OSMOREGULATION IN *CLIBANARIUS PADAVENTIS* DE MAN, UNDER HETEROSMOTIC CONDITIONS

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Many terrestrial and semi-terrestrial crabs are known to osmoregulate (Prosser *et al*., 1950; Krogh, 1939; Beadle, 1957). But very little is known of the regulation in the Paguridea. Gross (1955, 1957) found that *Birgus latro*, the robber crab, is a strong regulator in both concentrated and dilute sea-water. The common hermit crab of Madras, *Clibanarius padavensis*, which lives in the Adyar backwaters, is capable of wide salinity tolerance. In the estuarine waters where they live, the salinity ranges from 16% to 30·4% (Panikkar and Aiyar, 1937). *C. padavensis* is highly resistant, available in large numbers and easily kept alive under laboratory conditions. It was therefore felt that a study of the osmotic behaviour of *C. padavensis*, subjected to different salinities, would be of interest.

**MATERIAL AND METHODS**

Specimens of *C. padavensis* were collected from the Adyar estuary, Madras, and transferred to a large glass trough filled with full-strength sea-water to a depth of 1". 32‰ sea-water was taken as normal and diluted to 75, 50, 25, 15, 10 and 5‰ of its concentration with distilled water. The crabs were acclimatised to these concentrations in troughs containing 300 c.c. of the dilute sea-water which was changed every day. The crabs were fed once a week on fish fry and bits of prawn. Any mortality in each of the dilutions was noted.

The osmotic pressure of the body fluid was determined by the comparative melting point method of Jones (1941) and Gross (1954) as modified by Freeman and Rigler (1957). The procedure involves the comparison of the melting time of unknown samples of body fluid with that of a series of NaCl standard solutions. The body fluid was drawn into a capillary tube, with an inner diameter of 0·1 mm. by making a puncture on the arthrodial membrane at the base of the walking leg, after the animal without the shell
had been thoroughly dried between folds of filter-paper. Both ends of the tube were immediately sealed with sealing wax.

Similarly, solutions of NaCl whose strengths ranged from 0·05% to 3% were sealed in capillary tubes. The lengths of the body fluid and NaCl columns were kept constant. The samples were frozen with solid CO₂. Each of the samples was then allowed to melt in a pyrex vessel containing brine kept at a constant temperature of 2°C. with the help of a cooling unit. The brine was stirred with a hand stirrer. The capillary tube was held in a vertical position in order to view it better and a hand lens was used to observe the frozen sample column. The time taken for the last crystal to disappear was noted. The entire experiment was carried out in a cold room (15°C.) so that the rate of warming of the brine bath was slowed down to 5 minutes to every degree rise of temperature. The osmotic concentration of the body fluid is expressed in terms of % NaCl (Table I) and is calculated from the curve relating melting time to the strength of the standard NaCl solutions (Freeman and Rigler, 1957).

**Table I**

*Osmotic concentration of the body fluid in different dilutions of sea-water*

<table>
<thead>
<tr>
<th>Medium (% sea-water)</th>
<th>Ext. medium</th>
<th>Body fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>3</td>
<td>2·84</td>
</tr>
<tr>
<td>50</td>
<td>2·13</td>
<td>2·13</td>
</tr>
<tr>
<td>25</td>
<td>1·39</td>
<td>2·56</td>
</tr>
<tr>
<td>10</td>
<td>0·38</td>
<td>2·20</td>
</tr>
<tr>
<td>5</td>
<td>0·36</td>
<td>1·72</td>
</tr>
<tr>
<td>Tap water</td>
<td>0·30</td>
<td>2·61</td>
</tr>
</tbody>
</table>

The rate of loss or gain of salts by the crab was estimated by measuring the electrical conductivity of the external medium, using a Mullard Conductivity Bridge and a dip-type cell.

The chloride content of the body fluid and external medium was estimated in an attempt to see if there was any regulation of the body fluid chlorides in *C. padavensis*, acclimatised to sea-water and then transferred to distilled
water. Chloride titrations were performed by the method described by Wigglesworth (1937), as modified by Sanderson (1952). A Micrometer Syringe which gives an accuracy of ± 0.00005 ml. for volumes as small as 0.01 ml. was used. 0.05 ml. of body fluid was used for each titration.

RESULTS

*C. padavensis* thrived best when only half immersed in the medium. The animals were capable of living in salinities between 1.2% and 34.6%. Results of tolerance experiments show that survival is maximum in 75% to 25% sea-water (Fig. 1). By very gradual acclimatisation extending over a period of 2-3 months, it was possible to keep them alive in very low salinities of sea-water and in tap water for an indefinite period.

![Graph showing survival of C. padavensis in sea-water dilutions](image)

**Fig. 1. % Survival of C. padavensis in sea-water dilutions.**

- % Survival on direct transfer to lower dilutions.
- % Survival on slow acclimatisation to lower dilutions.

The osmotic pressure of the body fluid shows a significant difference from that of the external medium within very low salinities (Table I). In sea-water of 32.6% salinity and equivalent to 3% NaCl, the body fluid is kept slightly hypotonic to the medium, being equivalent to 2.84% NaCl. In 50% sea-water, however, the body fluid is isotonic with the external medium. In 25% sea-water and further dilutions, there is a tendency for the body fluid to remain in a hypertonic condition. The body fluid is at a maximum concentration of 2.56% to 2.61% NaCl, in 25% sea-water and in tap water. Figure 2
Fig. 2. Relation between osmotic pressures of internal medium of *C. padavensis* and the external medium, expressed in % NaCl. • Mean of 10 values.

shows the relation between the osmotic pressure of the internal and external media expressed in % NaCl.

The chloride level of the body fluid of the crab in sea-water and distilled water is kept more or less constant (Table II), in spite of the change in salt

**Table II**

*Chloride level of *C. padavensis* in sea-water and distilled water*

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of trials</th>
<th>Mean chloride (ml.)</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea-water</td>
<td>5</td>
<td>0.0371</td>
<td>± 0.000017</td>
</tr>
<tr>
<td>Body fluid in sea-water</td>
<td>5</td>
<td>0.0263</td>
<td>± 0.0017</td>
</tr>
<tr>
<td>Body fluid in distilled water</td>
<td>17</td>
<td>0.0224</td>
<td>± 0.0000116</td>
</tr>
</tbody>
</table>
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concentration of the medium. Therefore, the slightly hypotonic condition in sea-water becomes hypertonic in distilled water. Conductivity experiments show that animals which have been previously depleted of salts by treatment with ion-free distilled water for 19 hours, absorb salts from the external medium against a gradient, when transferred to sea-water.

DISCUSSION

It is evident that an active regulation is present in C. padavensis, by which means the animal maintains its body fluid hypertonic to the medium in 25% sea-water and further dilutions. In sea-water, the body fluid is kept hypotonic to the medium. Since the chloride level of the animal is maintained more or less constant both in sea-water and distilled water, it is probable that the osmotic concentration of the body fluid is adjusted by an active chloride regulation. Results of conductivity experiments, of a preliminary nature, support this view.

Birgus latro, the coconut or robber crab, controls its body fluid concentration by selecting water of appropriate salinity (Gross, 1955). Pachygrapsus crassipes, on the other hand, has been shown to be capable of absorbing water against a gradient in order to maintain the body fluid hypotonic to sea-water. Panikkar (1941) found that Leander serratus, L. squilla and Palæmonetes varians, the brackish-water prawn, maintain a blood hypotonic to sea-water and hypertonic to dilute sea-water. Panikkar and Viswanathan (1948) found the same ability shown by Metapenæus monoceros. To a lesser extent, M. dobsoni, Penæus indicus, P. carinatus and Eriocheir sinensis also maintain their internal concentrations hypotonic in sea-water and hypertonic in dilute sea-water (Panikkar, 1951). The behaviour of C. padavensis is also similar.

The present investigations have shown that C. padavensis is a hyper-osmoregulator in dilute sea-water, but where and how this mechanism works is not yet known. Gross (1957) has found that a correlation exists between regulatory ability and exoskeleton permeability in certain Decapod Crustaceans. Cambarus clarkii and Pachygrapsus which are good regulators show the lowest permeability value. Differential permeability of the body surface may offer an explanation for the hyper-regulatory condition in C. padavensis also.

It is also probable that the antenary glands play a part in salt regulation in G. padavensis. Prosser et al. (1955) claim that the green glands in Pachygrapsus are important ion regulators, though Jones (1941), and Gross (1957) have found that in Pachygrapsus the green glands are not osmoregulatory organs. Since the chloride level is more or less maintained even in dilute
sea-water, it is possible that there is an active retention of chlorides in the body by the animal. As pointed out by Panikkar (1951) "this ability to regulate is probably the most elaborate osmotic mechanism ever perfected by invertebrates". Nothing of the mechanism of osmoregulation is known yet in *C. padavensis*. It is hoped to deal with this mechanism of regulation in subsequent studies.

**Summary**

1. *C. padavensis* can tolerate wide salinity variations of 1.2% to 34%.
2. Survival rate is maximum in 75% to 25% sea-water, in *C. padavensis*.
3. *C. padavensis* maintains its body fluid hypotonic in sea-water and hypertonic in 25% sea-water and further dilutions. Isotonicity between the external medium and body fluid is established in 50% sea-water.
4. The chloride level of the body fluid is maintained more or less constant both in sea-water and distilled water.
5. The actual mechanism of regulation is not yet known, but it is probably by an active retention of chlorides in the body by the animal, coupled with differential permeability of the body surface.

**Acknowledgement**

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**References**

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