CHLORIDE ION REGULATION IN AN EYESTALK-ABLATED PRAWN *PENAEUS INDICUS* H. MILNE EDWARDS

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ABSTRACT

The removal of the eyestalks caused a substantial increase in the haemolymph-chloride level in batches of prawns, *Penaeus indicus*, kept in laboratory and, when the eyestalk extract was injected into them, the chloride level decreased again and reverted back to normal. The findings are deemed as a pointer to the possible role of eyestalk endocrines in ionic regulation.

INTRODUCTION

The hydro-mineral balance being an important factor in the adaptation of an organism to different salinities, the estuarine organisms on migration to lower salinities have to regulate their contents of salt and water. This process of osmoregulation has been known to be under the endocrine control. Earlier workers like Scudamore (1947), Carlisle (1955) and Bliss et al (1966) obtained evidence on the neuroendocrine influence on water regulation in the premolt stages of decapod crustaceans. Similar neuroendocrine influences had also been reported in the intermolt stages of crustaceans like *Procambarus clarkii* (Kamemoto et al 1966), *Hemigrapsus nudus* (Ramamurthi and Scheer 1967) and *Metapograpsus messor* (Kato and Kamemoto 1969).

However, only a few workers had considered the possible role of endocrine factors in controlling the levels of specific ions in the haemolymph of crustaceans. Since the ionic regulation is an index of the osmoregulatory abilities of the crustaceans, the neuroendocrine control of specific ions is a better approach to the study of the mechanism of osmoregulation in these animals. The chloride ion has a vital role in the maintenance of the ionic balance in crustaceans. The role of hormones in regulating the chloride level has been demonstrated in some freshwater crustaceans, viz., *Procambarus clarkii* (Kamemoto et al 1966, Kamemoto and Ono 1969), *Macrobrachium rosenbergii* (Kamemoto and Tullis 1972), *Potamon dehaani* and *Sesarma hematacheir* (Kamemoto 1976), *Caridina weberi* (Nagabhushanam and Jyoti 1977) and in *Barytelphusa guerini* (Venkatachari et al 1979). Similar studies have also been carried out by Kamemoto and Tullis (1972) on some marine forms, like *Spirontocaris marmoratus*, *Thalamita crenata* and *Metaprograpsus messor*.

The prawn *Penaeus indicus* is a good osmoregulator both in sea and in estuarine waters. An attempt was therefore made to study the effect of the eyestalk ablation on chloride changes in the haemolymph, in order to assess the possible hormonal control.

Material and Methods

Adults of *P. indicus* were collected from a few traditional impoundments adjacent to Cochin backwaters. Uniform-sized animals in the intermolt stages, having a length of 100 ± 10 mm, were selected. Sea water used was adjusted to a salinity of 25 ± 1 ppt., filtered and kept aerated. The animals were acclimatised to the above salinity for a period of 48 h, to maintain an isosmotic state before the start of the experiment. When being acclimatised they were not fed. The individual animals were maintained in circular plastic tubs of ten-litre capacity.

The experiments were designed mainly on the eyestalk-removal by cauterization technique. Four batches of twenty animals each were maintained. Of the twenty, ten were experimental animals and ten were controls with intact eyes. In each of the first batch of animals, one eyestalk was removed. Simultaneously, in the second batch, bilateral ablation was performed. In the third and fourth batches also both the eyes were removed, but the third batch were injected with eyestalk extract and the fourth with only distilled water.

After initiation of the experiment the first haemolymph sample was collected immediately. The next sample was collected after one hour and, subsequently, after 2nd, 4th, 6th, 8th, 12th, 16th, 20th and 24th hours, from animals of all the batches. Haemolymph was collected using a 1-ml tuberculin syringe equipped with a 22 guage needle from the suprabranchial as well as from the cardiac region. For injection experiments, fresh extract of eyestalk, of the same species, was obtained by homogenizing the eyestalk in glass distilled water and then centrifuging. A supernatent solution having a ratio of 2 eyestalks|0.2 ml of distilled water was injected (0.2 ml) into the ablated animal through the first abdominal segment. All the experiments were repeated four times, to obtain mean values.

Chloride concentration in the haemolyph was determined colorimetrically by the method of Schoenfeld and Lewellen (1964). Students *t*-test was applied to test the significance of the hourly variation of the chloride content.

Results

The variation of the chloride level in the haemolymph of the ablated animals was studied by comparing with the level in the control animals, and the results are presented in the Table 1. These observed differences in level show certain approximated patterns of variation, which are illustrated in graphical forms.

TABLE 1. *Chloride ion concentrations under different treatments.* (in milliequivalents/litre)

Hours	Unilateral ablation $\bar{X}_e$	Unilateral ablation $\bar{X}_c$	Bilateral ablation $\bar{X}_e$	Bilateral ablation $\bar{X}_c$	Bilateral ablation + Extract injection $\bar{X}_e$	Bilateral ablation + Extract injection $\bar{X}_c$	Bilateral ablation + Water injection $\bar{X}_e$	Bilateral ablation + Water injection $\bar{X}_c$
0	316 (15)	308 (6)	345 (13)	308 (7)*	332 (20)	306 (5)	331 (21)	308 (5)
1	346 (24)	313 (12)*	345 (25)	307 (11)*	354 (21)	310 (10)	333 (13)	310 (10)
2	359 (12)	316 (10)*	321 (25)	309 (12)	357 (4)	313 (11)*	363 (6)	313 (11)*
4	325 (10)	311 (8)	315 (9)	312 (9)	334 (7)	308 (7)*	316 (3)	308 (7)
6	315 (12)	307 (6)	336 (15)	315 (7)	331 (22)	307 (3)	335 (36)	307 (3)
8	325 (21)	306 (4)	325 (8)	318 (3)	317 (4)	309 (4)	331 (20)	309 (4)
12	321 (11)	309 (2)*	357 (11)	318 (4)*	324 (2)	310 (4)	351 (6)	310 (4)*
16	344 (10)	312 (4)*	369 (17)	312 (7)*	321 (10)	311 (2)	337 (4)	311 (2)*
20	331 (14)	311 (4)*	325 (10)	313 (3)	309 (0)	313 (1)*	336 (9)	313 (1)*
24	313 (13)	314 (4)	322 (13)	316 (2)	302 (11)	313 (2)	320 (9)	313 (2)

$\bar{X}_c$ = mean experimental value

$\bar{X}_c$ = mean control value

Numbers in parentheses are the standard deviations.

* Indicates difference of $\bar{X}_e$ and $\bar{X}_c$ significant at P = 0.05.

When the animals were unilaterally ablated, the chloride level increased gradually and reached significantly ($P < 0.05$) high value by the end of the second hour, and then on remained high, as compared to the values in the control animals, which were reckoned as the base for comparison (Fig. 1). A steep increase in the level was observed initially when both the eyese were ablated, and the level remained high in the following hours with slight fluctuations, reaching the maximum at the sixteenth hour after ablation. At the end of the experiment, during the twenty-fourth hour, the level fell to almost the normal value (Fig. 2).

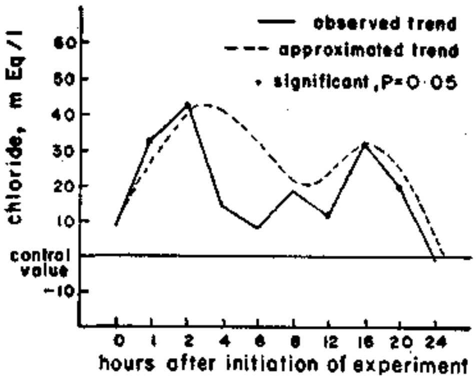

FIG. 1. Difference from the control in the variation of chloride levels in the haemolymph of an unilaterally ablated *P. indicus.*

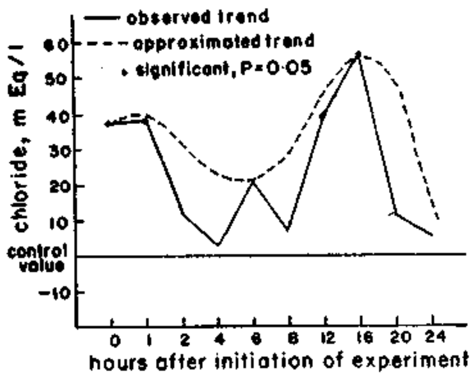

FIG. 2. Difference from the control in the variation of chloride levels in the haemolymph of a bilaterally ablated *P. indicus.*

In the extract-injected animals, as was in the case of the ablated animals discussed above, an initial increase in the chloride content was evident, but, afterwards, there was a gradual decline in the value, and, at the end of the experiment (24th hour), the values descended ($P < 0.05$) below the control level (Fig. 3).

The variations in the chloride levels of the bilaterally ablated and water-injected animals were almost similar to that of the bilaterally ablated animals. The values were higher than that of the control almost throughout the experiment. The effect of the treatment seemed, however, to peter out by about the end of twenty-fourth hour (Fig. 4).

DISCUSSION

Chloride ion is an osmotically important solute in the haemolymph of crustaceans. It has been proved earlier that the ionic movements are under a primary controller—hormonal action. Also, that the crustacean eyestalk possesses a haemolymph-chloride-regulating factor has been confirmed in a number of freshwater crustaceans. The removal of eyestalks resulted in a decrease in the chloride level in *Procambarus clarkii* (Kamemoto et al 1966). It was also reported that the brain and thoracic ganglion of this animal have a haemolymph-chloride-increasing factor. A similar substance was also described in other freshwater crustaceans like *Potamon dehaani, Sesarma hematocheir* and

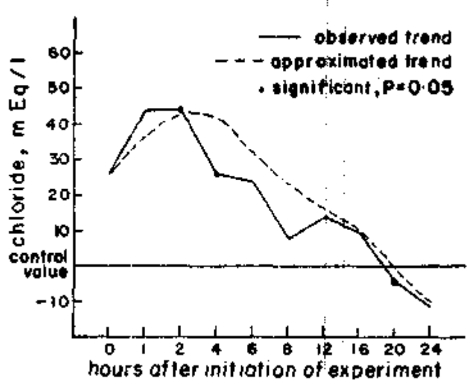

FIG. 3. Difference from the control in the variation of chloride levels in the haemolymph of a bilaterally ablated, extract injected *P. indicus*.

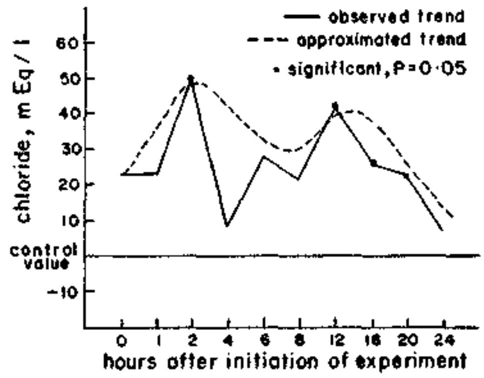

FIG. 4. Difference from the con- levels in the haemolymph of trol in the variation of chloride a bilaterally ablated water injected *P. indicus*.

Macrobrachium rosenbergii (Kamemoto and Tullis, 1972). Nagabhushanam and Jyoti (1977) concluded that the brain homogenates and thoracic-ganglion extracts could increase the haemolymph-chloride concentration in the freshwater prawn *Caridina weberi*. A haemolymph-chloride-elevating factor has also been described by Venkatachari et al (1979) in *B. guerini* in which the removal of this factor through eyestalk ablation had resulted in a drop in the haemolymph chloride and the replenishment of the factor through extract injection had elevated it to normal level.

The ionic regulation in the marine crustaceans under hormonal influence, however, revealed a picture different from that of the freshwater forms. Kamemoto and Tullis (1972) have observed the existence of a haemolymph-chloride-decreasing factor in the eyestalks of marine crustaceans such as *Spirontocaris marmoratus, Metapograpsus messor* and *Thalamita crenata*. In the present experiments, the chloride levels increased to high values after both unilateral and bilateral ablations. These high chloride values can be attributed to the removal of a proable chloride-decreasing factor that was present in the eyestalk, identical to that described by Kamemoto and Tullis (1972). In the unilaterally ablated animal, the chloride-decreasing factor still present in the other eye might be acting to prevent the imbalance caused by a total ablation. The chloride level approached that of the control by the twenty-fourth hour, seemingly because it took one day for the animal to recoupe. The higher values in the bilaterally ablated animals might be due to the total removal of the chloride-decreasing factor, which would let the action of the chloride-increasing factor from the brain and thoracic ganglion unchecked, as observed by Nagabhushanam and Jyoti. In the eyestalk-extract-injected, ablated *Penaeus indicus*, on the otherhand, the chloride level steadily decreased from the initial high values. This influence of the extract in lowering the level even beyond the base line (Fig. 3) shows the presence of a chloride-decreasing factor in the eyestalks. The water-injected animal also behaved similarly as the bilaterally ablated animal, supporting the above findings.

In the freshwater crustaceans, the chloride-regulating hormone of the eyestalk might increase the chloride uptake from the medium, or may influence a reabsorption of ions in the excretory organ, or may even mobilize ions from other tissues (Venkatachari et al 1979). In the euryhaline crustacean presently studied, the action of hormones may be converse to that mentioned above. The role of neuroendocrine structures in ionic balance and the mechanisms working behind needs further elucidation.

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