EXTRACELLULAR FLUID VOLUME CHANGES IN CARCINUS MAENAS DURING ACCLIMATION TO LOW AND HIGH ENVIRONMENTAL SALINITIES

By R. R. Harris and M. B. Andrews

Department of Zoology, University of Leicester, Leicester LE1 7RH, England

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SUMMARY

Changes in extracellular fluid (ECF) volume of Carcinus maenas (L.) were studied in vivo during acclimation to low and high environmental salinities. Initial investigations showed that there was a rapid equilibration into this compartment of the ECF markers used ([3H]inulin and [14C]hydroxymethyl inulin). Earlier reports of a relatively slow marker distribution, indicated from clearance curves, can be explained by high clearance rates occurring when frequent blood sampling was carried out. After transfer of the crabs to media hyposmotic to the haemolymph, ECF volumes decreased transiently to 74.8% of the initial volume, but within 40 h in 26% sea water original volumes were restored. Calculation of intracellular water contents suggests that a volume limitation phase precedes the regulatory return to the original volume. In hyperosmotic media, the ECF volumes increased significantly (to a maximum of 143%) but, in contrast to the response in hyposmotic conditions, showed only a partial return to the original volumes.

INTRODUCTION

Many euryhaline species show extensive and rapid changes in extracellular osmotic pressure when acclimating to different salinity environments. Adjustments to the osmotic concentration of their intracellular fluids are made to maintain isosmoticity with the extracellular fluid and thus regulate cell volume. Intracellular osmotic pressures are lowered in hyposmotic conditions by the release and/or removal by degradation of osmotic effectors. Similarly, they are raised in hyperosmotic conditions by the uptake or synthesis of these small molecules in the cells. In invertebrates, free amino acids are thought to be the regulatory osmotic effectors, although other solutes may be important (Gilles, 1979 for review).

These processes must occur at rates appropriate to produce intracellular osmotic pressure changes matching those taking place extracellularly, if there is to be no cellular shrinkage or swelling by osmotic transfer of water. The effectiveness of this cellular volume control can be investigated by measuring changes in body fluid compartment volumes during acclimation to different salinities. Recently, it has been found that some species, with limited capacities to regulate extracellular concentration, tolerate large changes in the volumes of their body fluid compartments (Arenicola:...
In these soft-bodied species, water enters both extra- and intracellular compartments. In acute osmotic stress, it is assumed that the cellular regulatory mechanisms are unable to cope with either the rate or amplitude of the extracellular osmotic pressure changes. It is not known if this leads directly to general cellular swelling and rupture causing cell death on a large scale or whether, as a result of tissue swelling, circulatory restriction occurs as a first stage, resulting in reduced blood flow to important organs and possible ischaemia. Consequent cell swelling due to tissue hypoxia may also be important (Macknight & Leaf, 1977).

This paper reports on experiments to measure the ECF volume of the euryhaline crab *Carcinus maenas* during acclimation to hypo- and hyperosmotic media. ECF volume was determined from the volume of distribution of the marker inulin. The rate of inulin distribution and some factors affecting its clearance from the ECF were investigated initially. A preliminary account of this work has been reported (Harris, 1976).

**MATERIALS AND METHODS**

Adult *Carcinus maenas* were obtained by trawling from the Wash (North Sea) and kept in circulating sea water (32–34‰ salinity) at 12 °C. Experimental animals (intermoult males) were fed weekly on mussel flesh.

Extracellular fluid (ECF) volumes of acclimating and acclimated animals were measured using [3H]- and [14C]hydroxymethyl inulin (Radiochemical Centre, Amersham). Co-identity of inulin space with ECF volume was assumed. Intracellular penetration of inulin, reported to occur gradually in some mammalian tissues (McIver & Macknight, 1974), was assumed to be negligible over the time course of these experiments. Initially, experiments were carried out to determine the rate of distribution of injected inulin. Fifty microlitres of radioactive inulin (0·05–0·10 MBq) in *Carcinus* Ringer (Pantin, 1948) were injected by Hamilton microsyringe into the infrabranchial sinus of an animal clamped vertically in a Perspex chamber containing sea water. Samples of haemolymph were taken from the pericardium by a catheter, aligned horizontally, consisting of a 19-gauge hypodermic needle connected to a short length of polyethylene tubing. The catheter was inserted through a rubber membrane glued over a pre-drilled hole in the dorsal carapace. The needle also served as an electrode for ECG recording with a stainless steel plate positioned beneath the animal as the other electrode. Single drops of haemolymph (~ 50 μl) were collected, during steady bleeding from the catheter, by an LKB Ultrorac fraction collector set in the single-drop count mode. Simultaneously the ECG was amplified by and recorded on a Tektronix 5110 oscilloscope and Rikadenki chart recorder. The release of drops from the catheter was marked on the chart by the event recorder.

The [3H]- and [14C]inulin activities of doubly labelled haemolymph samples were measured by scintillation counting (Nuclear-Chicago Mk III). Samples were digested in 0·5 ml NCS, neutralized with acetic acid and taken up in 10 ml of Triton X-100 fluor. Standards of the injectate were also counted (efficiency of counting ~58%). The ECF volume was calculated from the relation; ECF (ml) = \( Q_{\text{tot}}/C_t \), where \( Q_{\text{tot}} \) was the total activity (DPM) injected and \( C_t \) the haemolymph concentration of inulin (DPM ml\(^{-1}\)) at time \( t \).
For the measurement of rates of clearance, $[^3H]$- and $[^{14}C]$-inulin radioactivity of 1 ml samples of the external medium was determined. In these experiments animals were maintained in 250 ml of aerated sea water containing 100 mg l$^{-1}$ of the antibiotic Crystamycin (Glaxo) to reduce bacterial breakdown of inulin.

Haemolymph samples in both clearance determinations and ECF volume measurements were removed from the infrabranchial sinus via an arthrodial membrane, and radioactive markers were injected into the pericardium. This was thus the reverse of the experiments to determine the distribution of the marker. Rates of clearance, $V_{cl}$ (ml h$^{-1}$) were calculated using the relation $V_{cl} = k \cdot V_a$, where $k$ is the rate constant (h$^{-1}$) of haemolymph inulin concentration (radioactivity) decrease and $V_a$ the ECF volume (ml). Where the rate of appearance of inulin in the medium was measured, clearance was calculated from the equation $V_{cl} = \frac{Q_{t_1} - Q_{t_2}}{C_{t_1} - C_{t_2}}$, where $Q_{t_1}$ is the rate of appearance of inulin in the medium (DPM h$^{-1}$) between time 1 and 2, and $C_{t_1}$, the logarithmic mean concentration of inulin in the haemolymph (DPM ml$^{-1}$) between time 1 and 2 (Riggs, 1963). Individual clearance rates were expressed per 100 g wet weight day$^{-1}$. Animals were weighed on a Mettler top-pan balance to ±0.01 g after removal of water from the branchial chambers by shaking. Dry weights were determined by oven-drying at 105 °C.

Differences in ECF volumes or clearance rates measured in the same individuals before and after different experimental treatments were tested for significance by a paired sample $t$ test. Regression analysis of clearance curves was carried out by the method of least squares, using a Wang 720 C programmable calculator. All results are presented as the mean ± 1 S.E.M.; $n =$ number of determinations.

**RESULTS**

(i) **The initial distribution of injected inulin**

After injection of inulin into the infrabranchial sinus, haemolymph samples were removed about every 5–10 s from the pericardium for a period up to 600 s. A continuous record of sampling time and ECG was made in each case. Fig. 1 illustrates examples of curves of haemolymph inulin radioactivity showing that, after injection and a short delay, a peak of radioactivity appeared in the pericardium. These curves have been fitted by eye to individual points which have been omitted for clarity. Inulin activity declined to a relatively steady level after about 400 s. These animals showed continuous periods of normal heartbeat rate (89 ± 9 beats min$^{-1}$, $n =$ 19). The injected inulin was transported rapidly through the gills into the pericardium as indicated by the short interval between injection and the rise in inulin radioactivity in the pericardium. This interval included a period of delay due to flow through the sampling catheter.

Bradycardia and cardiac arrest, often observed in these animals (Taylor, Butler & Sherlock, 1973), affected the rate of distribution of the marker. When cardiac arrest occurred, the rate of bleeding was reduced and inulin appeared to increase in concentration in the pericardium at a slow rate (Fig. 1). When bradycardia occurred, a less abrupt rise in activity was observed.

In *Carcinus* the injectate did not pass the sampling point as a bolus when recirculated became widely distributed in the ECF since no clear secondary concentration...
peak, as observed in the mammalian circulation (Kelman, 1971) appeared. In these experiments, the volume of haemolymph bled from the animal was significantly large (6–10% of total volume) and for this reason they were generally terminated after 600 s. The later distribution of the marker inulin was investigated using other methods.

(ii) The later distribution and clearance of inulin

It has been suggested that distribution of inulin in the ECF of Carcinus is only complete about 10 h after injection. Although the bulk of the marker injected is rapidly distributed, diffusion into another compartment, representing about 10 ml 100 g⁻¹ wet weight, occurred at a slower rate as indicated by a steeper initial part of the curve of marker concentration plotted against time (Zanders, 1980).

In measuring ECF volume, the interval between marker injection and volume measurements needs to be long enough to attain a relatively steady state of tracer distribution. If this interval is long compared with the time scale of the changes in ECF volume under investigation, the nature and extent of these changes may not be detected. For this reason, 'kinetic methods' of ECF volume determination (Hickman, 1972), requiring the extrapolation of clearance curves to zero time to yield the haemolymph marker concentration, which assumes instantaneous distribution into the ECF, were not considered suitable.
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Fig. 3. Inulin clearance of 100 % sea-water-acclimated Cardium showing changes in the rate of haemolymph [*H] (or [14C]) inulin decrease (○), and medium appearance (●) during periods of hourly and ~12-hourly sampling. The low rate of appearance of inulin in nephropore-blocked animals is also shown (▲). C₀ = haemolymph inulin activity 1 h after injection; Cₜ = haemolymph inulin activity at any time t (dpm ml⁻¹); Qₜ = total medium inulin activity at time t (dpm); Qₜ₀ = total inulin injected (dpm).

Table 1. Rate constants (k) of haemolymph inulin concentration decrease in C. maenas
(Mean ± S.E.M., n = 10)

<table>
<thead>
<tr>
<th>Period</th>
<th>k h⁻¹</th>
<th>S.E.M.</th>
</tr>
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<tbody>
<tr>
<td>0-0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.6-0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-7</td>
<td>0.036</td>
<td>0.0011</td>
</tr>
</tbody>
</table>
| 7-96      | 0.007  | 0.0111  *
| 96-104    | 0.023  | 0.0033  **

** Significantly less than 1-7 h period and 96-104 h; P < 0.001.
* Significantly less than 1-7 h period; 0.01 < P < 0.002.

It was important, therefore, to investigate the presence of any such compartmentalization of the ECF as suggested by Zander (1980), and the rate of distribution of the ECF marker into each compartment. Animals were injected with inulin and haemolymph samples removed hourly for a period of 6-8 h. Subsequently, samples were taken at ca. 12 h intervals over 60 h. The animals were then sampled at hourly intervals for a final 6-8 h period. Fig. 2 shows the mean relative decrease of haemolymph inulin ([3H] or [14C]) counts plotted semi-logarithmically against time. The rate constants describing the exponential decrease in haemolymph inulin concentration were obtained from regression analyses of individual curves for the relevant periods of hourly and ca. 12-hourly interval sampling (Table 1).

The rate of decrease of haemolymph inulin concentration was highly significantly slower when animals were sampled at ca. 12 h intervals compared to the initial rate, when hourly samples were removed. It increased again when samples were removed at
a similar frequency later in the experiment. The latter rate constants were significantly lower than those determined in the initial period ($0.01 < P < 0.002$).

A major part of the rapid initial decrease in haemolymph concentration of marker may be due to increased rates of clearance. This possibility was investigated by determining rates of clearance as appearance in the medium of injected inulin. Medium samples were removed at the same time as the haemolymph samples. The increase of $[^3]$Hinulin activity, as a ratio of the total injected inulin ($Q_t/Q_{0t}$), is shown in Fig. 2 also. A more rapid rate of appearance of the marker in the medium during the hourly sampling periods was found. However, initially inulin appeared at variable rates. In some individuals a rapid release of inulin occurred, whereas in others the output was slower.

Mean rates of clearance (ml 100 g$^{-1}$ day$^{-1}$) calculated for all sampling periods are shown in Table 2, in comparison with rates calculated from the rate constants of haemolymph clearance (Table 1). The observed rate of appearance of inulin in the medium was significantly lower than the calculated rates in the initial sampling period ($0.01 < P < 0.05$). In the intermediate (ca. 12-hourly) period and in the final one clearance rates were not significantly different from the calculated rates ($P > 0.1$).

The more rapid rates of clearance of inulin from the haemolymph appeared to be due, at least in part, to increased outputs of marker into the medium. The main route of inulin loss in crustaceans is via the antennal gland (Riegel & Cook, 1975; Cameron & Batterton, 1978), although in other groups integumental routes of loss have been described (Kirschner, 1980). If urine release is prevented in Carcinus by sealing the nephropores with cyanoacrylate adhesive (Loctite), medium inulin activity increases at a very low rate (Fig. 2). Only $1.3 \pm 0.6\%$ of the total activity injected appeared in the medium after 22 h, compared with about $13\%$ after 24 h in normal animals. It would appear, therefore, that the main route of inulin loss is via the urine. The mean rate constant of clearance from the haemolymph was $0.024 \pm 0.004$ h$^{-1}$ during the initial 7 h. This was not significantly different from the rate in normal animals.

In Carcinus, frequent haemolymph sampling led to increased rates of clearance by the antennal gland. Since this increase also occurred when the ECF marker was most probably completely distributed (96+ h), the rapid initial decrease in haemolymph inulin activity must be due largely to increased clearance rates and, to a lesser extent, to a slower distribution into a less-accessible ECF compartment. The initially faster rates of clearance, measured by haemolymph inulin concentration decrease compared to medium appearance, may be due to differences in the rates of release of bladder urine. The results of nephropore blockage showed that a period of filling of the
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Table 3. Extracellular fluid volume (ECF) (ml 100 g⁻¹ wet weight) in Carcinus acclimated to 100% sea water. Calculations made after two different periods of marker (inulin) distribution (1·5 h and 400–600 s)

<table>
<thead>
<tr>
<th>Weight (g)</th>
<th>Mean (range)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>£WC/Ci</td>
<td>Mean S.E.M.</td>
<td></td>
</tr>
<tr>
<td>Q₁₀/C₁ = 1·5 h</td>
<td>32·96 0·93</td>
<td>42·4 (22–60)</td>
</tr>
<tr>
<td>Q₁₀/C₁ = 4–600 s</td>
<td>30·03 2·48</td>
<td>82·7 (62–106)</td>
</tr>
</tbody>
</table>

urinary bladder with urine containing inulin cleared from the haemolymph can occur before any release of the marker to the medium.

Animals acclimated to 26% sea water for 24 h showed rates of haemolymph clearance significantly faster than 100% sea-water-acclimated animals. In the initial hourly sample period, the mean rate constant was 0·066 ± 0·009 h⁻¹ (0·01 < P < 0·02). This faster rate was maintained even at low sampling frequency. The rate of clearance under these conditions was 29·0 ± 1·8 ml 100 g⁻¹ day⁻¹.

ECF volumes were determined using values of haemolymph inulin activities determined 1·5 h after injection. Table 3 shows the mean ECF values determined by this method and those estimated from inulin concentrations 400–600 s after injection during the early phase of marker distribution (see above). A t test showed that the mean weight-specific ECF volumes were not significantly different (P > 0·01), indicating that the distribution of inulin was almost complete within 10 min. Since the clearance experiments showed that no slow distribution into a major extracellular compartment occurred subsequently, a period of 1·5 h equilibration was chosen since it gave complete distribution and allowed for any period of cardiac arrest in handled animals. It is probable that the ECF volume determined 1·5 h after injection included a component representing part of the bladder volume. The experiments also showed that determination of ECF volume by the ‘kinetic method’ was impracticable in experiments where clearance rates would be changing markedly, as after transfer into a hypo-osmotic medium.

To determine changes in ECF volume of individual animals, differently radioactively labelled tracers of inulin were used. Successive measurements of ECF volume of control animals acclimated to 100% sea water with [³H]inulin initially, and hydroxymethyl [¹⁴C]inulin, subsequently, were made. The ratio of [³H]inulin space volume/ [¹⁴C]inulin space volume was 1·01 ± 0·02 (n = 42). No significant difference between the volumes of distribution of the two tracers in the haemocoel of *Carcinus* was shown (paired t test; P > 0·1), in agreement with the findings of Marlow & Sheppard (1970) in rats.

(iii) Changes in ECF volume in acclimating animals

Animals collected from and maintained in 100% sea water (32% salinity) were directly transferred to 26% (8·5% salinity) sea water. In addition, animals long-term-acclimated to 26% sea water (30+ days) were transferred to 100% sea water. One group of animals kept in 100% sea water was placed in distilled water and another
Fig. 3. Relative changes in ECF volume (inulin space as % initial) of *Carcinus* during acclimation to different salinity media. (▲) 100% sea-water-acclimated animals transferred to ion-free medium at time 0; (●) 100% sea-water-acclimated animals transferred to 26% sea water; (○) 26% sea-water-acclimated animals transferred to 100% sea water; (△) 100% sea-water-acclimated animals transferred to 162% sea water. Vertical bars indicate ± 1 S.E.M.; n > 6 at each interval.

from 100% sea water into 162% sea water (52% salinity). Thus the time course of ECF volume changes during acclimation to both hypo- and hyperosmotic media was followed for up to 180 h; except, obviously, in distilled water (16 h).

The mean changes in ECF volume (expressed as % initial) during acclimation are shown in Fig. 3. Paired $t$ tests were made on individual ECF volumes measured, as in the controls, before ([³H]) and after ([¹⁴C]) treatment. After transfer from 100% to 26% sea water, a significant decrease in ECF volume was measured at 16 h ($0.05 > P > 0.02$) and 30 h ($0.02 > P > 0.001$). Subsequently, there was a relatively rapid restoration of the original volume after about 40 h. The ECF volume decrease was maximal after 30 h (to 74.8% initial). For the remainder of the experiment after 40 h, no significant change in ECF volume was measured.

After transfer of acclimated animals from 26% to 100% sea water, an increase in ECF volume was detectable after only 4 h ($0.02 > P > 0.05$), and the largest increase in volume (143%) occurred later, after about 12 h ($0.02 < P < 0.05$). A markedly different pattern of response was observed during this acclimation to a hyperosmotic medium. In contrast to transfer from 100% to 26% sea water, the original ECF volumes were not restored even after 188 h ($0.02 < P < 0.05$), so that an increased ECF volume was maintained.

ECF volumes measured after transfer to distilled water showed a significant decrease after 8 h ($P > 0.001$) and remained reduced until the death of the animal at
Table 4. Relative changes in extracellular osmolality and calculated intracellular $H_2O$ content in Carcinus during acclimation to 26% sea water

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Intracellular $H_2O$ content</th>
<th>$\Pi_i$</th>
<th>$\Pi_t$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1.08</td>
<td>1.36</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>1.34</td>
<td>1.29</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>1.26</td>
<td>1.45</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>1.37</td>
<td>1.74</td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>1.04</td>
<td>1.73</td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>1.10</td>
<td>1.70</td>
<td></td>
</tr>
</tbody>
</table>

ca. 16 h, the maximum decrease being to 70.2% of the initial after 12 h. This was not significantly less than the volume after 30 h in 26% sea water (to 74.8).

Significant increases in ECF volume were observed in animals transferred from 100% to 162% sea water (to 125.6% initial after 6 h; 0.002 < $P$ < 0.01). Animals surviving 140 h in this medium had higher mean ECF volumes than the initial volumes, but these differences were not significant at the 5% confidence level ($P > 0.1$).

Thus marked changes in ECF volume (measured as inulin space) occurred during acclimation of Carcinus to hypo- and hyperosmotic media. In the former, decreases in ECF volume appeared to be transient, whereas in the latter, long-term increases in volume occurred. The rate at which these changes occur will depend on the rate of ECF osmolality change during acclimation, and their extent on the amplitude of osmotic pressure change. In Carcinus the $t_{0.9}$ of the exponential decrease of haemolymph osmolality to the new steady-state level in a hyposmotic medium is about 7 h (Margaria, 1931; Shaw, 1961). A large decrease in osmolality occurred during the acclimation to 26% from 100% sea water ($\Pi_i/\Pi_t = 1.70$, where $\Pi_i$ is the initial and $\Pi_t$ the final haemolymph osmolality).

The osmotic responses of the tissues of Carcinus after transfer to a hyposmotic medium were assessed by comparing the calculated intracellular water content with measured changes in extracellular osmolality, expressed as the reciprocal of the relative changes, in accordance with the Boyle-van't Hoff relationship $\Pi_i V_i \equiv \Pi_t V_t = \text{a constant}$ (Table 4). No account was taken of non-solvent water volume (Lucke & McCutcheon, 1932). At 4 h after transfer some water uptake by the tissues had taken place and at 12 h this uptake indicated osmometric behaviour. Subsequently, water uptake was limited and the tissue water content returned to a level similar to the initial value of about 40 h. The ECF volume reduction after 12 h was less than would be expected if osmotic equilibrium was reached between intra- and extracellular compartments suggesting some loss of solute from the cells. Later the tissues lost water (and presumably solutes) to regain their original volume. In hyperosmotic media (26–100% sea water; $\Pi_i/\Pi_t = 0.48$), a similar analysis (not shown) also
indicated a limitation of tissue shrinkage at 24 h, but later there was no significant increase in volume to the initial level.

**DISCUSSION**

Our finding that the ECF marker, inulin, was distributed rapidly into the major part of the ECF volume of the crab *Carcinus maenas* contrasted with those of Zanders (1980) and Gleeson & Zubkoff (1977), who have all proposed a slower distribution into a less-accessible part of the ECF. The latter investigators suggested that 40 min was necessary for the complete distribution of NaSCN. A rapid distribution of injected inulin is to be expected in intact animals where the integrity of the circulatory system is maintained. This is not true of isolated tissues or tissue slices where distribution into the ECF is by diffusion only.

In *Carcinus*, clearance of inulin by the antennal gland increased when haemolymph samples were removed frequently. High initial clearance rates resulted in a rapid decrease in haemolymph marker concentration giving, with the design of experiment chosen, the appearance of a two-compartment system where the initial clearance component could be mistaken for marker distribution into a less-accessible second compartment. It was clear that these faster rates of clearance accounted for most of the initial rapid decrease in haemolymph inulin concentration. The initial delay in output of inulin into the medium was probably due to its initial accumulation in the antennal gland during release of previously formed inulin-free urine. After attainment of inulin concentration steady states between urine and haemolymph the rates of haemolymph clearance and appearance of inulin in the medium were similar.

The mean ECF volumes of 100% sea-water-acclimated animals (32.96 ml 100 g⁻¹) were similar to those reported by Robertson (1960) of 32.6 ml 100 g⁻¹, and also, more recently, by Zanders (1980) of 31.1 ml 100 g⁻¹. However, they were larger than those reported by other workers (Binns, 1969; Siebers & Lucu, 1973) of about 19 ml 100 g⁻¹. Expressing the ECF volume per unit of body weight may give values which are different in different populations of the same species, where changes in exoskeleton weight occur during the moult cycle (Gleeson & Zubkoff, 1977). Also, there may be geographical variations in exoskeleton mass, while differences in nutritional state affect the haemocoelic volume, increasing it during starvation (Dall, 1974). Similarly, haemolymph volumes may change in relation to reproductive cycles as gonads develop or regress. These changes would be relatively slow. It was hoped the measurement of ECF volume in the same individuals, before and after acclimation to different salinity media, rather than comparing weight-specific volumes of different groups, would reduce some of the variability caused by these factors.

The estuarine and intertidal species *Carcinus maenas* showed a decrease and an increase in ECF volume during acclimation to hypo- and hyperosmotic media respectively. Since overall body volume changes are relatively small in crabs in these conditions (Schwabe, 1933), changes in ECF volume would be at the expense of the intracellular volume. Water movements into the cells during hyposmotic stress and out of them in hyperosmotic conditions occur, and there is negligible loss to or gain of fluid from the external medium. Other investigations (Siebers & Lucu, 1973) have shown that the ECF volume is relatively constant after acclimation (one month) to
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Salinities ranging from 11 to 49%. However, Zanders (1980) found a decrease in ECF volume in 25% sea-water-acclimated *Carcinus*. Changes in ECF volume of similar direction and magnitude to those of the present work were found to occur in *Pachygrapsus crassipes* after three days acclimation (Gross & Marshall, 1960). These workers showed that tissue water content changes reciprocally, and proposed that this was at the expense of the ECF volume.

However, the intracellular compartment is not the only route for or source of fluid during the ECF volume changes in *Carcinus*. Other extracellular compartments, particularly gut and antennal gland bladder, may be involved in these changes in volume. Riegel *et al.* (1974) found that the bladder volume of *Carcinus* was equivalent to about 10% of the haemolymph (ECF) volume. Some of the decrease in ECF volume in a hyposmotic medium may be due to an increased rate of filtration and storage of a larger volume of urine. However, under similar conditions a high rate of micturition has been observed (Norfolk, 1978). Since the bladder is thought to be almost completely emptied when voided (Talbot, 1980), a decrease of ECF volume as a result of bladder swelling would be short term. Osmotic movements of water between gut lumen and haemolymph may cause volume changes as a result of swelling or shrinking of the gut. Terrestrial species of crab have been reported to use gut fluid as a store of water during desiccation, suggesting that water can move between gut and haemolymph (Mantel, 1979). We assumed that the bulk of the volume change observed in *Carcinus* was due mainly to osmotically driven water movements between the ECF and intracellular compartments.

In many species, increases in tissue water content have been observed after acclimation to hyposmotic media (Shaw, 1958; Lang & Gainer, 1969; Gilles, 1977). The time courses of the muscle tissue water content changes in *Eriocheir sinensis* (Gilles, 1977) transferred to hypo- and hyperosmotic conditions showed large differences. In the former, the water content increased and was restored within 24 h, whereas in the latter a longer period was required (15 days) before the tissue regained lost water. A similar difference in response was found here for changes in ECF volume in *Carcinus*, although the rates of these changes, assuming they matched those of tissue water content, were slower than those of *Eriocheir*, particularly in hyperosmotic conditions.

It appears that in vivo the cellular volume regulatory mechanisms in *Carcinus* operate more slowly than expected from our knowledge of the osmotic responses of isolated muscle fibres exposed abruptly to hyposmotic media (Lang & Gainer, 1969; Kevers, Pequeux & Gilles, 1979). In vitro, there is a rapid initial limitation of cellular swelling (the release of K is implicated here) followed by almost complete volume regulation within 2 h. If similar rates of cellular volume regulation occurred in vivo, changes in ECF volume would clearly be minimal. Possibly, different tissues show different rates of volume regulation, and overall, as in vivo, the response rate is slower.

Marked changes in the concentrations of haemolymph proteins have been reported in euryhaline crabs (Gilles, 1977; Boone & Schoffeniels, 1979). However, in both hyposmotic and hyperosmotic conditions these changes do not appear to be related to ECF volume changes, as measured by tissue water content. The ECF volume changes reported here would result in changes in protein concentration, if there was no change in the quantity of protein in the ECF.

Spaargaren (1979) and Lockwood & Inman (1973) have suggested that a large ECF
volume is of adaptive significance to intertidal and estuarine species in that, for a particular rate of loss of solute when exposed to a dilute salinity, a species with a large ECF would show relatively smaller changes in ECF solute concentrations than one with a small ECF volume. Thus the tissues would be buffered against external salinity changes to a greater extent in the former species. A large ECF volume would also allow tissue swelling to occur without seriously affecting circulatory function. This would obviate the need for precise regulation of cell volume. However, a survey of crustaceans did not demonstrate significantly larger ECF volumes in estuarine species compared with marine species.

We have shown here that ECF volume changes do occur when animals are exposed to abrupt increases and decreases of salinity. A decrease in ECF volume may lead to a reduction of haemolymph flow where there are fine channels or spaces. For example, the ECF of isolated axons of Callinectes has been shown to become significantly reduced in hyposmotic media (Gerard, Dandrifosse & Gilles, 1974). The possibility that this reduction might be a significant factor in lethal hyposmotic stress has been discussed by Lockwood (1976). Furthermore, restricted circulatory flow to a particular organ could cause local tissue hypoxia. In isolated mammalian tissues, hypoxia is known to cause swelling (Macknight & Leaf, 1977), so that in hyposmotic stress, as a consequence of a reduced ECF volume, hypoxia could reinforce the primary effects of reduced extracellular osmotic pressure.

A more direct action of reduced extracellular osmolality has been shown by Pichon & Treherne (1976). They found that irreversible damage to the spike-generating mechanisms of isolated axon bundles from the stenohaline crab, Maia squinado, occurred after exposure to hyposmotic media. Although in vivo in Carcinus the decrease in haemolymph osmolality during acclimation to hyposmotic medium was not abrupt, a decrease below a critical extracellular concentration may cause similar lethal damage.

REFERENCES


Fluid volume and salinity in *C. maenas*


