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ELECTRICAL-POTENTIAL DIFFERENCE AND SODIUM ION FLUXES ACROSS THE INTEGUMENT OF *COROPHIUM VOLUTATOR* (CRUSTACEA; AMPHIPODA), A EURYHALINE HYPEROSMOTIC REGULATOR

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SUMMARY

1. Measurements of electrical-potential difference (TEP) and Na^+ efflux, across the integument have been made for *Corophium volutator* acclimated to 85 or 15 %SW.

2. The TEP of acclimated animals in 85 or 15 %SW is 1·4 mV or 11·1 mV respectively (haemolymph negative). For acclimated animals, unidirectional Na^+ efflux is 154·3 nmol mg^{-1} body weight h^{-1} in 85 %SW [efflux rate constant (k) = 0·70 h^{-1}] and approximately 35·5 nmol mg^{-1} h^{-1} in 15 %SW (k = 0·50 h^{-1} in 10 %SW).

3. The results indicate that Na^+ and Cl^- are passively distributed across the ion-permeable (gill) integument of acclimated animals in 85 %SW, but that active uptake of Cl^-, and possibly Na^+ also, occurs across the gills of acclimated animals in 15 %SW. The ion transport mechanisms appear to effect electroneutral transfers across the gill integumental epithelium.

4. *Corophium volutator* gill integument has a high permeability to ions; permeability to Na^+ (P_{Na}) is $7{\cdot}5\times10^{-8}$ m s^{-1}, and the ratio P_{Cl}/P_{Na} is 0·45, for animals acclimated to either salinity. The resistance of the gill epithelium of acclimated animals has been calculated to be $5{\cdot}3\times10^{-3}$ Ωm^2 and $2{\cdot}0\times10^{-2}$ Ωm^2 in 85 and 15 %SW respectively.

INTRODUCTION

The majority of euryhaline crustaceans are aniso-osmotic regulators over at least part of their salinity tolerance range. The strategies employed by these species to maintain salt and water balance whilst retaining permeable areas of integument (principally that of the gills) for exchange of dissolved gases and certain metabolites have been well documented (Lockwood, 1977; Kirschner, 1979). Osmoregulators generally maintain a transintegumental concentration gradient of NaCl between extracellular fluid (haemolymph) and medium. A significant electrical-potential difference (p.d.) also is developed across the integument of many species (Croghan,

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Curra & Lockwood, 1965; Lockwood & Andrews, 1969; Smith, 1969*a*; Lucu, 1977); this p.d. may be described in terms of the diffusional movements of Na^+ and Cl^- across the permeable surface of the gills (Croghan, 1976). Transintegumental electrochemical-potential gradients of Na^+ and Cl^- are maintained by utilizing active ion transport mechanisms situated within the epithelium of the gill integument (Kirschner, 1979).

Many studies on the nature of ion fluxes across the gill integument of osmoregulating crustacean species have considered ion movements with regard to certain electrical characteristics of the integumental epithelium (e.g. Croghan *et al.* 1965; Smith, 1969*a,b*), although few of these investigations relate to a euryhaline hyperosmotic regulator. *Corophium volutator* (Pallas) is a euryhaline amphipod (salinity tolerance range 2‰ to 50‰; McLusky, 1968) displaying considerable tolerance to rapid changes of environmental salinity (Taylor, 1984). *Corophium volutator* exhibits marked hyperosmotic regulation when acclimated to salinities below 20‰ (60 % seawater); at higher salinities haemolymph and medium tend increasingly towards iso-osmoticity (McLusky, 1968). A burrowing species, it characteristically inhabits muddy estuaries. This study investigates the p.d. and Na^+ fluxes across the body surface of *Corophium volutator*, particularly in relation to the environmental salinity, in an attempt to outline the electrochemical nature of Na^+ (and, indirectly, Cl^-) movements across the (gill) integument.

MATERIALS AND METHODS

General methods

Corophium volutator was collected from a small North Sea estuary near Skegness (Lincolnshire). The species was identified after Ingle (1969).

Experiments were performed at 12·0 ± 0·5 °C. Animals were acclimated at this temperature to dilutions of Skegness seawater (85 %SW or 15 %SW; where 100 %SW = 34·5‰ salinity) for at least 7 days prior to the experiments. Intermoult animals over 3 mm in body length (excluding ovigerous females) were used throughout. Pleopod ventilatory activity of all experimental animals was noted; if this became impaired the experiment was terminated and the results discarded.

Individual animals were weighed to ±0·05 mg on a DKP torsion microbalance. Mean experimental animal weight was 7·0 mg.

Haemolymph samples (0·2–0·5 μl) were collected using fine-tipped glass micropipettes; Na^+ concentration of a sample was determined by atomic absorption spectrophotometry (Techtron AA6) or Cl^- concentration by the first method of Ramsay, Brown & Croghan (1955).

The ionic compositions of the major experimental media are shown in Table 1. The osmotic pressure (OP) of all experimental media was determined on 0·2 ml samples using an Advanced 3W osmometer. Dilutions of artificial sea water (ASW) were used in experiments. In certain media major cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) or anions (Cl^-) were substituted by choline (Cho^+, as ChoCl) or benzenesulphonate (BeS^-, as NaBeS), ions respectively. Amiloride (Merck, Sharp & Dohme Ltd; donated by the Physiology Department of Leicester University) was used (0·1 mmol l^{-1}) to inhibit Na^+ transport (Kirschner, 1979). SCN^- (as NaSCN;

Table 1. *Composition of experimental media*

Ion	Ion concentration (mmol l^{-1})					
	ASW	ChoASW	BeSASW	NaASW	AFW	FWB
Na^+	460	66	460	460	2	
K^+	10	10	10		0·25	0·25
Ca^{2+}	10	10	10		2·5	2·5
Mg^{2+}	53	53	53		0·5	0·5
Cho^+		394		136		
Cl^-	536	536	142	536	3·0	1·0
SO_4^{2-}	28·75	28·75	28·75	28·75	2·5	2·5
HCO_3^-	2·5	2·5	2·5	2·5	0·25	0·25
BeS^-			394			

ASW, artificial sea water (after Pantin, 1948) (osmotic pressure = 1060 mosmol kg^{-1}).
AFW, artificial fresh water (osmotic equivalent of 1·0 %ASW; 12 mosmol kg^{-1}).
FWB, freshwater buffer.

0·05 mmol l^{-1}) was used as a Cl^- transport inhibitor (Kirschner, 1979). All experimental media were adjusted to pH 7·6 as necessary.

Data are expressed as ±1 standard error of the mean. The number of experimental animals (N) is given in parenthesis. The difference between sample means was tested for significance using Students t-test, differences were considered significant if $P < 0{\cdot}05$.

Theoretical considerations

In this study it is assumed that the silver-staining integument of *Corophium volutator* (0·99 $mm^2 mg^{-1}$ animal wet weight; Taylor, 1984), which is principally that of the gills, represents the only low resistance pathway for ion movements between haemolymph and external medium, and that the limiting barrier to such movements is the epithelium of this integument. Ion activity coefficients are assumed to be all equal to one another. The sign given to the p.d. across the integumental epithelium (transepithelial potential; TEP) is that of the haemolymph with respect to the medium.

At equilibrium there is equality of the electrochemical potential of an ion species (j) in the two phases [denoted here; in (haemolymph), out (medium)] separated by an epithelium (treated here as a single membrane); the Nernst equilibrium potential for that ion (E_j, in V) is

$$E_j = (RT/ZF) \ln([j]_{out}/[j]_{in}), \quad (1)$$

where [j] = ion concentration in denoted phase (mmol l^{-1}), R = gas constant (8·31 J $mol^{-1} K^{-1}$), T = absolute temperature (K), Z = ion valency (including sign), F = Faraday constant (96 500 C mol^{-1}).

The system of ion movements across and within the apical and basal membranes of an epithelium may be considered in terms of a theoretical model (Croghan, 1976; Schultz, 1979). In simple models all transepithelial ion movements (or at least all ion movements contributing to the development of the TEP) are assumed to be

passive and independent of the movement of other ions and of solvent water. Two such models are considered here (in terms of the major ion species Na^+ and Cl^- only) as alternative descriptions of ion movements across *Corophium* permeable integument.

The Goldman (constant field) model

A TEP may be developed across an epithelium displaying differential permeability between Na^+ and Cl^- (P_{Na}, P_{Cl} – the epithelial permeability to Na^+ or Cl^- respectively). If the epithelium is regarded as a single membrane the TEP [a diffusion potential; E (in V)] may be related to the ion gradients across the epithelium, thus (in terms of P_{Na}; after Hodgkin & Katz, 1949)

$$E = (RT/F) \ln \frac{[Na^+]_{out} + \alpha[Cl^-]_{in}}{[Na^+]_{in} + \alpha[Cl^-]_{out}}, \quad (2)$$

where α = ratio of Cl^- to Na^+, permeability (P_{Cl}/P_{Na}). The model assumes that there is a constant electric field in a homogeneous epithelium significantly permeable to Na^+ and Cl^- only, and that P_{Na} and P_{Cl} are constant.

The equivalent circuit model

Ions may be regarded as passing across a membrane through ion-selective channels, the current carried by the ion being proportional to the channel conductance and to the difference between the transmembrane p.d. and the ion equilibrium potential (Hodgkin & Horowicz, 1959). In a steady-state the sum of the respective ion channel currents in a membrane is zero, thus:

$$E^m = t_{Na} E^m_{Na} + t_{Cl} E^m_{Cl}, \quad (3)$$

where t_{Na}, t_{Cl} = transport number of Na^+ or Cl^- in the membrane (i.e. the proportion of total membrane conductance attributable to the Na^+ or Cl^- channel; here it is assumed that $t_{Na}+t_{Cl} = 1$), E^m = transmembrane p.d. (V), E^m_{Na}, E^m_{Cl} = equilibrium potential for the membrane of Na^+ or Cl^- (V). t_{Na} and t_{Cl} in the outer membrane of an integumental epithelium ($t^o_{Na(Cl)}$) may be estimated from the relationship:

$$t^o_{Na(Cl)} = \Delta E^{mo} / \Delta E^{mo}_{Na(Cl)}, \quad (4)$$

where ΔE^{mo}, $\Delta E^{mo}_{Na(Cl)}$ = finite 'instantaneous' changes in measured p.d., and calculated Na^+ (Cl^-) equilibrium potential, respectively across the membrane.

A known change in E^{mo}_{Na} or E^{mo}_{Cl} may be made experimentally by reducing the concentration of that particular ion species in the external medium by substitution with a non-permeating ion (i.e. one having $t^o = 0$) (Croghan *et al.* 1965; Smith, 1969*a*). In this study Na^+ was substituted by Cho^+ and Cl^- by BeS^-; t^o_{Na} or t^o_{Cl} was estimated, using equation 4, from the change in *Corophium* TEP (ΔE) following animal exposure to Cho ASW or BeS ASW respectively, assuming

$\Delta E = \Delta E^{mo}$. It is assumed that intracellular ion concentrations remained constant over the experimental period. Transport numbers determined in this manner are best termed 'apparent transport numbers' (Croghan *et al.* 1965).

Measurement of TEP

The TEP was measured using a pair of glass capillary microelectrodes. The microelectrodes were pulled from 0·65 mm bore tubing to a tip diameter of 10–20 μm, and were filled with 3 mol l^{-1} KCl by ethanol replacement (Tasaki, Polley & Orrego, 1954); tip resistance was approximately $10^6\,\Omega$. The experimental animal, cemented to a sharpened match head using a drop of superglue (Loctite Ltd), was mounted ventral side uppermost in a small Perspex chamber. Medium flowed through the chamber by gravity from one of several aerated reservoirs containing different media. Introduction of a new medium into the chamber was complete within 30 s of connecting the chamber inflow to a different reservoir. One microelectrode, mounted on a micromanipulator, was manoeuvred so that its tip pierced the ventrolateral cuticle of the animal through an intersegmental membrane, and was positioned in the haemolymph. The tip of the second (reference) microelectrode was immersed in the medium surrounding the animal. Each of the microelectrode pair was connected to a calomel (Hg/Hg_2Cl_2/saturated KCl) half-cell *via* a salt (3 mol l^{-1} KCl) bridge. The p.d. between the half-cells was measured using an electrometer with a high input resistance (Vibron 33B-2, Electronic Instruments Ltd; input resistance exceeds $10^{15}\,\Omega$) attached to a chart recorder. The asymmetry (tip) potential between the two microelectrodes was measured for each medium used in an experiment, then subtracted from the respective p.d. to give the TEP. Microelectrodes having ion-selective tips were rejected. Results where the asymmetry potential exceeded 5·0 mV were discarded. A brass plate in the base of the Perspex chamber was grounded; this held the microelectrode circuit at a relatively constant low potential relative to earth. The apparatus was grounded and enclosed by a Faraday cage.

The sequence of media used in a TEP measurement experiment followed one of two patterns.

(i) A progressive series of dilutions of ASW (100 %, 50 %, 20 %, 10 % ASW) plus artificial fresh water (AFW).

(ii) A sequence of media containing substitute ions (Cho^+, BeS^-) or trans-integumental ion transport inhibitors.

Generally a TEP was recorded over a 2-min period immediately after it had stabilized following a change in medium. In all experiments TEP was initially and finally measured in a reference medium (ASW for 85 % SW acclimated animals and 10 % ASW for 15 % SW acclimated animals); initial and final values were always within 1·0 mV of one another. Reference medium TEPs recorded over periods of up to 30 min were stable (±0·2 mV), indicating that there was no significant short-circuiting through the microelectrode penetration wound. Measurement accuracy was approximately ±5 % of the recorded TEP.

Measurement of Na^+ fluxes

Unidirectional sodium efflux (J^{Na}; nmol Na^+ mg^{-1} animal wet weight h^{-1}) was determined by washout of a ^{22}Na-loaded animal into unlabelled experimental medium, the radioactivity remaining in the animal being recorded against time.

^{22}Na was obtained as NaCl in aqueous solution from the Radiochemical Centre, Amersham. Radioactivity was assayed using a well-crystal scintillation counter (Panax Instruments Ltd). Cylindrical plastic minivials (8 ml capacity) were used as experimental chambers. All radioactive samples were counted in such vials, a single vial exactly fitting the well of the counter. ^{22}Na counting efficiency was 20·5 % throughout.

Animals were ^{22}Na-loaded for 24 h in 5 ml of aerated acclimation medium containing the isotope at a specific activity of 3·7 Bq $nmol^{-1}$ Na^+. After this period animals were fully ^{22}Na-loaded; i.e. animal specific activity was equal to that of the medium (Taylor, 1982).

Individual fully ^{22}Na-loaded animals were rinsed in unlabelled medium and transferred to a minivial. A 0·5 ml sample of experimental medium was pipetted into the chamber, and the animal counted (0·05 ml samples of ^{22}Na-loading media were also counted). Further medium was added to give a total volume of 8 ml. The experimental medium was changed (by careful pipetting) at time intervals, at which points the residual animal radioactivity was determined, the animal being counted in an initial 0·5 ml aliquot of the fresh medium. The interval between each medium change was timed from the addition of this aliquot. The experimental medium was aerated periodically.

Two types of experiment were performed:

(i) complete ^{22}Na washout of an animal in a single type of medium (ASW, 85 %ASW or 10 %ASW);

(ii) alternation of experimental media (at changes) between 85 %ASW/10 %ASW (85 %SW/15 %SW acclimated animals respectively) and either sucrose, or NaCl+sucrose, in FWB (the FWB media being iso-osmotic to animal haemolymph; see Table 4).

RESULTS

TEP measurements

The TEPs of *Corophium* exposed to various media are summarized in Fig. 1 and Table 2. Here it is regarded that TEPs recorded under non-steady-state conditions represent 'instantaneous' (formally steady-state) p.d.s; i.e. it is assumed that such TEPs are recorded before any significant changes in *Corophium* body fluid composition occur. The TEP of 15 %SW acclimated animals in 10 %ASW containing amiloride or SCN^- was identical to the TEP in 10 %ASW (amiloride experiments, $N = 8$; SCN^- experiments, $N = 6$). Similarly, the TEPs of 85 %SW acclimated animals in ASW, and ASW plus either inhibitor, were identical to one another (amiloride experiments, $N = 4$; SCN^- experiments, $N = 2$). These TEPs in inhibitor-containing medium did not change significantly over experimental exposure periods of 15–20 min.

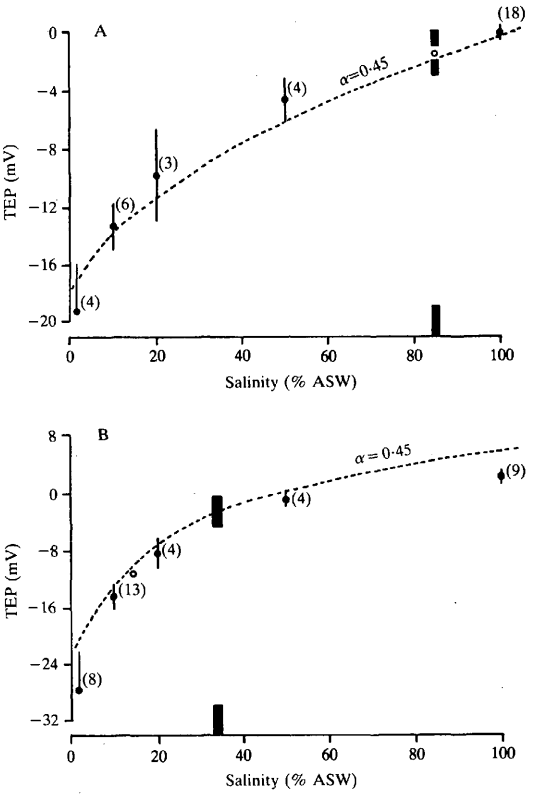

Fig. 1. TEP of *Corophium volutator* in relation to environmental salinity (where 1·0 %ASW ≡ AFW): (A) 85 %SW acclimated animals; (B) 15 %SW acclimated animals. A closed circle (●) indicates the mean TEP [±1 S.E.M. (N)] measured immediately following exposure to the denoted salinity. An open circle (○) indicates the TEP in the acclimation salinity, estimated by interpolation from adjacent experimental sample means. The solid bar indicates the point at which haemolymph and medium are iso-osmotic. The dotted line indicates the TEP-salinity relation predicted by the Goldman model (equation 2) where $\alpha = 0{\cdot}45$; this value of α gives the line of best fit (estimated by eye) of the model for animals acclimated to either salinity.

$[Na^+]_{in}$ and $[Cl^-]_{in}$ of *Corophium*, together with the equilibrium potentials (E_{Na}, E_{Cl}) across the integument, are shown in Table 3; the values are similar to those reported by McLusky (1968). In 85 %SW E_{Na} and E_{Cl} of acclimated animals are close to the TEP, suggesting that both ion species are passively distributed (i.e. in equilibrium) across the integument. However, it appears that Cl^-, and possibly Na^+, are out of electrochemical equilibrium across the integument of animals acclimated to 15 %SW.

able 2. *Changes in TEP of 85 %SW acclimated* Corophium volutator *following exposure to a range of experimental media*

Experimental medium Change in TEP (mV), relative to TEP in ASW			Mean TEP (mV) in ASW reference medium; see Fig. 1A	Ion transport number*	
ChoASW	BcSASW	NaASW		t^0_{Na}	t^0_{Cl}
−9·4 ± 0·7 (13)	+2·6 ± 0·35 (8)	−3·5 ± 0·4 (8)	−0·12	0·20	0·08

All TEP changes significantly different from zero (paired sample *t*-test).
* Estimated from equation 4.
For abbreviations see Table 1.

able 3. *Ion equilibrium potentials (E_z) across the integument of* Corophium volutator *acclimated to 85 %SW or 15 %SW*

Acclimation salinity (%SW)	$[Na^+]$ (mmol l^{-1})		E_{Na} (mV)†	$[Cl^-]$ (mmol l^{-1})		E_{Cl}(mV)†	TEP (mV)*
	$[Na^+]_{in}$	$[Na^+]_{out}$		$[Cl^-]_{in}$	$[Cl^-]_{out}$		
85	435 ± 14 (15)	393	−2·5	496 ± 19 (6)	458	+2·0	−1·4
15	178 ± 15 (11)	67	−24·0	169 ± 22 (6)	79	+18·7	−11·1

$[Na^+]_{in}$, $[Na^+]_{out}$, concentration of Na^+ in haemolymph and medium respectively; $[Cl^-]_{in}$, $[Cl^-]_{out}$, same for Cl^-.
* TEP estimated from Fig. 1.
† E_z calculated from equation 1.

In *Corophium* the TEP becomes increasingly negative as external ion concentrations (i.e. environmental salinity) are progressively lowered. The Goldman model (equation 2) approximates most closely to this relationship between TEP and external salinity, in both 85 %SW and 15 %SW acclimated animals, when $\alpha = 0{\cdot}45$ (Fig. 1); $[Na^+]_{in}$ and $[Cl^-]_{in}$ are assumed to remain constant at the levels of acclimated animals (Table 3).

The magnitude and sign of TEP changes following ion substitutions were dependent on the ion species substituted. The transport numbers t^o_{Na} and t^o_{Cl} were estimated for 85 %SW acclimated *Corophium* (Table 2). The change in TEP following animal exposure to NaASW was small compared to the TEP change following exposure to ChoASW, indicating that the transport numbers t^o_K, t^o_{Ca} and t^o_{Mg} are considerably lower than t^o_{Na}. The largest values of ion transport numbers in the outer membrane of *Corophium* integumental epithelium appear to be t^o_{Na} and t^o_{Cl}, although it is conceivable that a significant proportion of the membrane conductance is attributable to ion channels selective for H^+, HCO_3^- or OH^-. These 'apparent' transport numbers may underestimate considerably actual values, as Σt_j (here, $t^o_{Na}+t^o_{Cl} = 0{\cdot}28$) should equal unity. This large discrepancy might be ascribed to limitations of the experimental system; it is possible that intracellular ion concentrations changed significantly during the experiments (t_j is strictly a function of [j] in the membrane), and also that the substitute ions were not entirely non-permeating or inert (Smith, 1969*a*).

Unidirectional Na^+ efflux (J^{Na})

Measurement of ^{22}Na washouts of *Corophium* in a single medium type showed that the decrease in animal radioactivity was described by a single exponential function;

$$Q_t = Q_o e^{-kt}, \tag{5}$$

where Q_o = initial animal radioactivity (counts min^{-1}; c.p.m.), Q_t = animal radioactivity at time t (c.p.m.), t = time (h), k = efflux rate constant (h^{-1}). The rate constant (k) was estimated as the slope of a plot of $\ln(Q_t/Q_o)$ against time. Typical plots of this type are shown in Fig. 2. In experiments where the medium composition was changed at intervals, k in each medium was estimated from equation 5 with Q_o and Q_t representing animal radioactivity at the commencement and the end, respectively, of exposure to each experimental medium.

The magnitude of k was dependent on animal wet weight (W; in mg); k decreased with increase in animal weight. Therefore, rate constants were weight-corrected to those of a 7·0-mg animal ($k_{7\cdot0}$) according to the relation (after Taylor, 1982):

$$k_{7\cdot0} = 0{\cdot}61\, k/\exp(-0{\cdot}64 \ln(W) + 0{\cdot}75). \tag{6}$$

The weight-corrected Na^+ efflux rate constants for *Corophium* exposed to various media are summarized in Table 4.

Table 4. *^{22}Na efflux rate constants (k) of* Corophium volutator *in experimental media*

Acclimation salinity (%SW)	Rate constant (h^{-1})				Change in rate constant relative to that in iso-osmotic sucrose (h^{-1})
	ASW	85 %ASW	10 %ASW	Iso-osmotic sucrose*	150 mmol l^{-1} NaCl+sucrose*
85	0·76 ± 0·09 (16)	0·70 ± 0·07 (12)	0·89 ± 0·12 (4)	0·36 ± 0·03 (16)	+0·10 ± 0·035† [+0·10]‡ (6)
15	0·88 ± 0·08 (3)		0·50 ± 0·03 (12)	0·34 ± 0·05 (15)	+0·35 ± 0·07† [+0·26]‡ (6)

* FWB media made iso-osmotic to haemolymph; 85 %SW acclimated animals – 910 mosmol kg^{-1}, 15 %SW acclimated animals – 340 mosmol kg^{-1} (cLusky, 1968).
† Significant change in k (paired-sample *t*-test).
‡ Values in parentheses represent changes in k predicted by Goldman model (equation 7); predicted changes are not significantly different from observed changes.
All rate constants are weight-corrected to those of a 7·0-mg animal (equation 6).

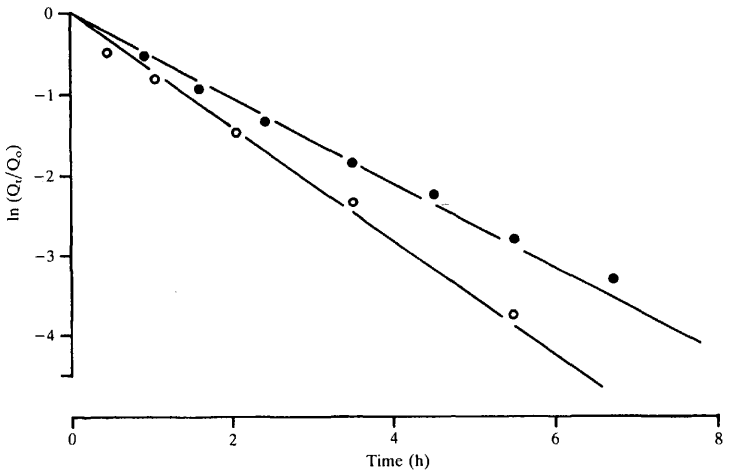

Fig. 2. Semi-logarithmic plots of decreasing *Corophium volutator* ^{22}Na radioactivity against time. Each plot relates to a single animal, where Q_o is initial animal radioactivity (c.p.m.) and Q_t is animal radioactivity at the denoted time (c.p.m.). Open circles (○); 85 %SW acclimated animal, efflux into ASW. Closed circles (●); 15 %SW acclimated animal, efflux into 10 %ASW.

The rate constant k has two major components, transintegumental Na^+ efflux and Na^+ loss in the urine. In iso-osmotic, and hyperosmotic, media (in Table 4, all except 10 %ASW), urine production rate of *Corophium* (and hence urinary Na^+ loss) is likely to be comparatively low. In these media k may be regarded as representing mainly transintegumental Na^+ efflux (k_{int}). The k_{int} of 85 %SW and 15 %SW acclimated animals increases significantly when $[Na^+]_{out}$ rises; such changes in unidirectional Na^+ flux across the integument may be related to concomitant changes in TEP (House, 1963; Smith, 1969*b*). If Na^+ movement is diffusional, the ratio of k_{int} in two different media [denoted (i) and (ii)] may be described as a function of the respective TEPs using the Goldman model (here assuming $[Na^+]_{in}$ is constant)(after House, 1963).

$$\frac{k_{int}(i)}{k_{int}(ii)} = \frac{D(i)}{D(ii)}, \qquad (7)$$

where (for each medium) $D = (FE/RT)/[1-\exp(-FE/RT)]$. Here, medium (ii) may be identified as a sucrose solution iso-osmotic to the haemolymph, and medium (i) as 150 mmol l^{-1} NaCl with sucrose to the same osmolarity (see Table 4). The expected TEP in these two media may be calculated for 85 %SW and 15 %SW

acclimated animals from equation 2 ($\alpha = 0{\cdot}45$), assuming sucrose has no independent effect on the TEP. Values of k(i)−k(ii), predicted from equation 7 using experimentally-determined k(ii), are shown in Table 4; these are not significantly different from the observed changes in k (k_{int}). Values of k_{int} in ASW may be predicted similarly if medium (i) is reidentified as ASW; predicted k_{int} in ASW is $0{\cdot}58\,h^{-1}$ and $0{\cdot}68\,h^{-1}$ for 85 %SW and 15 %SW acclimated animals respectively (neither is significantly different from observed k, Table 4).

Total body sodium ion content of individual *Corophium* (B^{Na}; nmol Na^+ mg^{-1} animal wet weight) was estimated from the relation:

$$B^{Na} = Q_o/(WX_m^{Na}), \tag{8}$$

where Q_o = initial animal radioactivity (i.e. that of a fully ^{22}Na-loaded animal) (c.p.m.), X_m^{Na} = specific activity of loading medium (c.p.m. $nmol^{-1}$ Na^+). B^{Na} is $220{\cdot}5 \pm 7{\cdot}9$ nmol mg^{-1} for 85 %SW acclimated animals ($N = 14$) and $71{\cdot}0 \pm 2{\cdot}2$ nmol mg^{-1} for 15 %SW acclimated animals ($N = 11$). The values are significantly different from one another, and reflect the difference between $[Na^+]_{in}$ of the acclimatory types (Table 3). J^{Na} may be determined as:

$$J^{Na} = kB^{Na}. \tag{9}$$

Thus steady-state J^{Na} may be estimated for *Corophium*; for 85 %SW acclimated animals J^{Na} (in 85 %ASW) is $154{\cdot}3$ nmol $mg^{-1}\,h^{-1}$, and for 15 %SW acclimated animals J^{Na} (in 10 %ASW) is $35{\cdot}5$ nmol $mg^{-1}\,h^{-1}$.

P_{Na} may be estimated, using the Goldman model, from the following relationship (House, 1963):

$$P_{Na} = \frac{J_{int}^{Na}[1 - \exp(-FE/RT)]}{A_p(FE/RT)[Na^+]_{in}}, \tag{10}$$

where J_{int}^{Na} = diffusional transintegumental flux (mol $mg^{-1}\,s^{-1}$), A_p = surface area of permeable integument ($9{\cdot}9\times10^{-7}\,m^2\,mg^{-1}$), ($[Na^+]_{in}$; mol m^{-3}). Identifying J_{int}^{Na} as Na^+ efflux into iso-osmotic sucrose, P_{Na} may be estimated as $7{\cdot}5\times10^{-8}\,m\,s^{-1}$ for *Corophium* acclimated to either 85 %SW or 15 %SW. P_{Cl}/P_{Na} appears to be approximately 0·45 for both acclimatory types, thus P_{Cl} may be estimated as $3{\cdot}4\times10^{-8}\,m\,s^{-1}$. Although k appears to be weight-dependent, there is evidence to suggest that A_p is not (Taylor, 1984); thus P_{Na} may be higher in smaller individuals than larger ones.

DISCUSSION

The Goldman model provides a reasonable description of experimentally induced changes in TEP of *Corophium volutator*, suggesting that it may be a simple diffusion potential generated principally by independent passive Na^+ and Cl^- movements across a gill integument of constant P_{Na} and P_{Cl}. $[Na^+]_{out}$-dependent changes of Na^+ k_{int} appear to reflect changes in the nature of diffusional Na^+

movements across the integument, with no significant residual flux components which might be ascribed to an 'exchange-diffusion' (Ussing, 1952) Na^+ transfer mechanism. Electrochemical-potential differences of Na^+ and Cl^- across *Corophium volutator* integument (apparent here in the case of 15 %SW acclimated animals, where such differences favour passive ion loss from the haemolymph, across the integument) are likely to be maintained by ion transport (uptake) mechanisms situated within the integumental epithelium, as is the case for other hyperosmotically-regulating crustacean species (Kirschner, 1979). Externally-applied ion transport inhibitors have no significant effect on the TEP of animals acclimated to either 85 %SW or 15 %SW. This supports the suggestion that the TEP is a diffusion potential with any Na^+ and Cl^- transport mechanisms (at least those located on the outer epithelial membrane) effecting overall electroneutral ion transfers.

G ($S\,m^{-2}$), the ion partial conductance of the integumental epithelium, may be estimated using the Goldman model from the relation (for ion j) (Dawson, Croghan, Atwater & Rojas, 1983):

$$G_j = \frac{P_j CZF\{([j]_{out} - [j]_{in})C\exp[C] + ([j]_{out} - [j]_{in}\exp[C])(1 - \exp[C])\}}{E(1 - \exp[C])^2}, \quad (11)$$

where $C = ZFE/RT$ ([j]; $mol\,m^{-3}$).

For acclimated *Corophium volutator*; in 85 %SW, G_{Na} is $123\,S\,m^{-2}$ and G_{Cl} is $64\,S\,m^{-2}$, and in 15 %SW G_{Na} is $33\,S\,m^{-2}$ and G_{Cl} is $17\,S\,m^{-2}$. Epithelial resistance of acclimated animals [as $1/(G_{Na}+G_{Cl})$; probably a minimum estimate] is $5{\cdot}3\times10^{-3}\Omega m^2$ and $2{\cdot}0\times10^{-2}\Omega m^2$ in 85 %SW and 15 %SW respectively. This is of the same order of magnitude as resistance reported for certain other epithelial tissues, which are regarded as 'leaky' (as opposed to 'tight') epithelia; i.e. they are of comparatively low resistance (Diamond, 1962; Smith, 1969*a*; Fromter, 1972). Smith (1969*a*) suggested that in such situations, where transintegumental ion fluxes are comparatively rapid, 'apparent t^o' should be regarded as a gross estimate of transport number for the whole epithelium (t^e). G may be formally related to t^e, where for any ion $t^e = G/(\Sigma G_j)$; thus t^e_{Cl}/t^e_{Na} should equal G_{Cl}/G_{Na}. In fact for 85 %SW acclimated *Corophium volutator* t^e_{Cl}/t^e_{Na} (in ASW) is 0·4, a value not dissimilar to the respective G_{Cl}/G_{Na} (in 85 %SW) of 0·52 calculated using the Goldman model. The limiting junctional complexes of the epithelial cells, as opposed to the cell membranes, may be the major site of diffusional ion movements across low-resistance epithelia (Fromter, 1972). These junctional complexes may constitute a single barrier for ions to cross, which might tentatively be considered as supporting evidence for the application of the Goldman model to describe passive ion movements across *Corophium volutator* integument.

Corophium volutator k_{int} is markedly higher than comparable values for other euryhaline amphipod species (these are of the order 0·04–0·12 h^{-1}: Sutcliffe, 1968, 1975), suggesting that P_{Na} of this species is unusually high. P_{Na} of *Corophium volutator* is higher than P_{Cl}, as appears to be the case for crustacean integumental epithelia in general (Croghan *et al.* 1965; Smith, 1969*b*; Lucu, 1977).

There is no evidence in this study to suggest that *Corophium volutator* is able to alter its integumental permeability to Na^+ or Cl^- after acclimation, or immediately following exposure, to different salinities. However, J^{Na} does alter following acclimation as a function of changes of $[Na^+]_{in}$ (i.e. B^{Na}). These changes would be likely to result in an adaptive reduction of any passive integumental (and urinary) Na^+ loss during periods of hyperosmotic regulation at low environmental salinity. *Corophium volutator* exhibits only a limited capacity to reabsorb ions from primary urine (McLusky, 1968), and also appears to have a highly water-permeable integument (Taylor, 1982). Hence, a significant proportion of J^{Na} when haemolymph is hyperosmotic to the medium (e.g. animals in 10%ASW) might be apportioned to urinary Na^+ loss.

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