THE INFLUENCE OF EYESTALK ABLATION ON THE REGULATION OF HAEMOLYMPH SODIUM CONCENTRATION IN THE PRAWN *PENAEUS INDICUS* H. MILNE EDWARDS

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ABSTRACT

Removal of the eyestalks of the prawn *Penaeus indicus* resulted in an increase in the haemolymph-sodium level. This increase was more evident in bilaterally ablated animals. Injection of eyestalk extract into destalked animals helped them to restore the level almost similar to that of normal animals.

INTRODUCTION

Crustaceans are charaterized by a wide range of osmoregulatory powers, which are developed based on the environment to which the animals are adapted. The capacity to regulate the ions aids the prawns in the successful completion of their life history in the wide-differing aqua-regions, viz., the sea and the estuary. The ionic ability seems to be a purely adaptive feature that may change markedly during development according to environmental needs (Dall 1981).

The involvement of endocrine factors in hydromineral regulation in decapod crustaceans have been demonstrated experimentally from early works of Scudamore (1947) to more recent works of Charmantier et al (1981). Early workers like Carlisle (1955) on *Carcinus* sp. and Bliss et al (1966) on *Gecarcinus lateralis* succeeded in establishing the possible role of endocrine factors in water uptake during molting process. Kamemoto et al (1966) and Tullis and Kamemoto (1971), working on the crabs, *Metapograpsus messor* and *Thalamita crenata,* respectively, have concentrated their efforts on the control of salt and water balance by the brain and thoracic ganglion. Schreiner et al (1969) recorded the importance of the abdominal ganglion in water balance in *Homarus* sp. Information is scanty on the neuroendocrine factors controlling specific ions in the haemolymph of crustaceans. The sodium regulation in an eyestalkless crab, *Uca pugilator,* was studied by Heit and Fingerman (1975).

In the present work the possible role of the eyestalk in regulating the important anion such as sodium was investigated. The Indian white prawn, *Penaeus indicus,* was chosen as the experimental animal due to its good osmoregulatory capacity both in estuarine and marine environment.

MATERIAL AND METHODS

The adult *Penaeus indicus* for the present study were collected from traditional impoundments adjacent to Cochin backwaters. The animals in the intermolt stage and of uniform size having a length of about 100 $\pm$ 10 mm were selected. Sea water used was adjusted to a salinity of 25 $\pm$ 1 ppt., filtered and kept aerated. The animals were acclimatized to the above salinity for a period of 48 h to maintain an isosmotic state before starting the experiment. When being acclimatized, they were not fed, 'save for cannibalism'. The individual animals were maintained in circular plastic tube of 10-l capacity.

The experiments were designed mainly on the eyestalk removal technique.. Four batches of twenty animals each were maintained. Of this twenty, ten were experimental and ten control with intact eyes. In the first batch of animals one eyestalk was removed. Simultaneously, in the second batch, both eyestalks were ablated. In the third and fourth batch also both eyestalks were removed and they were injected with eyestalk extract and distilled water respectively. After initiation of experiment, the first haemolymph sample was collected immediately. The next sample was after 1 h. and then subsequently after 2nd, 4th, 6th, 8th, 12th, 16th, 20th and 24th h from animals of all batches. Haemolymph was collected using a 1-ml tuberculin syringe equipped with a 22-guage needle; from the suprabranchial and also from the cardiac region. The experiments were repeated four times. Analysis of sodium was carried out immediately to avoid the use of anticoagulant. Eyestalk extract for injection was prepared in the ratio of 2 eyestalks|0.2 ml of distilled water and each ablated animal was injected 0.2 ml on the first abdominal segment.

Sodium concentration was determined using Elico digital flame photometer model No. CL 22D. Students t-test was used to analyse the significance of the variation of sodium levels from the control animals.

RESULTS

The variations of the sodium ionic level in the haemolymph were studied by comparing the levels in the experimental animals with that of control animals and its is presented in Table 1.

In the unilaterally ablated animal the haemolymph showed a decreased sodium ionic level in the beginning, which increased to a high level and maintained high then onwards with slight fluctuations. The values remained high throughout the period with significant difference at the sixteenth hour (Fig. 1).

The ionic level of sodium in the haemolymph of bilaterally ablated animal was higher than that of control up to the sixteenth hour after ablation and, thereafter, the values fell, but remained high compared to control animals. Most values under this treatment were significant at $P = 0.05$ level (Fig. 2).

TABLE 1. *Sodium ion concentration in the haemolymph of* P. indicus *under different treatments.*

(in milli equivalents|litre)

Hours	Unilateral ablation		Bilateral ablation		Bilateral ablation + extract injection		Bilateral ablation + water injection	
	$\bar{X}_e$	$\bar{X}_c$	$\bar{X}_e$	$\bar{X}_c$	$\bar{X}_e$	$\bar{X}_c$	$\bar{X}_e$	$\bar{X}_c$
0	422 (33)	430 (4)	413 (24)	428 (6)*	457 (5)	431 (4)*	400 (4)	431 (4)*
1	422 (16)	426 (11)	464 (12)	430 (12)*	433 (21)	430 (10)	459 (3)	430 (10)*
2	447 (11)	435 (10)	448 (4)	437 (11)	455 (11)	435 (8)	462 (23)	436 (8)
4	432 (10)	430 (7)	468 (18)	431 (6)*	446 (13)	431 (7)	453 (11)	431 (6)
6	437 (12)	430 (5)	462 (8)	429 (2)*	442 (16)	430 (6)	440 (4)	430 (6)
8	443 (27)	433 (5)	464 (4)	428 (3)*	432 (31)	428 (8)	430 (7)	428 (8)
12	441 (17)	434 (5)	452 (5)	428 (5)*	432 (25)	433 (5)	438 (2)	432 (5)
16	439 (5)	430 (4)*	457 (5)	430 (5)*	431 (17)	421 (6)	442 (9)	420 (6)
20	436 (7)	431 (5)	442 (7)	431 (5)	430 (15)	431 (3)	439 (9)	431 (3)
24	435 (6)	430 (5)	440 (5)	430 (5)*	436 (15)	432 (5)	437 (16)	432 (4)

$\bar{X}_e$ - Mean experimental values

$\bar{X}_c$ - Mean control values

* = Difference $\bar{X}_e$ - $\bar{X}_c$ significant at P = 0.05.

Numbers in parentheses are the standard deviations.

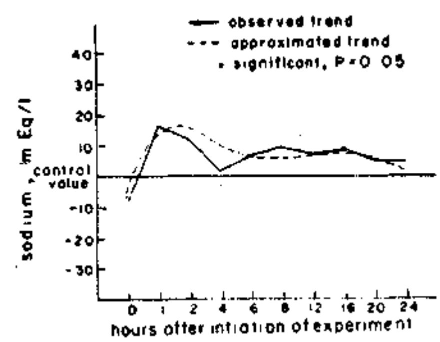

FIG. 1. Difference from the control in the variation of sodium levels in the haemolymph of an unilaterally ablated *P. indicus*.

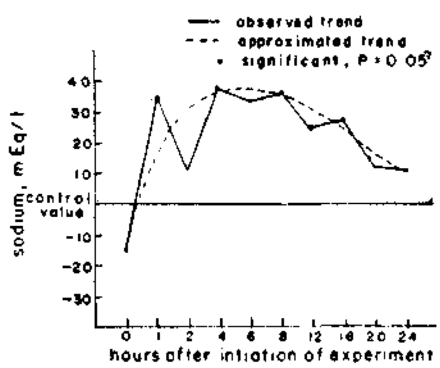

FIG. 2 Difference from the control in the variation of sodium levels in the haemolymph of a bilaterally ablated *P. indicus*.

In the bilaterally ablated, eyestalk-extract-injected animal, the difference in the sodium levels of the experimental and control animals was insignificant ($P > 0.05$), except at the initial observation. The levels in the experimental animals resembled those of control towards the terminal part of the experiment (Fig. 3).

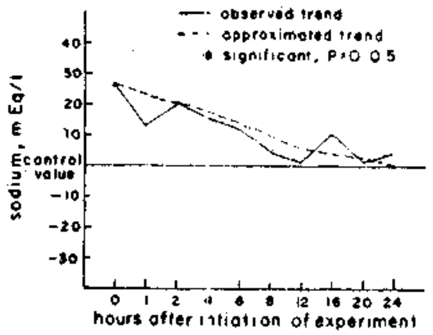

FIG. 3. Difference from the control in the variation of sodium levels in the haemolymph of a bilaterally ablated extract injected *P. indicus*.

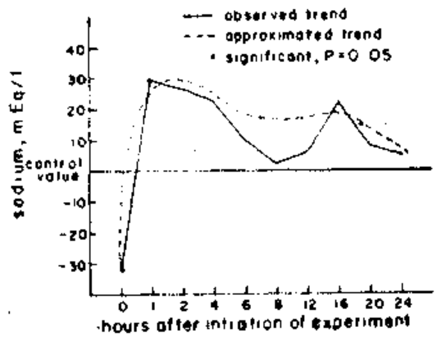

FIG. 4. Difference from the control in the variation of sodium levels in the haemolymph of a bilaterally ablated water injected *P. indicus*.

When water was injected to a bilaterally ablated animal, most of the observations were higher than the control (Fig. 4).

Discussion

The most important ions involved in the maintenance of osmotic balance are the sodium and chloride. The exchange of these ions results from passive diffusion in an isosmotic medium as has been shown in *Carcinus maenas* (Shaw 1955, 1961; Zanders 1980), *Hemigrapsus sp.* and *Pachygrapsus crassipes* (Dehnel and Carefoot 1965; Rudy 1966). Webb (1940) and Shaw (1961) have suggested that a small amount of active uptake of sodium ion is involved in its distribution between the medium and the haemolymph in crab *Carcinus maenas*.

The regulation of ions seems to be in response to a common factor like haemolymph-sodium concentration, the total ion concentration, or the osmotic pressure of the haemolymph. This process may be under a primary controller which could be hormonal (Zanders 1981). Heit and Fingerman (1975) had already established the role of the eyestalk hormone in controlling the sodium concentration of the haemolymph in *Uca pugilator*. They found that bilateral eyestalk removal from crabs in hyposmotic medium resulted in a significant drop in the haemolymph levels. When they were in an isosmotic medium the drop was not significant. Eyestalk-extract injection restored the sodium levels of eyestalk-less crabs to that of intact ones. Further, the authors have confirmed a sodium-decreasing hormone in the eyestalk and concluded that the effectiveness of the eyestalk extract in increasing the sodium level decreased as the sodium concentration of the medium increased. In the present study, it was revealed that the removal of the eyestalk resulted in an increase in the haemolymph concentration of sodium ions. An initial lowering was evident under all treatments except the extract injection. This may probably be due to the shock of ablation. In the case of unilaterally ablated prawns the haemolymph values did not show wide fluctuations compared to bilaterally ablated animals. The sodium-decreasing hormone present in one eye may be acting to prevent the imbalance caused by ablation. In the case of bilateral ablation the sodium level increased significantly from low values to figures greater than the control. The removal of a sodium-decreasing factor causes an increase in the sodium content of haemolymph. Moreover, the treatment might also aid the dominance of sodium-increasing factors, which might be synthesized in other parts of endocrine system like cerebral and thoracic ganglia of the animal. Kamemoto et al (1966) described a similar haemolymph sodium-increasing factor in the brain of *Procambarus clarkii.* In the destalked animals injected with extract, the sodium concentration which was high initially dropped gradually and behaved almost similar to that in normal animals. The sodium concentration in these animals clearly indicated that the level came down only because the extract was injected. This revealed the capacity of the eyestalk hormone in decreasing the haemolymph value. The destalked animals injected with water behaved like the bilaterally ablated animal, but, in them, the deviation of the sodium values from the control was restricted. The eyestalk removal thus results in the loss of capacity for sodium regulation.

No reliable information is available about the mechanism of eyestalk hormones in ionic regulation. The sodium controlling hormones may be acting on the body surfaces and excretory organs. At the body surfaces it would decrease the outward permeability of the epithelial cells while at the same time promoting the active uptake of sodium across the gills by stimulating a Na^{+}-K^{+} activated ATPase such as was found in the gills of the terrestrial crab, *Cardiosom quanhumi,* by Quinn and Lane (1966). Sodium retention may also be taking place in the excreatory organs.

Sodium regulation in *Penaeus indicus* assumes importance due to its life phases in the diverse salinities. Further investigation is warranted to show how the hormones deploy the ion regulating systems.

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