SODIUM EXTRUSION BY THE SEA-WATER-ACCLIMATED FIDDLER CRAB *UCA PUGILATOR*: COMPARISON WITH OTHER MARINE CRUSTACEA AND MARINE TELEOST FISH

BY DAVID H. EVANS, KERRY COOPER AND MARGARET B. BOGAN

*Department of Biology, University of Miami, Coral Gables, Florida*

(Received 1 July 1975)

**SUMMARY**

1. Measurements of the blood Na concentration and transepithelial electrical potential (T.E.P.) across *Uca pugilator* acclimated to sea water indicate that Na is maintained out of electrochemical equilibrium with sea water. The resulting net Na influx as well as the sodium gain due to ingestion of the medium must be balanced by extrarenal Na extrusion.

2. The small T.E.P. (—0.7 mV) and the ‘transport numbers’ of Na and Cl indicate that the permeability to these ions is equivalent.

3. Removal of external K results in a significant stimulation of unidirectional Na efflux that is dependent upon external Na but is not inhibited by ouabain.

4. Transfer of *Uca* to K and Na-free sea water results in a 54% decline in unidirectional efflux, which is not due to T.E.P. changes. Readdition of 25 mM-K stimulates Na efflux much more than can be accounted for by changes in the T.E.P. Readdition of 25 mM-Na to potassium-free sea water does not change the Na efflux.

5. The results indicate that Na extrusion by *Uca* is via a Na/K exchange mechanism which partially inhibits Na/Na exchange. Cessation of Na/K exchange (in K-free sea water) removes this inhibition and allows rapid Na/Na exchange. It is not known whether Na/K and Na/Na exchange are via the same or parallel carrier systems.

**INTRODUCTION**

Although the basic pattern of NaCl balance by marine teleost fish has been intensively investigated (see reviews by Maetz, 1971, 1973, 1974; Maetz & Bornancin, 1975; Potts, 1975), the mechanisms of NaCl balance of hyporegulating marine crustacea have been relatively little studied. Croghan (1958a) showed that *Artemia salina* (hereafter referred to as *Artemia*) maintains water balance in sea water by ingesting the medium as do teleost fish (Maetz, 1974) and this has now been confirmed for *Artemia* (Smith, 1969b) and demonstrated in *Metapenaeus bennettiae* (Dall, 1967), *Uca pugilator* (Hannan & Evans, 1973) and *Parartemia xietziana* (Geddes, 1975b). Since the antennal excretory gland of marine crustacea is unable to produce a hyper-
osmotic urine (Lockwood, 1967; Prosser, 1973), it appears that extrarenal mechanisms exist to extrude NaCl to offset the sum of the oral influx and any net diffusional gain across the permeable surfaces of the animal. The site of this extrarenal NaCl extrusion in hyporegulating crustacea has not been precisely determined. Croghan (1958b) proposed that NaCl extrusion is across the branchiae of *Artemia*, and Geddes (1975b) has recently come to the same conclusion while studying ionic regulation in *Parartemia zietziana*. In support of this hypothesis, Copeland (1967) described mitochondria-rich cells in the metepipodite segments of the branchiae of *Artemia*. However, Dall (1967) has suggested that the gut is the site of NaCl extrusion by *Metapenaeus bennettae*, since the rate of efflux of $^{22}$Na from the cephalothorax actually decreases after *M. bennettae* is salt-loaded.

Little is known about the actual mechanisms for the excretion of NaCl from hyporegulating crustacea. Potts & Parry (1964) proposed that the prawn *Palaemonetes varians*, when acclimated to sea water, extrudes Na electrogenically. The resulting internal electronegativity maintains blood Cl concentration in electrochemical equilibrium with sea water despite the concentration gradient favouring net influx of Cl. Croghan (1958b, c) proposed that both Na and Cl are excreted by the branchiae of *Artemia* and that much of the Na and Cl efflux is via a Na/Na and Cl/Cl exchange diffusion mechanism which provides for no net extrusion of these ions. This proposition was supported by Croghan’s finding (1958c) that the efflux of $^{22}$Na falls rapidly after *Artemia* is transferred from sea water to distilled water. Croghan (1958c) also showed that substitution of K for Na in the sea-water medium results in a net loss of haemolymph Na from *Artemia*. This would seem to indicate that some Na efflux is via a Na/K exchange system and more recent determinations show that *Artemia* homogenates contain significant quantities of an ATPase which is activated by Na and K, and is sensitive to ouabain, and that the concentration of the enzyme increases when *Artemia* is raised in hypersaline solutions (Augenfeld, 1969). Thuet, Motais & Maetz (1968) supported the concept of Na/Na and Cl/Cl exchange diffusion across the branchiae of *Artemia* and hypothesized that a specific Na exchange diffusion carrier is present since the stimulation of Na efflux by external Na displays what appears to be Michaelis-Menten saturation kinetics. Smith (1969a, b) proposed that Na movements across *Artemia* branchiae are largely diffusive and that haemolymph Na is maintained in electrochemical equilibrium with sea water by the internal positivity of the organism (23 mV). In addition, he proposed that the internal positive potential is the result of a large Na permeability relative to Cl permeability. He also showed that the immediate fall in Na efflux after transfer of *Artemia* to Na-free solutions (choline sea water) could be accounted for by a depolarization of the electrical potential between the haemolymph and the medium to an inside negative potential (−36 mV). Smith (1969b) also calculated that changes in the electrical potential across a membrane can give rise to changes in passive Na fluxes which display what appear to be Michaelis-Menten saturation kinetics, and he concluded that there is no need to postulate active Na extrusion by *Artemia* or a Na/Na exchange diffusion system. However, he proposed that Cl extrusion is indeed active and that a considerable proportion of the Cl efflux is through a Cl/Cl exchange diffusion system.

The intertidal crab *Uca pugilator* maintains its blood NaCl concentration below that of sea water (Green *et al.* 1959) and ingests the medium to offset the osmotic loss
Table 1. Ionic concentrations of 'natural' and experimental seawater solutions (all concentrations are in mM)

<table>
<thead>
<tr>
<th>Solution</th>
<th>Na</th>
<th>Cl</th>
<th>K</th>
<th>Mg</th>
<th>Ca</th>
<th>SO₄</th>
<th>HCO₃</th>
<th>Choline sulphonate</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-free SW</td>
<td>460</td>
<td>536</td>
<td>0</td>
<td>53</td>
<td>10</td>
<td>28</td>
<td>2.5</td>
<td>0</td>
</tr>
<tr>
<td>K and Na-free SW</td>
<td>2.5</td>
<td>571</td>
<td>0</td>
<td>53</td>
<td>10</td>
<td>28</td>
<td>2.5</td>
<td>0</td>
</tr>
<tr>
<td>25 mm-K, Na-free SW</td>
<td>2.5</td>
<td>571</td>
<td>25</td>
<td>53</td>
<td>10</td>
<td>40</td>
<td>2.5</td>
<td>501</td>
</tr>
<tr>
<td>K-free, 25 mm-Na SW</td>
<td>5</td>
<td>571</td>
<td>0</td>
<td>53</td>
<td>10</td>
<td>39</td>
<td>2.5</td>
<td>501</td>
</tr>
<tr>
<td>'50%' Na SW</td>
<td>230</td>
<td>536</td>
<td>10</td>
<td>53</td>
<td>10</td>
<td>28</td>
<td>2.5</td>
<td>230</td>
</tr>
<tr>
<td>'50%' K SW</td>
<td>460</td>
<td>531</td>
<td>5</td>
<td>53</td>
<td>10</td>
<td>28</td>
<td>2.5</td>
<td>0</td>
</tr>
<tr>
<td>'50%' Cl SW</td>
<td>460</td>
<td>268</td>
<td>10</td>
<td>53</td>
<td>10</td>
<td>28</td>
<td>2.5</td>
<td>0</td>
</tr>
<tr>
<td>'Natural' SW</td>
<td>500</td>
<td>590*</td>
<td>11*</td>
<td>57*</td>
<td>11*</td>
<td>30*</td>
<td>2.4*</td>
<td>0</td>
</tr>
</tbody>
</table>

• Concentrations calculated from Prosser (1973).

of water (Hannan & Evans, 1973). The present study deals with the mechanisms of sodium extrusion by this species and compares these mechanisms with those described for Artemia and marine teleost fish.

MATERIALS AND METHODS

(1) Collection and maintenance of animals

Specimens of both sexes of Uca pugilator (Bosc) (hereafter referred to as Uca) (1-3 g) were collected in the intertidal region of Biscayne Bay, Florida, and maintained in a 30 gal aquarium containing a crushed coral bottom and with bottom filtration. They were acclimated to natural sea water (Table 1) (maintained by the addition of either distilled water or Instant Ocean Sea Salts) for at least one week before experimentation. The temperature of the acclimation tank and the experimental solutions was 23 ± 1 °C.

(2) Experimental solutions

All experimental solutions were modifications of Pantin’s formulation for artificial sea water (Pantin, 1962) (Table 1).

(3) Determination of sodium concentrations

Individuals were weighed on a Mettler Model 1200 balance and then homogenized in a known volume of distilled water in a Virtis Model 23 Homogenizer. The Na concentration of an aliquot was determined with an Instrumentation Laboratory Model 143 Flame Photometer with an internal lithium standard. The total body Na of the crab was calculated by correcting for the volume of the homogenate and the weight of the crab. To determine the Na concentration of the haemolymph, samples were withdrawn at the base of a walking leg with a microcapillary tube and immediately diluted with a known volume of 15 mM-Li solution. The Na concentration of the diluted sample was determined on the Flame Photometer. Total body Na is expressed in μmole/g and haemolymph Na concentration in mM.
(4) Measurement of electrical potential

A procedure modified from Evans, Carrier & Bogan (1974) was used to determine the electrical potential (transepithelial potential or T.E.P.) between *Uca* haemolymph and various external solutions. The internal recording bridge (3 M-KCl, 2% Agar-filled polyethylene, I.D. 0.011 in. tubing) was placed into haemolymph through a 19 gauge needle which had been inserted through the left anterior quarter of the dorsal carapace. The needle was left in place and the site of insertion was coated with silicone grease to avoid leakage of haemolymph and possible shorting of the measuring circuit. The animal was clamped in place with a laboratory clamp on a ring stand and was partially submerged in the appropriate solution so that the legs, mouth, anus and gills were submerged, while the majority of the dorsal carapace and the site of the insertion of the needle were above the water. This type of restraint of the experimental animal prevented short-circuiting of the T.E.P. and facilitated handling of the animal during changes of the experimental media without interfering with leg or respiratory movements. The recording bridge and a similar bridge in the experimental medium were inserted into separate 3 M-KCl, 2% Agar-filled Polyethylene I.D. 0.055 in. tubing which terminated in separate 3 M-KCl-filled test tubes. The electrical potential between these bridge terminals was measured with matched calomel electrodes connected to either a Bausch and Lomb VOM recording multimeter (10^6 Ω input impedance) or to a Keithley Model 616 digital multimeter (10^13 Ω input impedance). No differences were noted between potentials measured with the different multimeters. Electrode asymmetry was measured by placing a 3 M-KCl, 2% agar-filled polyethylene, I.D. 0.055 in. tube between the two test tubes containing the calomel electrodes. Bridge and tip asymmetry was checked by placing both internal recording bridge terminals in the same sea-water medium. All measured T.E.P.s were corrected for electrode and bridge asymmetry. All potentials are expressed as haemolymph relative to medium.

(5) Determination of transport members

Croghan, Curra & Lockwood (1965) and Smith (1969a) proposed that the 'transport number', first described by Hodgkin & Horowicz (1959), could be used as a measure of relative ion permeabilities of a given membrane. The 'transport number' can be defined by the equation (Smith, 1969a):

\[ T_i = \frac{\Delta V_m}{\Delta V_t} \]

where \( T_i \) is the 'transport number' of the ion \((i)\), \( \Delta V_m \) is the change in T.E.P. measured after a change in the ionic concentration gradient of ion \((i)\) and \( \Delta V_t \) is the theoretical T.E.P. change (calculated from the Nernst Equation) if ion \((i)\) is the only permeant ion.

The use of the 'transport number' as a measure of relative permeabilities for various ions rests on the assumption that the T.E.P. between the haemolymph of *Uca* and the external medium is predominantly the result of the differential permeability of the epithelial membrane to various ion species that are diffusing down their respective
Sodium extrusion by sea-water-acclimated *Uca pugilator*

concentration gradients. Thus, the T.E.P. in any given medium would be defined by the Hodgkin & Katz (1949) derivation of the Goldman constant field equation:

\[ V_m = \frac{RT}{F} \ln \left( \frac{P_K (K_{in}) + P_{Na} (Na_{in}) + P_{Cl} (Cl_{out})}{P_K (K_{out}) + P_{Na} (Na_{out}) + P_{Cl} (Cl_{in})} \right) \]  

(2)

where \( V_m \) is the T.E.P., \( R, T, F \) have their usual meaning \((RT/F = 0.026 \text{ V at } 23^\circ \text{C})\), \( P_K, P_{Na}, P_{Cl} \) are the membrane's permeability to these ions and \( (K_{in}), (K_{out}) \), etc., are the concentrations (more properly activities) of the relevant ions in the medium (out) and the haemolymph (in). Thus, the role of a particular ion in generating the T.E.P. will be proportional to the product of its relative permeance and its relative ionic gradient across the membrane. If equation (2) defines the T.E.P. across a *Uca* then the change in \( V_m \) measured after a change in the concentration of one of the ions in the external medium will be proportional to the product of the membrane's permeability to that ion and the concentration gradient for the ion. Thus, to a first approximation, the relative permeance of a given ion (Na or Cl in this example) can be calculated by the following relationship (Hodgkin & Horowicz, 1959):

\[ \frac{P_{Na}}{P_{Cl}} = \frac{T_{Na}/T_{Cl}}{Na_{out}/Cl_{out}}. \]  

(3)

In order to determine the ‘transport numbers’ of Na, Cl and K across the epithelium of *Uca*, the T.E.P. was recorded in sea water and then measured again immediately after the external medium had been changed to either ‘50%’ Na sea water, ‘50%’ Cl sea water or ‘50%’ K sea water (see Table 1). The T.E.P. in sea water was rechecked between each experimental solution, and the change in T.E.P. between that measured in the experimental medium and in the immediately preceding sea-water solution was noted. Using the Nernst equation, \( \Delta V \) would be \(-20 \text{ mV} \) for ‘50%’ Na or K sea water and \(+20 \text{ mV} \) for ‘50%’ Cl sea water, since these solutions were actually 46% of the natural sea-water concentration.

(6) **Determination of the rate of efflux of Na**

Long-term effluxes were monitored by placing crabs into a radioactive sea-water bath overnight (approximately \(10 \mu \text{Ci } ^{22}\text{Na} \text{ per } 500 \text{ ml of sea water} \)). After at least 12 h, the radioactive crabs were removed, rinsed in non-radioactive sea water for 15 min and placed into individual beakers containing 100 ml of non-radioactive sea water. At 30 min intervals between 2 and 4 h, 25 ml samples of the sea water were removed and assayed for radioactivity in a Packard ‘Armac’ scintillation detector attached to a Packard Tri-Carb Model 2001 scintillation spectrometer. At the end of the experimental time period, a final 25 ml sample of the bath and the crab itself were assayed for radioactivity. The rate constant \( (K) \) of efflux was then calculated from the following formula:

\[ K = 1/T \ln \frac{Q_o/Q_t}{1}, \]

where \( K \) is expressed as a fraction of the exchangeable sodium pool exchanged per hour, \( T \) is the length of the experimental period in hours, \( Q_o \) is the radioactivity in the
crab at the start of the experimental period and $Q_t$ is the radioactivity in the crab at the end of the experiment. $Q_t$ is measured directly, but $Q_o$ is the sum of the final radioactivity in the crab and the total radioactivity in the bath at the end of the experiment. The unidirectional Na efflux was then calculated as the product of the rate constant ($K$) and the total body sodium and expressed in $\mu$-mole Na g$^{-1}$ hr$^{-1}$. In some experiments, after an initial period in sea water, the crab was transferred into one or a series of ion-free or ion-substituted media as indicated in the Results and Discussion. In these experiments the rate constant of Na efflux was determined for the time period in each of the experimental solutions. In one series of long-term efflux experiments the crabs were injected with $^{22}$Na (2 $\mu$Ci per crab, carried in 2 $\mu$l of distilled water) before being placed in sea water and then into other media.

In order to determine the effect of rapid changes in the ionic concentration of the external medium on the sodium efflux, a flow system described by Evans, Mallery & Kravitz (1973) was used. The only modification of this system was that the total volume of the circulating bath was 140 ml. In these experiments the crabs were injected with $^{22}$Na (3–5 $\mu$Ci per crab, carried in 3–15 $\mu$l of distilled water), placed into non-radioactive sea water for approximately 15 min, to allow equilibration of the isotope in the haemolymph, and then transferred to the experimental sea-water bath. After the initial period in sea water (5–10 min), the crab was transferred into one or a series of ion-free or ion-substituted media as indicated in the Results and Discussion. The crabs remained in each of these experimental media for 5–15 min.

### RESULTS AND DISCUSSION

1. **Total body Na content and haemolymph Na concentration**

   The total body Na content of *Uca* in sea water (500 mM-Na) is $219 \pm 11$ $\mu$-mole g$^{-1}$ (8) (mean ± standard error, number of animals in brackets). The sodium concentration of *Uca* haemolymph is $454 \pm 10$ mM (11). Thus, *Uca* is able to maintain its haemolymph Na concentration some 46 mM below that of its environment. These data corroborate earlier work by Green et al. (1959), who showed that *U. pugilator* maintains its haemolymph at 328 mM-Na when acclimated to sea water containing 397 mM-Na. However, *Uca* is unable to maintain the substantial Na concentration gradient described for *Artemia*, which can maintain its haemolymph Na concentration at 172 mM when acclimated to sea water whose Na concentration is 469 mM (Smith, 1969a).

2. **Electrical potential difference in sea water**

   The electrical potential between the haemolymph of *Uca* and sea water (trans-epithelial potential or T.E.P.) is $-0.7 \pm 0.1$ mV (67). This slightly haemolymph-negative T.E.P. is in sharp contrast to that found in *Artemia* which maintains its haemolymph 23.4 mV positive to sea water (Smith, 1969a). Given the blood:sea water Na gradient measured across *Uca* in the present study, one can calculate (using the Nernst equation) the Na equilibrium potential which could account for the observed Na gradient. This potential would be $+2.3$ mV. Although the measured T.E.P. is only 3.0 mV displaced from the sodium equilibrium potential, we observed a blood positive electrical potential across *Uca* in only 3 out of 67 determinations. Therefore
Sodium extrusion by sea-water-acclimated Uca pugilator

Fig. 1. Effect of 50\% reduction of external concentrations of Na, or K on the T.E.P. measured between Uca haemolymph and external solution. During the periods indicated the crab was transferred to a solution whose Na, K or Cl had been reduced by approximately 50\%. At the points marked with a ★ the potential between the two calomel electrodes was recorded by shunting the circuit via a 3 M-KCl, 2\% agar bridge between the two test tubes containing the calomel electrodes. See Evans et al. (1974) and present text for details. Figure is redrawn from a recorder plot for a single crab.

Table 2. Changes in the T.E.P. ($V_m$) measured across Uca pugilator and transport numbers for reductions in external ion concentrations by 50\%:

<table>
<thead>
<tr>
<th>Ion</th>
<th>$V_m$ (mV)</th>
<th>$T_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>$-1.8 \pm 0.2$ (13)</td>
<td>$0.09 \pm 0.04$ (13)</td>
</tr>
<tr>
<td>K</td>
<td>$-0.02 \pm 0.11$ (13)</td>
<td>$0.0008 \pm 0.006$ (13)</td>
</tr>
<tr>
<td>Cl</td>
<td>$+2.2 \pm 0.2$ (13)</td>
<td>$0.11 \pm 0.01$ (13)</td>
</tr>
</tbody>
</table>

on the basis of the inequality of the measured and Nernst Potential (Gutknecht, 1970), it appears that Uca is maintaining its blood Na concentration out of electrochemical equilibrium with sea water. In addition to the slight net gain of sodium down the electrochemical gradient, Uca must also extrude Na which is gained via oral ingestion of the medium. Hannan & Evans (1973) showed that Uca ingests the medium at a rate of 600 µl 100 g$^{-1}$ h$^{-1}$. Since their sea water was also 500 mM-Na, this ingestion represents a net gain of 3 µ-mole Na g$^{-1}$ h$^{-1}$ which must be balanced by some type of extrusion mechanism.

(3) 'Transport numbers' for Na, Cl and K

In order to estimate the relative ionic permeabilities of the epithelium of Uca, we determined the 'transport numbers' for Na, K and Cl by reducing in turn the external concentration of each ion by approximately 50\% and noting the change in the T.E.P. (Fig. 1, Table 2). If our data are compared with similar determinations on Artemia (Smith, 1969a), two discrepancies are readily evident. In Artemia, $T_{Na}$ is 0.65 whereas $T_{Cl}$ is only 0.04, which indicates that the epithelium of Artemia is many times more permeable to Na than it is to Cl (Smith, 1969a). Our data indicate that, contrary to the
situation in *Artemia*, *Uca* maintains an approximately equal $T_{Na}$ and $T_{Cl}$. In addition, while the sum of $T_{Na}$, $T_{Cl}$ and $T_{K}$ of *Artemia* is 0.69, the sum of the 'transport numbers' for the same ions in our experiments is only 0.20. Croghan et al. (1965) showed that, in theory, the sum of the 'transport numbers' for the permeant ions should be 1.0. That this criterion is not met by either our study or Smith's (1969a) indicates either that other permeant ions play a substantial role in the origin of the potential across *Artemia* and *Uca*, or that equation (2) does not define the origin of the T.E.P. across these species. Neither study can differentiate between these alternatives, but it is unlikely that equation (2) strictly defines the membrane potential, because of the constraints of the constant field theory and because it is possible that some electrogenic ionic transport may also be playing a role in the origin of the T.E.P. Thus, the 'transport numbers' of complex epithelia should really be termed 'apparent transport numbers' (Croghan et al. 1965) and should be used only as an estimate of relative ionic permeabilities rather than quantitative measurements of actual permeabilities.

Using equation (3) we can estimate the relative permeabilities of *Uca* epithelium to Na, Cl and K. Thus $P_{Na}/P_{Cl} = 0.97$ and $P_{Na}/P_{K} = 2.25$ and $P_{Na}/P_{Cl}/P_{K}$ is therefore 1:0:1:0:4. It appears, therefore, that the relative Na and Cl permeabilities of *Uca* epithelium are distinctly different from those of *Artemia*. Smith (1969a) found that in *Artemia* the ratios are 1:0:0:03:0.6. It should be noted that since $T_{K}$ approaches zero, our calculated relative $P_{K}$ must be regarded as a gross approximation. Nevertheless, it appears that, like *Artemia* (Smith, 1969a), *Uca* maintains a $P_{K}$ that is lower than $P_{Na}$. Evidence in teleosts suggest that in this group $P_{K}$ is substantially greater than $P_{Na}$ (Potts & Eddy, 1973; Kirschner, Greenwald & Sanders, 1974).

(4) *Unidirectional Na efflux and P*$_{Na}$

The rate constant ($K$) for the unidirectional efflux of Na from *Uca* which were loaded with the radioisotope overnight is 0.15 ± 0.02 h$^{-1}$ (23). This is considerably below the rate constant described for *Artemia*: 0.42 h$^{-1}$ (Thuet et al. 1968), 0.69 h$^{-1}$ (Smith, 1969b). Since the total body Na of *Uca* is 219 µ-mole g$^{-1}$, the unidirectional efflux of Na from *Uca* is 33 µ-mole g$^{-1}$ h$^{-1}$ which is somewhat below that described for *Artemia*: 43 µ-mole g$^{-1}$ h$^{-1}$ (Thuet et al. 1968), 68 µ-mole g$^{-1}$ h$^{-1}$ (Smith, 1969b) but of the same order as that found in various species of marine teleosts (Maetz, 1974). The unidirectional Na efflux can be used to calculate $P_{Na}$ directly as long as the sea-water concentration of Na and electrical potential are known and two assumptions are made: (1) the animal is in steady state, such that Na influx and efflux are equal and (2) oral ingestion and diffusional gain are the only pathways of Na influx into *Uca acclimated* to sea water. The membrane's permeability to a specific ion (cm/s) can be calculated by the formula (Dainty, 1969):

$$J_{in} = -P_{j} \frac{zFV_{m}/RT}{1 - \exp(zFV_{m}/RT)} C_{j}^{0},$$

(4)

where $J_{in}$ is the diffusional influx (µ-mole cm$^{-2}$ s$^{-1}$), $C_{j}^{0}$ is the concentration (more properly activity) of the ion in sea water, $V_{m}$ is the electrical potential across the membrane, and $z, F, R, T$ have their usual meaning ($zF/RT = 39$ coulomb/joule (volt$^{-1}$) at 23 °C for a monovalent ion). If we assume that the gill epithelium is the major site of passive Na fluxes (Hannan & Evans (1973) showed that 86% of the water
Sodium extrusion by sea-water-acclimated Uca pugilator

Influx into Uca was branchial) and take the gill surface area of Uca to be 6.24 cm²/g (Gray, 1957), the unidirectional Na flux into Uca (corrected for oral ingestion) is 1.28 × 10⁻³ μ-mole cm⁻² s⁻¹. Since the electrical potential across Uca is only -7 × 10⁻⁴ V, the factor

\[
\frac{zFV_m/RT}{1 - \exp(zFV_m/RT)}
\]

is -1.015. \(P_{Na}\) is therefore apparently 2.45 × 10⁻⁶ cm/s. Smith (1969b) calculated that \(P_{Na}\) for Artemia is 2.8 × 10⁻⁶ cm/s and hence it appears that Uca's Na permeability is only some 9% of the Na permeability of Artemia. However, one must be cautious when applying equation (4) to a unidirectional Na influx, since one component of that influx may be a Na/Na ionic exchange which would give an overestimate of \(P_{Na}\) (see assumption 2 above). In fact, it will be shown subsequently that Na/Na exchange does take place in Uca epithelium so that our value for \(P_{Na}\) is somewhat overestimated (see below).

(5) The effect of potassium-free sea water (K-free SW)

Since Na/K exchange is involved in active sodium transport across a variety of tissues (Ussing & Thorn, 1973; Askari, 1974) and in particular hyporegulating marine teleosts (Maetz & Bornancin, 1975), it is of interest to investigate the effect of removal of K from sea water on the efflux of Na from Uca. If Na extrusion by Uca is via a Na/K exchange mechanism then removal of external K should result in a reduction of Na efflux as is found in some species of teleost (Maetz, 1969; Motais & Isaia, 1972; Evans et al. 1973). In experiments with ²²Na-loaded animals (long-term effluxes) transfer into K-free SW after 2 h in sea water increases the rate constant of the unidirectional Na efflux from 0.15 ± 0.04 h⁻¹ (8) to 0.37 ± 0.04 h⁻¹ (8). Using the rapid transfer technique we found that within 1-15 min after the transfer of ²²Na-injected Uca to K-free SW (Fig. 2) the unidirectional efflux of Na increases to 602 ± 66% (40, range 163%-166%) of the sea-water efflux.

This 'K-free sea-water effect' (stimulation of unidirectional Na efflux) does not at first seem to support the model for Na extrusion mediated via a Na/K exchange system. However, studies on squid axon (Caldwell et al. 1960; Baker et al. 1969; DeWeer, 1974), erythrocytes (Garrahan & Glynn, 1967a, b, c, d; Garrahan & Garay, 1974), frog striated muscle (Keynes, 1966; Sjodin, 1971) and marine teleosts (Maetz, 1969; Motais & Isaia, 1972) indicate that under certain conditions the carrier system mediating Na/K exchange can be transformed into a carrier with a variety of ionic transfer modes such as a reversed mode (K/Na exchange which generates ATP), Na/Na exchange, K/K exchange and uncoupled electrogenic Na transport. The supporting evidence for this model is extensive (for a recent review see Glynn & Karlish, 1975). The proposition that Na/K to Na/Na transformation takes place is based upon the finding that (under certain conditions), in squid axon, erythrocyte and frog muscle, unidirectional Na efflux continues unabated (or actually increases) after removal of K from the 'trans' side of the membrane and that this Na efflux is dependent upon 'trans' Na and is inhibited by external application of ouabain (see references above). In addition, Maetz (1969) showed that the K-stimulation of Na efflux from the flounder, Platichthys flesus (which is seen immediately after transfer of the sea-water-acclimated fish to K-enriched tap water), is reduced in the presence of Na. Motais & Isaia (1972) found that transfer of the eel Anguilla anguilla to K-free
Fig. 2. Effect of K-free SW on unidirectional efflux of Na from *Uca*. External medium was changed during the time period delineated by the arrows. Numbers in parentheses are relative fluxes. Figure is a tracing from a recorder plot for a single crab.

SW does not alter the efflux of Na even though the unidirectional Na efflux in normal sea water is reduced by 16% when ouabain is added. In addition, they showed that ouabain is much more effective in reducing the K-stimulated Na efflux in K-enriched tap water than the Na efflux in sea water and that it has no effect on Na efflux when *A. anguilla* is placed in K-free SW. They postulated that, under the conditions of normal sea water, unidirectional Na efflux across the teleost gill is via a carrier system which mediates both Na/K and Na/Na exchange. Whereas the former component is ouabain-sensitive, the latter is apparently ouabain-insensitive. When K is removed from sea water, the carrier transforms to the single, ouabain-insensitive Na/Na mode; in Na-free solutions (K-enriched tap water) the carrier mediates only ouabain-sensitive Na/K exchange (Motais & Isaia, 1972).

The 'K-free sea-water effect' on Na efflux from *Uca* may therefore be the result of a similar type of Na/K to Na/Na transformation of the carrier system (see appendix for alternative propositions). To test this we examined the sensitivity of the 'K-free sea-water effect' to external Na. *Uca* were transferred to K and Na-free SW, and rapid changes in Na efflux and electrical potentials were noted in separate experiments. In K and Na-free SW the electrical potential increases from $-0.6 \pm 0.2$ mV to $-7.9 \pm 0.7$ mV (15) while the flux falls to $45 \pm 3\%$ (32) of the sea-water efflux (Figs. 3 and 4). Using equation (5) (see Appendix) one can calculate that this measured increase in the internal negativity of *Uca* would result in a 15% reduction in passive Na efflux, far less than that measured. It is therefore obvious that the 'K-free sea-water effect' is indeed dependent upon external Na (exclusive of potential effects) and is presumably the result of a stimulation of Na/Na exchange after the removal of external K. Since the Na efflux in K and Na-free SW is less than 50% of the Na efflux in sea water, it appears that either Na/Na exchange is already taking place in sea-water-acclimated *Uca* (parallel to Na/K exchange) and is stimulated by the removal of K (and cessation of...
of Na/K exchange), or else a considerable portion of the Na efflux in sea-water-acclimated *Uca* is running through a Na/K carrier system that is transformed into a Na/Na carrier when external K is removed. One cannot differentiate strictly between these two alternative models but evidence presented below indicates that Na/K exchange is a relatively small component of Na efflux under the conditions of normal sea water. However, it should be noted that both models suppose that in the presence of external K the rate of Na/Na exchange is limited – the maximal rate is only possible when external K is removed. Thus, functionally, K is inhibiting Na/Na exchange under normal conditions.

(6) *K*-stimulated Na efflux

Although the above data indicate that Na/K to Na/Na transformations are possible in *Uca* epithelium, they are not direct evidence for a Na/K exchange system itself. If Na/K exchange is one of the components of Na efflux from *Uca* then re-addition of K after both Na and K have been removed from the external medium should stimulate Na efflux. To test this we transferred *Uca* to 25 mM-K, Na-free SW (see Table 1) after an initial transfer from sea water to K and Na-free SW. The efflux and potential changes were studied in separate experiments. In 25 mM-K, Na-free SW the electrical potential across *Uca* is $-6.0 \pm 0.9$ mV (8 animals) while the Na efflux is $72 \pm 7\%$ of the control, sea-water Na efflux (Fig. 3). Although the slight measured drop in internal negativity could account for minor stimulation (5\%) of Na efflux from the rate seen in K and Na-free SW (see above), it is obvious that the re-addition of K to the external medium stimulates Na efflux ($P \ll 0.005$ compared to K and Na-free SW efflux) much more than can be accounted for by changes in the electrical potential. These data support the proposition that the epithelium of *Uca* is capable of carrying on Na/K exchange. However, since addition of 25 mM-K (2.5 times sea-water K concentration) results in an Na efflux that is still less than the Na efflux in sea water, it is apparent that Na/K exchange is a relatively minor component of normal Na efflux across *Uca* epithelium. Thus, one must conclude that the substantial drop in Na efflux
Fig. 4. Effect of K and Na-free SW and K-free, 25 mM-Na SW on the unidirectional efflux of Na from *Uca*. External medium was changed during the time period delineated by the arrows. Numbers in parentheses are relative fluxes. Figure is a tracing from a recorder plot for a single crab.

seen in K and Na-free SW (see above) is secondary to the cessation of both Na/Na and Na/K exchange and that Na/Na exchange does take place in *Uca* epithelium even in the presence of external K.

K-stimulated Na efflux has also been described in teleosts (Maetz, 1969; Motais & Isaia, 1972; Evans *et al.* 1973; Potts & Eddy, 1973; Greenwald, Kirschner & Sanders, 1974); however, only Evans *et al.* (1974) were able to show that this stimulation was exclusive of changes in the electrical potential. In addition, Thuet *et al.* (1968) showed that the sodium efflux from *Artemia* in 1 M-KCl is 93% of the efflux in sea water.

It should be noted that since our data show that Na/Na exchange is one component of Na flux across *Uca* epithelium, our calculation of a $P_{Na}$ of $2.45 \times 10^{-5}$ cm/s (see above) is obviously an overestimate and that *Uca* is therefore even less permeable to Na than proposed. The true $P_{Na}$ is difficult to estimate since we have not measured the Na influx in K and Na-free SW; it is probably of the order of $1.5 - 2.0 \times 10^{-6}$ cm/s since the efflux declines by 30% (exclusive of electrical potential) when *Uca* is placed in K and Na-free SW (when Na/K and Na/K exchanges cease).

(7) **Relative affinity of Na and K for the Na/K exchange carrier**

Although our data support the proposition that both Na/K and Na/Na exchange are taking place in *Uca* epithelium when the animal is in sea water, the carrier system must have a much greater affinity for K than for Na since Na/K exchange is apparently taking place in the presence of a relatively huge concentration of the potentially competing ion, Na (500 mM-Na vs. 10 mM-K). To test this proposition directly, in experiments parallel to those just described, *Uca* were transferred from sea water to K and Na-free SW and then to K-free, 25 mM-Na SW (Table 1). In the latter solution, the potential decreases from $-7.9$ mV to $-5.6 \pm 0.9$ mV (7) while the Na efflux actually declines slightly from 46% of the sea-water efflux to $42 \pm 3\%$ (6) of the sea-water efflux (Fig. 4). Thus, 25 mM-Na does not stimulate Na efflux whereas 25 mM-K produces a significant stimulation. One can only conclude that the carrier has a much greater affinity for external K than Na. The addition of 25 mM-Na to K and Na-free SW depolarizes the electrical potential to the same degree as addition of 25 mM-K.
to K and Na-free SW. These data indicate that $P_Na$ approximates $P_K$, rather than being greater than $P_K$ (section 3).

(8) The effect of ouabain on the 'K-free sea-water effect'.

Since Na/Na exchange is ouabain-sensitive in squid giant axons (Baker et al. 1969), erythrocytes (Garrahan & Glynn, 1967a) and frog striated muscle (Sjodin, 1971) but ouabain-insensitive in teleosts (Motais & Isaia, 1972) we investigated the sensitivity to ouabain of the 'K-free sea-water effect' of Uca. Using the long-term efflux technique (Fig. 5) with $^{23}$Na-injected crabs and the rapid transfer technique we could find no diminution of the 'K-free sea-water effect' when concentrations of ouabain from $10^{-4}$M to $10^{-3}$M were added to the external medium. It appears then that either Na/K activated ATPase is not involved in the 'K-free sea-water effect' or that the enzyme has become ouabain-insensitive during the transformation to Na/Na exchange.

SUMMARY AND CONCLUSION: Na/Na AND Na/K EXCHANGE ACROSS UCA EPITHELIUM

Our data indicate that unlike Artemia (Smith, 1969a, b) and marine teleosts (Potts & Eddy, 1973; Kirschner, Greenwald & Sanders, 1974), Uca maintains a relatively similar permeability to Na and Cl across its epithelium. The small potential across Uca is a result of this lack of differential permeability as well as the absence of significant electrogenic active transport of either ion. Like some teleosts (Evans et al. 1973, 1974) but unlike Artemia (Smith, 1969a, b) and other teleosts (Kirschner et al. 1974), Uca is faced with a net diffusional gain, down their respective electrochemical
gradients, of both Na and Cl, which must be extruded along with ingested NaCl transported across the gut. Our data indicate that this extrusion of Na is via a Na/K exchange carrier. In addition, a substantial portion of the unidirectional Na efflux from _Uca_ is coupled to Na influx in a Na/Na exchange diffusion system. There are indications that similar Na/K and Na/Na exchanges can take place (under certain circumstances) in squid giant axons, erythrocytes, and frog muscle cells (Glynn & Karlish, 1975); in these cases it appears that Na/K exchange is the normal mode but that the ouabain-sensitive carrier transforms into a carrier mediating Na/Na exchange if no K is present. Although we have implied that a similar type of carrier transformation could account for the 'K-free sea-water effect' that we have described for _Uca_, in fact, we have no direct evidence for this proposition. It could also be proposed that the Na/K and Na/Na exchanges that we have described are via parallel, functionally linked, but separate carrier systems. However, it is important to note that in either case Na/Na exchange is limited by the presence of Na/K exchange and is therefore substantially stimulated when K is removed from the sea water.

Despite our inability to differentiate between the same or parallel carrier systems for Na/K and Na/Na exchange across _Uca_ epithelium, it is interesting to propose a model for each alternative:

1. **Na/K and Na/Na exchange via a single carrier system**

   If the two ionic exchange pathways are running through the same carrier then we must propose that the presence of a K ion at a 'potassium site' on the carrier molecule somehow inhibits more than one 'Na site' so that, when K is removed, not only is the former 'K site' transformed into a 'Na site', but many formerly shielded 'Na sites' also become functional. This would account for the substantial stimulation of Na efflux after removal of external K. In this case one would propose that the 'K-free sea-water effect' would be ouabain-sensitive.

2. **Separate, but functionally linked, Na/K and Na/Na carrier systems**

   If the two efflux pathways for Na are parallel, but not identical, then we must propose that somehow Na/K exchange acts as a 'brake' on Na/Na exchange. Removal of external K (and therefore cessation of Na/K exchange) would remove the 'brake' and allow full expression of the rapid Na/Na exchange. Perhaps in this case, Na/Na exchange would not be ouabain-sensitive. Motais & Isaia (1972) showed that sodium efflux from the teleost, _Anguilla anguilla_, in potassium-free sea water is insensitive to ouabain, and our data indicate that the 'K-free sea-water effect' in _Uca_ is also insensitive to even high concentrations of ouabain (10^{-8} M).

   While the ouabain-insensitivity of the _Uca_ 'K-free sea-water effect' would seem to support our second model (separate carrier systems for Na/K and Na/Na exchange), one could also propose that transformation of a Na/K carrier into a Na/Na carrier would abolish the carrier's sensitivity to ouabain. Our data, unfortunately, do not allow us to distinguish between these two possibilities.

   It should be noted that if Na/K and Na/Na exchanges are via separate carrier systems then our description of the relative affinities of Na and K for the carrier (see (7) above) must apply to their respective carriers rather than to a single carrier capable of mediating Na/K and Na/Na exchange.
This research was supported by NSF Grants GB 36423 and BMS 75-00091 to D.H.E.

REFERENCES


**APPENDIX**

One might argue that the ‘K-free sea-water effect’ is secondary to effects on the diffusional loss of Na from Uca. The diffusional efflux of an ion species across a membrane is defined by the following formula (Dainty, 1969; Smith, 1969b):

$$ J_{out} = -P_f \frac{zFV_{m}/RT}{1 - \exp (zFV_{m}/RT)} C_f \exp (zFV_{m}/RT), $$

where the various factors have the same meaning as in equation (4) except that $C_f$ is the concentration of the ion (in this case Na) in the body fluids. Thus, the substantial
Increase in $J_{\text{Na}}^{\text{out}}$ which is seen in K-free SW could be the result of change in $V_m$ (depolarization to an inside positive T.E.P.), or an increase in either $P_{\text{Na}}$ or $C_{\text{Na}}^i$.

(1) Depolarization of the T.E.P.

Smith (1969a, b), Potts & Eddy (1973), Evans et al. (1974), House & Maetz (1974), and Kirschner et al. (1974) have shown that changes in the T.E.P. can result in substantial changes in unidirectional Na efflux from Artemia and various species of teleosts. However, our data indicate that, in Uca, the 'K-free sea-water effect' is not the result of changes in diffusional loss of Na secondary to depolarization of the T.E.P. We recorded the changes in the T.E.P. during transfers from sea water to K-free SW and found that the potential did not change ($-0.4 \pm 0.2$ mV in sea water and $-0.6 \pm 0.1$ mV in K-free SW, 17 animals). In fact one can calculate from equation (5) the voltage change necessary to account for a 6-fold increase in unidirectional, passive Na efflux. This amounts to a change in T.E.P. of more than 5 V.

(2) Increase of $C_{\text{Na}}^i$

To account for a 6-fold increase in Na efflux as a result of an increase in $C_{\text{Na}}^i$, one would have to postulate that the haemolymph concentration increased 6-fold (to approximately 3 M) within 15 minutes after the removal of external K. This seems highly unlikely although the uncoupling of a Na/K exchange system could result in a decline in Na extrusion, which in turn could give rise to a slight increase in blood Na concentrations. However, our data indicate that Na/K exchange is a relatively small component of the unidirectional Na flux.

(3) Increase of $P_{\text{Na}}$

$P_{\text{Na}}$ would also have to increase 6-fold to account for the 'K-free sea-water effect' which we have observed in Uca. Although we have no direct evidence that an increase of $P_{\text{Na}}$ does not take place, the dependence of the 'K-free sea-water effect' on external Na supports the proposition of an increase in Na/Na exchange rather than an increase in $P_{\text{Na}}$. 