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Neuroendocrine Control of Water Content and Calcium Concentration in the Crab *Ocypode macrocera* (H. Milne-Edwards 1852) (Brachyura, Ocypodae)

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Abstract: The present study is focused to see the effect of crustacean neuroendocrine organs on the water and calcium metabolism which is very much important for the osmoregulatory functions. Since the experiments were carried out to investigate the control of water contents and calcium concentration in the crab, *Ocypode macrocera*. The animals were collected from the shore of the Bay of the Bengal near Annan Koil one among the biggest landing centers of south east coast of Tamil Nadu, India. The data revealed that water content in the hepatopancreas and thoracic muscle of the control crab were 70.16 and 79.86%, respectively, whereas in the experimental ones, the values were 80.32 and 87.44% after eyestalk removal and 54.52 and 66.98% after eyestalk extract injection. Calcium concentration in both the hepatopancreas and thoracic muscle of the control crab were 2.16 and 2.14 mg g^{-1}, respectively, whereas in the experimental animals the values were 2.76 and 3.52 mg g^{-1} in the eyestalkless crabs and 1.52 and 1.57 mg g^{-1} after eyestalk extract injection, respectively. Hence it was observed the % of water content is more in eyestalk less crabs as compared to that of control and injected. The roles of neurosecretory secretions, which control these parameters, were discussed. The ability for *Ocypode macrocera* to adapt rapidly and maintain homeostasis in a wide range of abnormality supports the fact that *Ocypode macrocera* are a suitable species for land-based aquaculture in ponds as well as critical condition where rapid fluctuation in salinity can occur.

Key words: Neuroendocrine control, calcium metabolism, water content %, eyestalk ablation, eyestalk injection, hormonal regulation

INTRODUCTION

The mechanism of osmotic and ionic regulation has received much attention since a long time ago. The neuroendocrine control of salt and water has been studied in various species of crabs (Baumberger and Olmsted, 1928; Abramowitz and Abramowitz, 1940; Scudamore, 1942, Scudamore, 1947; Carlisle, 1955a, 1957; Knowles and Carlisle, 1956; Bliss *et al*., 1966; Kamemoto *et al*., 1966; Kato and Kamemoto, 1969; Heit and Fingerman, 1957; Nagabhushanam and Jyoti, 1977; Girard and Maissiat, 1984; Charmantier *et al*., 1984; Souza and Moreira, 1987). Bauchau (1948) found that the increased growth rate of Eriocheir sinensis could be recognized to a larger volume increase at each moult in the eyestalkless animal, following upon a greater intake of water, than occurs in normal animals, even when the animals are starved, it increase linearly at each moult about three times as much as unoperated or eye stalked controls. A normal *Carcinus lateralis* increases in volume by about 80% at each moult; the increase is nearly 180% in eyestalkless animals due entirely to greater water content. When Sinus gland extracts was injected into eyestalkless animals it counteract the abnormal water uptake and when injected into normal *Carcinus lateralis*, less water than usual is taken up at the moult (Carlisle, 1957). The crayfish *Procambarus clarkii* was found to be able to regulate the body water through the continuous release of an eyestalk neurosecretion (Averett, 1970; Kamemoto and Tullis, 1972). Calcium deposition and metabolism has frequently been suggested to be under some form of direct hormonal control (Kyer, 1942; Scudamore, 1942; Carlisle, 1955b). After eyestalk removal of *Crangon vulgaris*, the exuvium has less calcium than the exuvium of an intact individual (Koller, 1930; Guyselman, 1950, 1953). Removal of sinus glands from *Hemigrapsus nudus* leads to a normal increase in hepatopancreas ash and calcium levels

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(Kincaid and Scheer, 1952). It is apparent therefore that some factors in the eyestalks are involved in the regulation of water and calcium metabolism. The grapsid crab, *Ocypode macrocera*, is a semi-terrestrial species and has not been subjected to such experiments. It is thought to be important to investigate the mechanism controlling the water content and calcium concentration of this crab.

MATERIALS AND METHODS

Collection and acclimation of crabs: Adult individuals of the crab, *Ocypode macrocera*, were collected from the shore of the Bay of the Bengal near Annan Koil, South east coast of Tamil Nadu, India (Lat 11°29'N; 79°46'E). The crabs were brought to nearby laboratory by means of large buckets along with some moistened sand. The crabs were maintained in the laboratory provided a large and natural illuminated room by following the method of Ahmed and Sukar (1987). The animals were transferred to large troughs of area 2 m^3 with moistened sand with saline water and were fed with fish meat, bivalve and dull crabs during the period of acclimation. Everyday sand was being moistened with saline water. The animals were left undisturbed during the acclimation period except at the time of food.

Eyestalk extract injection: The crabs were then divided into three groups of 20 each. The crabs of the first group were used as a control and of the second group the eyestalks were removed, while the third group was injected by eyestalk extract as described by Simpkins (1973).

Tissue collection and analysis: All the animals were dissected and the tissues of the hepatopancreas and the thoracic muscle were isolated and weighed. The percentage of water was determined by drying the tissues in an oven at 105°C till constant weight of tissues were obtained. Calcium was determined by the precipitation method (Trinder, 1964). Since the mortality rate was high beyond the tenth day, the estimations were confined to the eighth day of the experiment.

Statistical analysis: All quantitative measurements were expressed as Means±SD for control and experimental animals. The data were analyzed using one way Analysis of Variance (ANOVA) and the results were considered statistically significant if the p value is less than or equal to 0.05.

RESULTS AND DISCUSSION

The water content of the liver in the control group ranged from 70.16-69.5% throughout the experiment (Table 1), whereas after eyestalk removal, the values increased significantly (p<0.01) from the first 24 h until the 8th day of the experiment. Similarly the water content of the muscle in the control group ranged from 79.86- 81.70% throughout the experiment, but after eyestalk removal the value increased significantly (p<0.01) from the first 24 h until the 8th day of the experiment when the value reached 95%. On the other hand, the water content in the injected crabs was reduced significantly (p<0.01) in the hepatopancreas starting from the first 24 h until the 8th day of the experiment. Water content of the muscle showed the same trend. The increase in the body size as a result of molting has been observed by Huxley (1879). The total increase in the volume is a result of the absorption of water through the integument and hills (Baumberger and Olmsted, 1928; Drach, 1939; Needham, 1946). The swelling consequent upon this absorption of water may play a large part in opening up the old shell along the lines of dehiscence and hence be of importance in the actual mechanism of the molt process (Skinner, 1985). The removal of both eyestalks and both sinus glands causes a considerable shortening of the normal intermoult period and thus induces preparation for the molt (Smith, 1940; Abramowitz and Abramowitz, 1940; Scudamore, 1947; Edwards, 1950; Bliss, 1953a; Nakatani and Otsu, 1979; McConaugha and Costlow, 1987). Consequently the increase in the water content of the tissues is expected after eyestalk removal as a result of increased frequency of the molting process. Water uptake recommences immediately after exuviations, probably lasting through stage A. The water absorbed appears not only in the hemolymph but also in the body tissues,

Table 1: Water content and Ca concentration in control, eyestalkless crabs and injected crabs tissues

		Liver					Muscle				
Parameters	Time (day)	Control	Stalkless crab	p-value	Injected crab	p-value	Control	Stalkless crab	p-value	Injected crab	p-value
Water content (%)	1st	70.16±5.8	80.32±4.1	<0.01	54.52±4.6	<0.01	79.86±1.8	87.44±4.8	<0.01	66.98±4.5	<0.01
	4th	70.9±5.9	82.3±3.7	<0.01	50.8±2.4	<0.001	80.9±2.2	92.6±2.7	<0.001	64.8±3.7	<0.001
	8th	69.5±5.0	92.1±2.6	<0.001	45.5±4.3	<0.001	81.7±2.1	96.4±1.6	<0.001	61.9±2.8	<0.001
Ca conc.	1st	2.16±0.7	2.76±0.5	<0.1	1.52±0.4	<0.01	2.2±0.5	3.52±0.4	<0.01	1.57±0.5	<0.01
(mg g^{-1} wet weight)	4th	1.8±0.2	3.06±0.3	<0.001	1.11±0.2	<0.001	2.66±0.5	3.8±0.2	<0.001	1.15±0.2	<0.001
	8th	1.9±0.3	3.5±0.3	<0.001	0.96±0.1	<0.001	2.48±0.3	4.03±0.3	<0.001	1.1±0.3	<0.01

Values are Mean±SD of 5 replicates, p: Level of significance especially the hepatopancreas, integument epithelium and the muscle (Passano, 1969). Since the plasma or hemolymph ion content of a wide range of animals varies from about 300 mOsmol kg^{-1} for freshwater species to >1000 mOsmol kg^{-1} for marine species (Prosser, 1973). Renaud (1949) believed that cholesterol plays a large part in the imbibitions of water during molting. Moreover, Sinha and Mooswi (1978) found that eyestalk ablation caused significant increase in cholesterol concentration of *Ocypode macrocera*. Therefore, the endocrine control of water balance may be related to the control of lipid metabolism.

On the other hand, the injection of eyestalk extract slows down the molting process and thus prevents the greater intake of water resulting in lower content of water in the tissues (Scudamore, 1947; Passano, 1953; Guyselman, 1953). In the hepatopancreas, calcium concentration in the control group was around 2.5 mg g^{-1} wet weight throughout the experiment but after eyestalk removal the values increased significantly ($p<0.01$) from the first 24 h, until it reached 3.2 mg g^{-1} in the eighth day of the experiment. In the muscle, calcium concentration of the control group was 2.4 mg g^{-1} but after eyestalk removal the values increased significantly ($p<0.01$) from the first 24 h till the eighth day when the value reached 3.9 mg g^{-1}.

In the injected crabs, hepatopancreas calcium decreased significantly ($p<0.01$) as compared with the control from the first 24 h and reached 0.96 mg g^{-1} in the eighth day. Muscle calcium showed the same trend since it decreased significantly ($p<0.01$) from the first 24 h till it reached 1.1 mg g^{-1} on the eighth day.

The relation between calcium and moulting in Crustacea has been studied by several investigators (Numanoi, 1934, 1939; Drach, 1939; Kleinholz, 1940, 1942). Certain deposits rich in calcareous material (the gastroliths and hepatopancreas) have been suggested as internal reserves from which calcium for the new skeleton was drawn. Bliss (1953a, b) found that if the eyestalks of the land crab *Gecarcinus lateralis* are removed, this throws the animals precipitously into the proecdysis stage, where the main portion of hepatopancreas mineral reserves consists of calcium and magnesium phosphates. On the other hand, injection of eyestalk extracts as observed earlier delay the molting process and hence there is no further need for accumulation of inorganic reserves to meet the coming ecdysis. This seems to be in agreement with the finding of Numanoi (1939), who found that the gastroliths of *Sesarma haematocheir* enlarged as ecdysis neared and disappeared after the moult. These changes were correlated with periodic fluctuations in the level of blood calcium, indicating transport of calcium from the exoskeleton to the gastroliths before ecdysis and in the reverse direction after ecdysis.

The cherished relation of this store calcium indicates that both processes may be mediated by the same mechanism. Although, the connection may be more complicated than is apparent, a speculative interpretation would connect the inhibition of ecdysis with retention of calcium in the exoskeleton and absence of the hormone would indicate molting and migration of calcium to the internal depot. Transfer of calcium in the reverse direction after completion of ecdysis might be mediated either by the reappearance of the same hormone in the circulation or by some other agent (Kleinholz, 1942).

Thus it is clear that some regulatory factor is involved in the water and calcium metabolism. Hence further study is necessary to find out the exact mechanism.

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