

Neuroendocrine control of water content and calcium concentration in the crab *Sesarma bouleengeri* Calman (Brachyura, Grapsidae)

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Abstract

Experiments were carried out to investigate the control of water contents and calcium concentration in the crab, *Sesarma bouleengeri* Calman, from the Shatt al-Arab River, at Basrah, Iraq. The data revealed that water contents 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 mg/g and 2.14 mg/g, respectively, whereas in the experimental animals the values were 2.76 mg/g and 3.52 mg/g in the eyestalkless crabs, and 1.52 mg/g and 1.57 mg/g after eyestalk extract injection, respectively. The roles of neurosecretory secretions, which control these parameters, are discussed.

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 Ommsted 1928; Abramowitz and Abramowitz 1940; Scudamo 1942, 1947; Carlisle 1955, 1957; Knowles and Carlisle 1956; Bliss *et al.* 1966; Kamemota *et al.* 1966; Kato and Kamemota 1969; Heit and Fingerman 1957; Najabushanam and Jyoti 1977; Girard and Maissiat 1984; Charmantier *et al.* 1984 and Souza 1987).

Bauchau (1984) found that the increased growth rate of *Eriocheir sinensis* could be ascribed to a larger volume increase at each moult in the eyestalkless animal, consequent upon a greater intake of water, than occurs in normal animals, even when starved, increase linearly at each moult about three times as much as unoperated 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. Sinus gland extracts injected into eyestalkless animals 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; Kamemota and Tullis 1972).

Calcium deposition and metabolism has frequently been suggested to be under some form of direct hormonal control (Kyer 1942; Scudamore 1947; Carlisle 1956). 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 (Kincaid and Sheer 1952).

It is apparent therefore that some factors in the eyestalks are involved in the regulation of water and calcium metabolism. The grapsid crab, *Sesarma bouleengeri*, is a semiterrestrial 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

Adult individuals of the crab, *Sesarma bouleengeri* Calman, were collected from the Shatt al-Arab River, Basrah, Iraq (30° 30' N, 47° 56' E) during June 1987. The temperature of the air and water were 36 C and 29 C respectively. The crabs were maintained in large aquaria provided with air. The volume of water was adjusted so that the animals were just submerged. The crabs were fed with some phytoplankton and meat. The water of the aquaria was changed daily. The animals were left undisturbed for three days. The crabs were then divided into three groups of fifty. The crabs of the first group were used as a control, and of the second group the eyestalks were removed, while the third group were injected by eyestalk extract as described by Simpkins (1973).

All the animals were dissected and the tissues of the hepatopancreas and the thoracic muscle were isolated and weighed by a Mettler balance. 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.

Results and Discussion

The water content of the liver in the control group ranged from 69.5% – 70.16% throughout the experiment (Table 1), whereas after eyestalk removal, the values increased significantly ($P < 0.01$) from the first 24 hours until the 8th day of the experiment when the value reached 92%. 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 hours until

Table 1. Water content (%) and Ca-concentration (mg/g wet weight) in control, eyestalkless crabs and injected crabs. Values are mean \pm S. D. (of 5 replicates). P: level of significance.

	Time	Liver					Muscle				
		Control	Stalkless crab	P	Injected crab	P	Control	Stalkless crab	P	Injected crab	P
Water Content (%)	1 st day	70.16 \pm 5.8	80.32 \pm 4.1	< 0.01	54.52 \pm 4.6	< 0.01	79.86 \pm 1.8	87.44 \pm 4.8	< 0.01	66.98 \pm 4.5	< 0.01
	4 th day	70.9 \pm 5.9	82.3 \pm 3.7	< 0.01	50.8 \pm 2.4	< 0.001	80.9 \pm 2.2	92.6 \pm 2.7	< 0.001	64.8 \pm 3.7	< 0.001
	8 th day	69.5 \pm 5	92.1 \pm 2.6	< 0.001	45.5 \pm 4.3	< 0.001	81.7 \pm 2.1	96.4 \pm 1.6	< 0.001	61.9 \pm 2.8	< 0.001
Ca Concentration (mg/g wet weight tissue)	1 st day	2.16 \pm 0.7	2.76 \pm 0.5	< 0.1	1.52 \pm 0.4	< 0.01	2.2 \pm 0.5	3.52 \pm 0.4	< 0.01	1.57 \pm 0.5	< 0.01
	4 th day	1.8 \pm 0.2	3.06 \pm 0.3	< 0.001	1.11 \pm 0.2	< 0.001	2.66 \pm 0.5	3.8 \pm 0.2	< 0.001	1.15 \pm 0.2	< 0.001
	8 th day	1.9 \pm 0.3	3.5 \pm 0.3	< 0.001	0.96 \pm 0.1	< 0.001	2.48 \pm 0.3	4.03 \pm 0.3	< 0.001	1.1 \pm 0.3	< 0.01

the 8th day of the experiment when the value reached 96%. 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 hours 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 moulting has been observed by Reaumur (1712, 1718) and Huxley (1879). The total increase in the volume is a result of the absorption of water through the integument and gills (Baumberger and Olmsted 1928; Drach 1936, 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 moult process (Skinner 1984). The removal of both eyestalks or both sinus glands causes a considerable shortening of the normal intermoult period and thus induces preparation for the moult (Smith 1940; Abramowitz and Abramowitz 1940; Scudamore 1947; Edwards 1950; Bliss 1953; Nakatani and Otsu 1979, and McConaughy *et al.* 1987). Consequently the increase in the water content of the tissues is expected after eyestalk removal as a result of increased frequency of the moulting 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, especially the hepatopancreas, integument epithelium, and the muscle (Passano 1969).

Renaud (1949) believed that cholesterol plays a large part in the imbibition of water during moulting. Moreover, Sinha and Mooswi (1978) found that eyestalk ablation caused significant increase in cholesterol concentration of *Sesarma boulengeri*. 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 moulting process and thus prevents the greater intake of water resulting in lower content of water in the tissues (Scudamore 1947; Passano 1953; Coyselman 1953). In the hepatopancreas, calcium concentration in the control group was around 2 mg/g wet weight throughout the experiment, but after eyestalk removal the values increased significantly ($P < 0.01$) from the first 24 hours, until it reached 3.5 mg/g in the eighth day of the experiment. In the muscle, calcium concentration of the control group was 2.4 mg/g but after eyestalk removal the values increased significantly ($P < 0.01$) from the first 24 hours till the eighth day when the value reached 4.03 mg/g.

In the injected crabs, hepatopancreas calcium decreased significantly ($P < 0.01$) as compared with the control from the first 24 hours and reached 0.96 mg/g in the eighth day. Muscle calcium showed the same trend since it decreased significantly ($P < 0.01$) from the first 24 hours till it reached 1.1 mg/g on the eighth day.

The relation between calcium and moulting in Crustacea has been studied by several investigators (Numanoi 1934, 1939; Orach 1939, and Kleinholz 1940, 1941). 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 moulting 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 intimate relation of this reserve 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 moulting 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 1941).

Thus it is clear that some regulatory factor is involved in the water and calcium metabolism.

Acknowledgments

I am extremely thankful to Dr. S. D. Salman of the Marine Science Centre for reading the manuscript and for his constant help and encouragement.

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