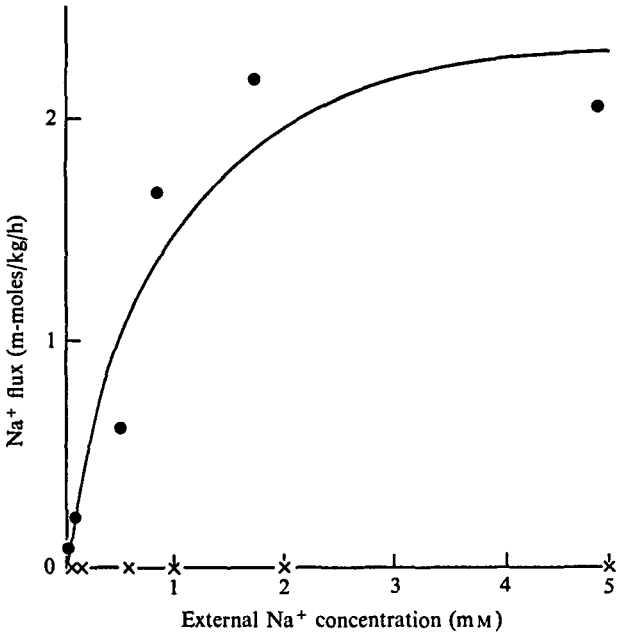

Fig. 10. A comparison of sodium efflux in depleted *C. tentans* predicted from equation (3) using TEP measurements from Fig. 9 (assuming passive sodium movement) with observed efflux (taken from Wright, 1975*b*; Fig. 1). —●—, observed efflux (S.E.'s omitted); ×, predicted efflux (assuming diffusive influences only).

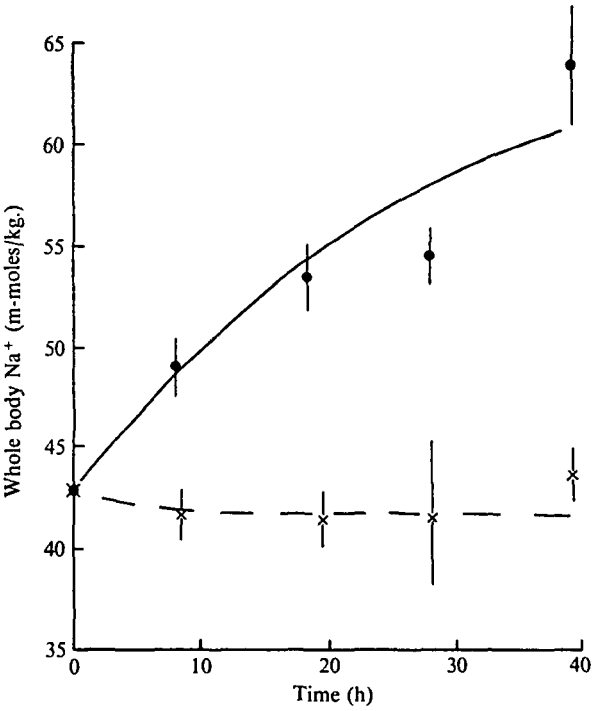

Fig. 11. Total body sodium followed in depleted *C. tentans* after immersion in (2 mM-NaCl + 10 mM-LiCl). —●—, control animals in 2 mM-NaCl only; –×–, animals in (2 mM-NaCl + 10 mM-LiCl). Each point represents the mean for 3 groups of 8 larvae.

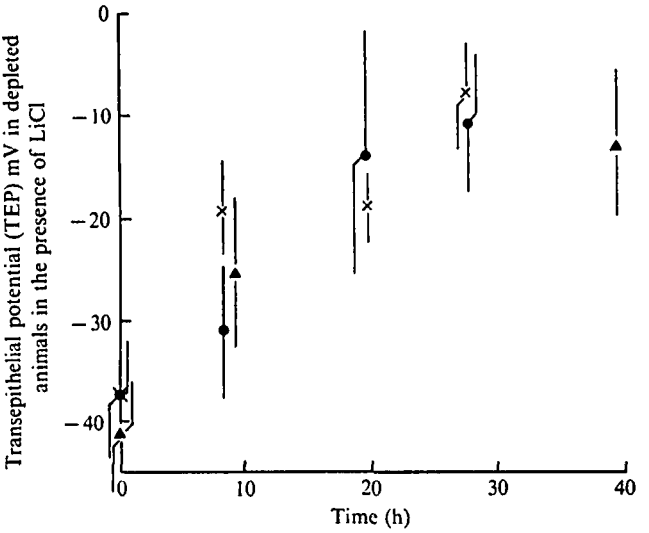

Fig. 12. Transepithelial potential difference measurements made in depleted *C. tentans* exposed to (2 mM-NaCl + 10 mM-LiCl), and to 2 mM LiCl; ●, controls* (2 mM-NaCl); ×, (2 mM-NaCl + 10 mM-LiCl)*; ▲, (2 mM-LiCl). * animals taken from batches used for measuring total body sodium, Fig. 11. In all cases each point represents the mean ± S.E. for 6 to 11 individual TEP determinations. Sign of TEPs refers to haemolymph with respect to external medium.

uptake was possible from the NaCl/LiCl mixture, the TEP changes in these animals were similar to the controls (Fig. 12). A third batch of animals placed in 2 mM-LiCl displayed similar TEP changes (Fig. 12), although whole body lithium content was not followed.

DISCUSSION

Work on isolated epithelia and whole body preparations has led to the suggestion of a number of possible systems for sodium transport in freshwater animals. A consideration of certain features is useful in placing the data presented here within the framework of existing models.

Models for isolated epithelia are, essentially, modifications of Koefoed-Johnsen & Ussing's (1957) frog skin model, and involve at least one cationic pump on the inner and outer borders of the epithelium. A saturable electrogenic sodium pump has been postulated at the outer, apical surface of such complex vertebrate epithelia (Biber & Curran, 1970), for which lithium may also compete (Frazier, 1964; Biber & Curran, 1970) and cause a TEP to be developed (Herrera, Egea & Herrera, 1971). Brodsky & Schilb (1966), working on turtle bladder membrane, note that in sodium-free mucosal solutions the serosal (blood) side becomes electronegative to the mucosa. When a sodium-rich mucosal solution is used, the serosal side becomes less electropositive to the mucosal side. Increasing serosal electropositivity with increase in external sodium is also noted in the frog skin (Brown, 1962). Net sodium transport across the turtle bladder membrane into the blood is associated with increased electropositivity of the serosal side followed by a period when this side becomes less electropositive. This may be considered in the light of work by Solinger *et al.* (1968), who showed that inhibition of the sodium pump in the turtle bladder by ouabain was

accompanied by a reversal of TEP. Both effects may be explained in terms of a electrogenic cation pump superimposed upon an electrogenic anion pump. The two pumps are apparently independent.

There are, then, two features widely found in freshwater vertebrate epithelia which could contribute to a model for *C. tentans*: an electrogenic sodium pump secreting sodium into the blood, and a high degree of independence of anion and cation transport. It seems that an electrogenic sodium pump causing transepithelial separation of charge in *C. tentans* is the best explanation of data from Figs. 2–7. When net sodium movement is restricted, so too is a change in TEP (Figs. 4 and 5). TEP also remains stable in the absence of sodium in the external medium. Data from Figs. 2–7 compare fairly closely with similar data for *Aëdes aegypti* (Stobbart, 1974). Probably the most significant difference concerns TEP changes in 1 mM-Na_2SO_4, where a significant shift in TEP in *Aëdes* was associated with net sodium uptake from this solution. The stability of TEP in depleted *C. tentans* placed in 1 mM-Na_2SO_4 (Fig. 5) probably reflects the greater inhibition of sodium movement by SO_4^{2-} in this species, and is consistent with the conclusions drawn here.

Despite some possible mutual enhancement (Wright, 1975*b*) it seems that the sodium and chloride pumps are capable of working quite independently of each other. Probably the best evidence of this comes from a consideration of net chloride uptake from NaCl and KCl (Figs. 2 and 6). Unlike the turtle bladder, carriage of charge by chloride does not appear to be implicated in *C. tentans* at these external chloride concentrations. It is possible that stabilization of TEP during net uptake from KCl could be due to a 'balance' of charges carried out by K^+ and Cl^-. However, such a model would presuppose a separate electrogenic potassium pump, electrically equal to the chloride pump, and electroneutral chloride movement seems a more likely explanation. Dietz *et al.* (1967) found that a wide variation in external chloride concentration, in the absence of sodium, made no difference to the TEP in *Ambystoma tigrinum*. They concluded that chloride is transported in a non-electrogenic manner over a wide range of external concentrations. In both *C. tentans* and *A. tigrinum* it seems likely that the mechanism concerned is a closely coupled anion/anion exchange.

It seems, then, for depleted *C. tentans* placed is solutions containing the major ions (Na^+, Cl^- and K^+) at concentrations of 2 mM the TEP data can be explained in terms of a sodium pump working electrogenically, with chloride (and possibly potassium) being transported by an electroneutral pathway. The term 'electrogenic' is used here to describe a mechanism causing a very localized separation of charge, and is not intended to imply a strict quantitative link between TEP and ionic movement. The possibilities of electrogenic Cl^- movement, or indeed electroneutral Na^+ movement at external concentrations other than 2 mM, have not been eliminated and are discussed later.

Data from Figs. 2, 6 and 8, although limited, suggest that the total amount of chloride taken up from NaCl was greater than that taken up from KCl, despite similar initial net uptake rates for chloride. It is interesting to note that such a phenomenon has been described in *Rana esculenta* (Krogh, 1937), *Aëdes aegypti* (Stobbart, 1967) and *Ambystoma gracile* (Alvarado & Dietz, 1970). The latter authors suggest that the Na : Cl ratio may be of some importance in ionic control, as suggested by Shaw (1960) for *Astacus*. Although it is possible that TEP changes during net NaCl uptake could

create a more favourable gradient for chloride accumulation than in KCl, it is unlikely that this effect would be very great, due to the largely electroneutral and independent nature of the chloride pump. No significant overshoot in TEP during net NaCl uptake is seen in *C. tentans*, as was noted in the turtle bladder membrane (Brodsky & Schilb, 1966).

Stobbart (1967) noted that, in *A. aegypti*, increased chloride uptake was more effective in enhancing sodium uptake than vice versa. The effect was manifest as an increase in sodium influx, and a decrease in outflux. It has already been noted that the chloride uptake rate is similar in both KCl and NaCl. In an earlier paper (Wright, 1975*b*) it was implied that chloride does stimulate sodium influx to some extent. As net sodium uptake from 1 mM-Na_2SO_4 is very small (Fig. 4), the sodium efflux at this concentration may be taken as more or less equal to influx (Wright, 1975*b*, Fig. 3). Comparing these values with similar data for sodium fluxes and net uptake in NaCl (Wright, 1975*b*), it appears that compared with sulphate, chloride promotes a very much increased sodium influx, although, unlike the situation in *A. aegypti*, efflux is also slightly increased. As was noted earlier (Wright, 1975*b*), it is always difficult to decide on a baseline for assessing ionic interactions. For example, it is impossible to say where inhibition of sodium movement by sulphate 'ends', and enhancement by chloride 'begins'. Certainly the lack of sulphate penetration would create an unfavourable electrical gradient for inward sodium movement. It must be remenbered, however, that this may have little effect on *active* sodium movement and, bearing this in mind, it is interesting to consider the data relating TEP to the external sodium concentration.

In depleted animals there is a non-linear change in TEP with increasing external sodium, the haemolymph becoming less electronegative (Fig. 9). The stability of the TEP in *C. tentans* below 1 mM-NaCl may be due to a low sodium influx, perhaps coupled with a non-electrogenic mode of operation of the pump, such as a closely linked cation/cation exchange. In this respect *C. tentans* is similar to *Austropotamobius pallipes*, where stability of TEP was noted until the sodium concentration of the external medium rose above 2 mM (Croghan *et al.* 1965). In *C. tentans* above an external sodium concentration of 1 mM the TEP becomes concentration dependent. The most likely explanation for this appears to be a change in mode of operation of the pump involving the introduction of the electrogenic component, which is apparent in the net uptake experiments (Figs. 2–7). It is possible that an increase in sodium permeability may also be a contributory factor. At higher external NaCl concentrations, the rate of change of TEP in *C. tentans* falls off and diverges from the line followed in Na_2SO_4. In the amphibians *Rana pipiens* (Kirschner, 1970) and *Ambystoma tigrinum* (Dietz *et al.* 1967) there is a similar effect, and in fact the blood becomes less electropositive at higher NaCl concentrations. This effect has also been noted in isolated frog skin (Linderholm, 1951). Kirschner (1970) attributes this effect to an increase in permeability to chloride at high NaCl concentrations. As in *C. tentans* there is a continued tendency towards a more positive TEP at higher external NaCl concentrations (Fig. 9), it appears that despite a possible chloride influence at higher NaCl concentrations, transport of charge by sodium still remains 'ahead' of chloride. It may be noted that the levelling off of the TEP at higher NaCl concentrations starts at concentrations where the electrochemical gradient is still opposed to chloride accumulation. This could be explained by a chloride pump operating in an electro-

genic mode at such concentrations. However, further work would be needed to elucidate this.

Table 3 indicates that *C. tentans* is almost completely impermeable to sulphate. This is similar to the situation in *A. tigrinum* (Dietz *et al.* 1967). As TEPs at very low concentrations of both NaCl and Na_2SO_4 are similar, it would appear that the effective chloride permeability at these low concentrations is also very low. It seems reasonable to assume, for depleted animals, that the logarithmic part of the graph relating external sodium (as Na_2SO_4) to TEP is due to electrogenic sodium movement. Although separation of charge appears to be unimpeded, the low influx and net sodium uptake rates from Na_2SO_4 suggest that SO_4^{2-} strongly inhibits the release of Na^+ from the inner side of the membrane.

Although in both steady-state and depleted animals the TEP below 1 mM-NaCl remains stable, in depleted larvae the TEP is displaced by about -35 mV. This displacement of TEP is opposite in sign to that obtained by Dietz *et al.* (1967) for *A. tigrinum*. In *C. tentans* this probably represents a sodium diffusion potential, the mobility of chloride being reduced more than that of sodium during the depletion process. Between 1 mM and 5 mM-NaCl there is a significant correlation between TEP and external sodium ($P < 0{\cdot}01$), which may represent electrogenic sodium movement at these concentrations. Above 5 mM-NaCl, the TEP again goes negative, and, at very high external NaCl concentrations coincides with the TEP for depleted animals. This, again, may be due to an alteration in the relative permeabilities of sodium and chloride.

As *C. tentans* has been reported from salinities of these high values (Palmen & Aho, 1966), clearly more work on sodium and chloride movements at such external concentrations would be valuable. Consideration of flux-concentration data in the light of observed TEPs (Fig. 10) indicates that the mechanism for sodium accumulation in *C. tentans* is clearly very different from *Artemia* (Smith, 1969*a*, *b*), although again a study of this relationship in *C. tentans* exposed to dilutions of seawater would provide a more valid comparison, and would seem to be a useful area of expansion of this investigation.

It appears from Figs. 11 and 12 that lithium competes with sodium for the same electrogenic mechanism. Once again, this compares with larval *A. tigrinum* (Dietz *et al.* 1967) where '...of the alkali and alkaline earth metals tested, only sodium and lithium generated a concentration dependent TEP (transepithelial potential)'.

Overall, then, the TEP data presented here, for *C. tentans* is broadly similar to that collected for whole animal preparations of freshwater amphibians (Dietz *et al.* 1967; Alvarado & Dietz, 1970; Alvarado & Stiffler, 1970), the larva of *A. aegypti* (Stobbart, 1974) and the isolated crayfish gill (Croghan *et al.* 1965).

A more detailed model of TEP components in *C. tentans* is difficult without access to the inner side of the membrane. However, if the transporting membrane proves to be a syncytium as in *Aëdes* larvae (Sohal & Copeland, 1964), the mechanism may be rather more simple than in complex vertebrate epithelia.

Reference to whole body and haemolymph sodium, potassium and chloride levels shown in Figs. 2, 6 and 8 indicates that these ions are considerably reduced in depleted animals. Sodium, potassium and chloride loss represent approximately 40, 32 and 60% of the body totals respectively. In both steady-state and depleted larvae it

appears that approximately 60% of the body chloride is contained in the haemolymph. As haemolymph and whole body chloride are restored proportionally, there is obviously free exchange of haemolymph and tissue chloride. Haemolymph potassium in depleted animals was not significantly different from steady-state animals (Wright, 1975*a*). This is probably indicative of the small haemolymph potassium compartment (apparently only 10% of total body potassium) in these animals. During net uptake from 2 mM-KCl, total body potassium was slowly restored over a period of 50 h or more. However, during net uptake, a rise in haemolymph potassium was noted within a period of 5 h and this was apparently sustained even after restoration of total body potassium. It is possible that this replaced some of the haemolymph sodium.

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