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IONIC REGULATION IN RELATION TO THE MOULT CYCLE OF *CRANGON VULGARIS* (FABR.) (CRUSTACEA, NATANTIA) FROM BRACKISH WATER

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ABSTRACT

Investigations on the ionic regulation of the hæmolymph during the moult cycle of *Crangon vulgaris* (Fabr.) (Crustacea, Natantia) were made in a salinity of 10 ‰. Sodium showed a minimum immediately after ecdysis and a maximum in the intermoult stages, while potassium, which also is held at a level higher than the medium, showed a minimum in the intermoult stages and an increase prior to and after ecdysis. Calcium showed, contrary to species from fully marine areas, a minimum after ecdysis and then a rapid increase in calcium ion concentration and a slow decrease in the later intermoult phase and in the premoult stages. Magnesium, generally held at a very low concentration, showed an increase at ecdysis. Chloride varied in the same way as sodium. The changes in ion concentrations during the moult cycle can mostly be explained by an increased permeability and an increased water intake before the ecdysis and a swelling of the animal after ecdysis before the new exoskeleton has hardened.

INTRODUCTION

Studies of the ionic changes during the moult cycle of crustaceans have been performed on only a few species from marine environments; on *Leander serratus* (Drach, 1944), *Panulirus argus* (Travis, 1955), *Homarus americanus* (Donahue, 1953), *H. vulgaris* (Glynn, 1968) and *Carcinus maenas* (Robertson, 1960; Adelung, 1971). All these investigations were made on animals collected in fully marine areas. Similar investigations on animals from brackish waters seems to be confined to those of Lockwood & Andrews (1969) on *Gammarus duebeni* and de Leersnyder (1967) on *Eriocheir sinensis*. The earlier investigations indicate that in general the pre-moult period is characterized by a rise in the osmotic pressure of the blood, caused by an increase in the blood proteins and in the concentrations of many of the major ions. The increase in ion concentration is accompanied by a subsequent increase in the uptake of water. The means by which the fluid and ions are taken in (or out) across the body surface or gut wall are not known. Both active ion transport and the colloid osmotic pressure of the blood proteins could be responsible.

The extensive physicochemical changes which take place during the moult cycle must exert a still greater influence on brackish water animals than on pure marine animals because of the necessity for the animals to keep their blood osmotically stable. Kinne (1953) noticed for instance a lower moult frequency at extreme salinities for *Gammarus duebeni.*

Crangon vulgaris is a good osmo and ion regulator, hyper-osmotic in salinities below 23-25‰ and hypo-osmotic above this salinity (Hagerman, 1970b, 1973). Thus in the Øresund and in the Baltic *C. vulgaris* is always keeping its blood concentration above that of the external medium.

The aim of this study is to survey the changes in the major ions in the *Crangon* hæmolymph during the moult cycle, and to discuss the possibilities for the species to regulate and maintain its ionic composition during these severe and sensitive phases of their growth.

This paper forms part of a larger investigation on the ecophysiology of *Crangon vulgaris* (Hagerman, 1970a, b; 1971, 1973).

MATERIAL AND METHODS

Specimens of *Crangon vulgaris* were collected in shallow water (0-4 m) in the Øresund north of Helsingør (salinity 10-15‰). The animals were placed singly in small containers (diam. 12 cm), and left to acclimatize to the experimental salinities for at least three days. All measurements were made on animals acclimatized to 10‰, + 10°C. Days of moulting were noted, all moultings occurred at night. The moult cycle for natantians has been discussed by Passano (1960) and his terminology (modified after Drach, 1944) has been used here.

Before sampling the animals were gently dried on soft filter paper and placed on a special surgery-table. Hæmolymph was sucked directly from the heart by means of a special glass-syringe, and placed under liquid paraffin. It was possible to get 20-35 μl hæmolymph from a 40-50 mm long specimen. This volume was then transferred to subsamples by means of Drummond microcaps, 5 or 10 μl, and prepared for further analysis.

Measurements of sodium, potassium, calcium and magnesium were made on a Unicam SP 90 Serie 2 atomic absorption spectrophotometer. The hæmolymph samples were diluted 1000 × (Na^+), 100-200 × (K^+), 100 × (Ca^{2+}) and 510 × (Mg^{2+}). The results were recorded on a Goerz Servogor Re 511 recorder. Calcium was measured after addition of EDTA or lanthanumchloride.

Chloride was measured on 5 or 10 μl samples with an Aminco-Buchler-Cotlove direct reading chloridometer.

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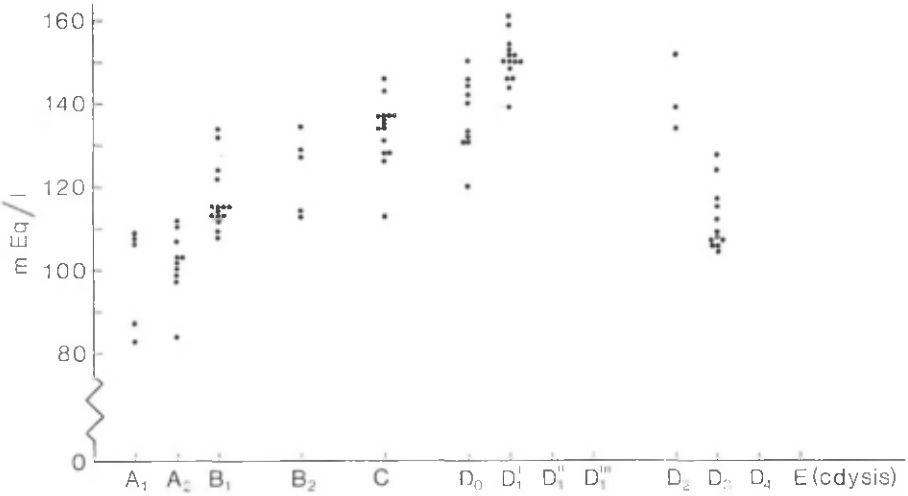

FIG. 1. Variation in sodium hæmolymph concentration during the moult cycle of *Crangon vulgaris*.

RESULTS

The regulation of sodium, the major cation, during the moulting cycle is shown in Fig. 1. After a very low concentration immediately after ecdysis, when the blood has a sodium concentration almost isotonic to the outer medium, the sodium concentration shows a steady increase in stages A_2, B_1 and B_2. The concentration lies within 120-150 mEq/l in the intermoult stages, i.e. a rather great hyperosmoticity with regard to the outer medium. In the premoult stage D_3 (and D_4) the sodium concentration is rapidly falling in the hæmolymph, no doubt due to an intake of water as a prelude to the ecdysis (provided the active uptake of Na^+ is held constant). Close to ecdysis, cracks in the old carapace might increase the surface permeability, and thus increase both water inflow and sodium outflow as a result of normal osmotic processes. No comparable investigations on the regulation of sodium during the moulting cycle exists. So far, all previous investigations have been performed in salinities above 30‰ For *Gammarus duebeni* measured in 33‰ salinity, Lockwood & Andrews (1969) found that recently moulted individuals had a sodium content some 20 % higher than inter-moult individuals. Robertson (1960) noted that the sodium content of *Carcinus maenas* from fully marine areas also increased at the time of moult, while Adelung (1971) for the same species found a constant sodium concentration throughout the entire moulting cycle.

The regulation of the major anion, chloride, is shown in Fig. 2. The regulation of this ion in the moulting cycle resembles the regulation of sodium. This is also reasonable; as chloride ion concentration is usually considered to passively follow changes in sodium ion concentration (Lockwood, 1968). Thus chloride shows a minimum immediately after ecdysis (stage A) and then a steady increase in ion concentration in stage B and a stable level in stage C-D_2 (the inter-moult stage). The decrease in the premoult stage D_{3-4} is not so evident as for sodium, but still recognizable. In the investigation of ionic regulation during the moult cycle of the fully marine *Homarus vulgaris*, Glynn (1968) found no variation for chloride.

The fluctuations in calcium hæmolymph concentration during the moult cycle are shown in Fig. 3. Calcium shows some rather astonishing features not found so far in other crustaceans. Calcium is during the whole moulting cycle held at a niveau above the outer medium, fluctuating between 8-15 mEq/l (medium = 5 mEq/l). A minimum (7-10 mEq/l) is reached immediately after ecdysis in stage A_1-B_1, followed by an increase to 12-15 mEq/l between stage B_2-C. Thereafter Ca^{2+} slowly falls up to ecdysis. The regulation of calcium in stage D is quite the opposite to what has been found for *Homarus vulgaris* (Glynn, 1968) and *Carcinus maenas* (Robertson, 1960, Adelung, 1971), but a closer examination of the functioning of Ca^{2+} in the physiology of a crustacean shows both types of regulation to be equally possible. According to Glynn (op. cit.) the hepatopancreas calcium content rises considerably in pre-ecdysis stage D and the post-moult fall in hæmolymph calcium is parallelled by a similar change in the hepatopancreas calcium. It is thus reasonable to assume that the decrease in hæmolymph calcium of *Crangon* in stage D is reflected by an increase in hepatopancreas concentration, i.e. a transfer of calcium to a better storage site. From here it could then be released immediately after ecdysis to calcifye the new cuticle. In a study of the moult cycle of *Panulirus argus* (Travis, 1955) it was shown that calcium was stored partly in the hepatopancreas and partly by formation of gastroliths (mineral deposits in the gastric epithelium). Whether this is the case also for *Crangon vulgaris* will be subject of a special investigation. All previous investigations on the Ca^{2+} regulation during the moult cycle seem to be confined to animals adapted to full strength sea water. For animals more or less isotonic to the medium, the calcium regulation may be easier as less work is done in total ionic regulation. Fluctuations in outer salinity exert a greater influence on the hæmolymph calcium than on calcium stored in the hepatopancreas or formed into gastroliths. Further investigations will reveal if the pattern of calcium regulation during the moult cycle in high salinities, i.e. above 30‰ is the same as in 10‰ or if it is similar to that of *Carcinus* (Adelung, op. cit.).

The magnesium content of the hæmolymph at the different moult stages is recorded in Fig. 4. From a rather high level immediately after moulting (stage A) the magnesium ion concentration falls slowly to a minimum in stage D_0-D_1. In stage D_2-D_4 there is a considerable increase in magnesium ion concentration.

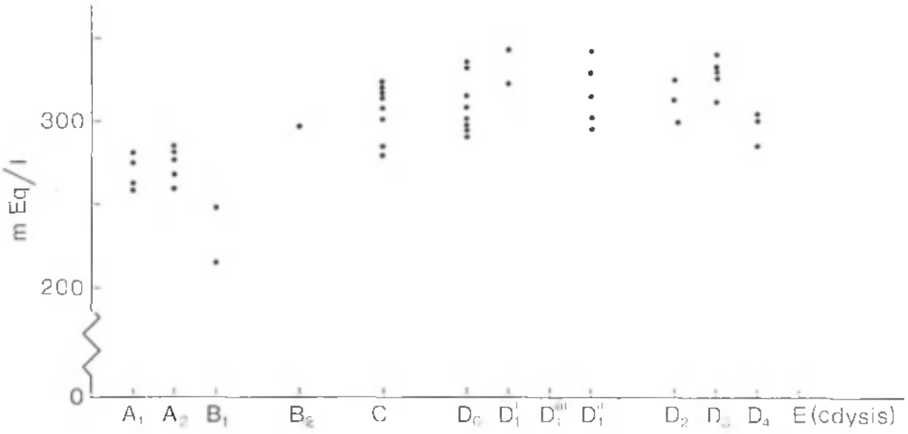

FIG. 2. Variation in chloride hæmolymph concentration during the moult cycle of *Crangon vulgaris*.

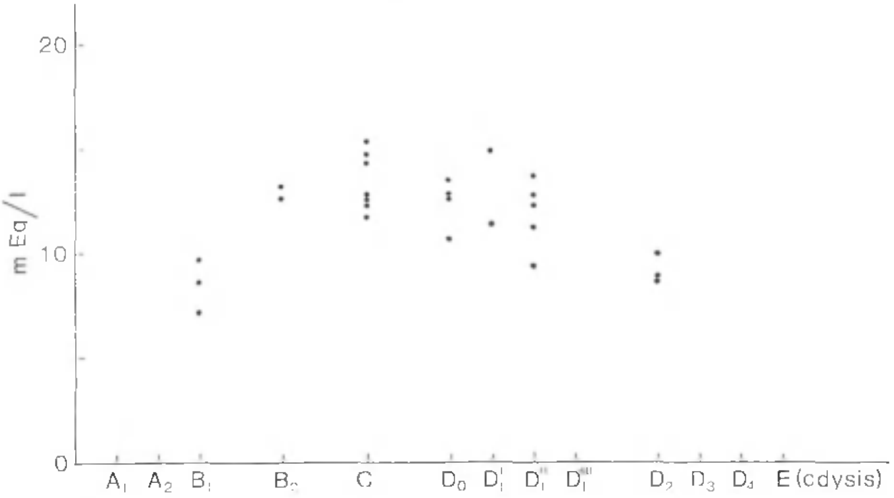

FIG. 3. Variation in calcium hæmolymph concentration during the moult cycle of *Crangon vulgaris*.

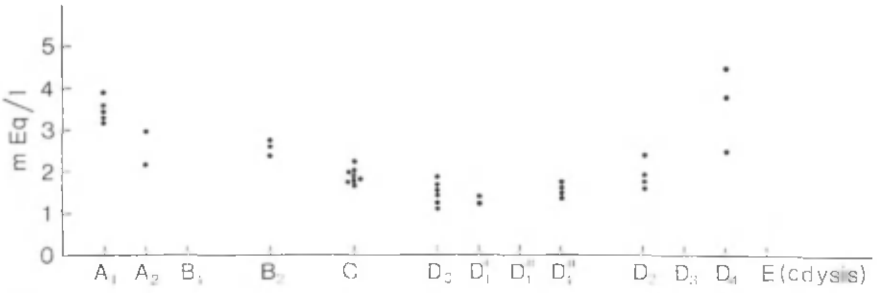

FIG. 4. Variation in magnesium hæmolymph concentration during the moult cycle of *Crangon vulgaris*.

Throughout the entire moult cycle the magnesium ion concentration is held at a niveau very far below the outer medium. The increase in Mg^{2+} content prior to and immediately after ecdysis could be due to either a decrease of the efficiency in the excretion of magnesium from the hæmolymph or to an increased uptake of water in the pro-ecdysis stage D_{3-4}. Probably the latter is the case, as a decrease in the magnesium excretion in the urine has not been shown to have a significant effect on the hæmolymph concentration just prior to ecdysis of crustaceans (Glynn, 1968). Difficulties of obtaining enough urine from *Crangon* to analyse its composition prevented an answer to this question.

It is well-known that the magnesium level and the behaviour of decapods are closely correlated: A high magnesium level means a low activity, and a low magnesium level indicates an active animal. The calcium/magnesium ion ratio affects the exitability of the nerve tissue (Robertson, 1953). It is thus evident that the temporary decrease in this ratio immediately before, on and after ecdysis must contribute to the very characteristic immobilization when *Crangon* moults.

The hæmolymph magnesium concentration is very low in *Crangon* even when the fluctuations during the moult cycle are considered. In his recent publication, Adelung (1971) mentions that the magnesium in the blood of *Carcinus* lies at about 65 % of the surrounding medium. In *Crangon* the corresponding figure lies around 15-35 %. Considering the ecology of *Crangon* and the general view of a low magnesium content correlated with a high activity, it may be suggested that *Crangon* has a higher relative activity than *Carcinus*. The very low magnesium ion concentration in the *Crangon* hæmolymph is comparable to that of certain squids and cuttlefishes (Nicol, 1967).

The regulation of potassium during the moult cycle is shown in Fig. 5. During the entire moulting cycle potassium is held significantly above the surrounding medium of 10‰ sea water, the potassium ion concentration in the hæmolymph lying between max. 16 and minimum 4.5 mEq/l. Highest potassium concentration is recorded immediately after ecdysis in stages A_1-B_2 whereafter it decreases to the minimum in stages D_0-D_1 and increases again closer to ecdysis.

According to Robertson (1957) the potassium ion concentration in the blood of *Carcinus* is above the concentration of the outer medium. This was not found by Adelung (1971) who instead found a potassium concentration isotonic to sea water in *Carcinus* during the whole moult cycle. In a later paper Robertson (1960) again showed that the potassium concentration in the plasma was higher than that of the outer medium and that the potassium ion concentration was highest prior to and just after ecdysis (stage D_{2-3} and B) and lowest in the intermoult stages. If the measurements of Adelung (1971) are analyzed in detail a small decrease in potassium level can be seen in the intermoult stages. It is well known that most of the osmoregulating decapods tend to accumulate potassium in relation to the sea water concentration (Nicol, 1967) and as this accumulation is so pronounced for *Crangon*, it is reasonable to assume that *Carcinus* also ac-

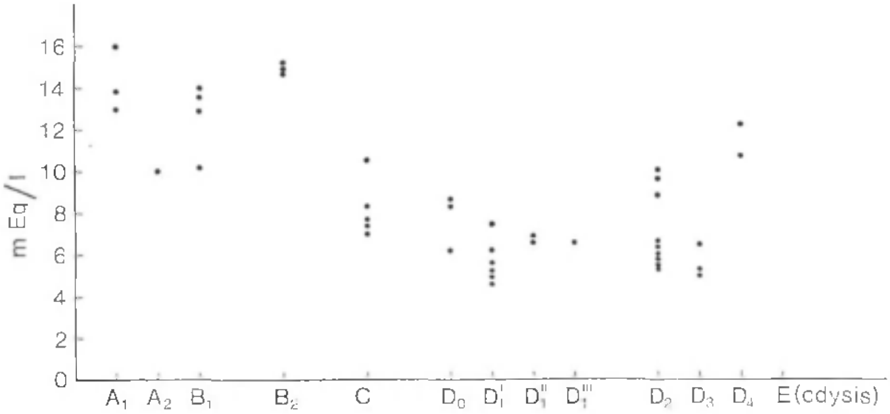

FIG. 5. Variation in potassium hæmolymph concentration during the moult cycle of *Crangon vulgaris*.

cumulates potassium in the same way. This is still more probable as the two species can co-exist in the same biotopes and show the same horizontal and vertical migrations during the year. The findings of Adelung (op. cit.) are thus difficult to explain.

DISCUSSION

The moult is from several aspects the most sensitive phase in the life of crustaceans, and mortality is often rather high at this time. Mechanical difficulties arise in withdrawing from the old cuticle, physiological problems arise from difficulties in osmotic and ionic regulation, and finally the animal is an easier prey for predators until the new cuticle is hardened enough to make movements such as escape or attack possible.

For an osmoregulating, homoiosmotic animal like *Crangon vulgaris* or *Leander serratus* living in brackish water, the moulting problems on the physiological level must dominate. The increased permeability, the water intake and the changed ionic content, all mean a heavy load to the excretory organs and to the cells. These latter must during the moult tolerate greater changes in their surrounding fluids than in the inter-moult phases. The cells might protect themselves from these changes by a variation in the concentration of intracellular amino-acids; so that when the osmotic concentration of the blood decreases, so also the cellular concentration of amino-acids decreases, a process which is fully reversible (Duchateau, Florkin & Jeuniaux, 1959).

On moulting large amounts of calcium are needed for the mineral component of the new cuticle. This is derived from the hæmolymph and from eventual reservoirs in the hepatopancreas and in gastroliths, but mainly by intake of water and probably by production of a hypotonic urine (Hagerman, 1973). The changes in magnesium ion concentration in the hæmolymph is probably due to this uptake of water and the increased permeability associated with moult. The significance of the increase in potassium ion concentration prior to and during moult is obscure, but is similar to that reported for other marine and freshwater invertebrates (Drilhon, 1935; McLennan, 1955).

The difficulties in keeping sodium and chloride at their normally high level (Hagerman, 1973) in a medium of 10‰ during the moult are evident. During moulting both ions decrease in concentration to an almost isosmotic level, no doubt a result of the increased permeability and swelling. The decrease in concentrations of these ions indicate that the transport mechanisms is fully loaded the whole time and unable to compensate for their loss.

If anything goes wrong, for instance if the moult is prolonged due to difficulties in casting the old cuticle or if the hardening of the new cuticle takes too long a time, *Crangon vulgaris* will rapidly die due to osmotic loss of important ions. It may be suggested that one of the factors determining the distribution of *C. vulgaris* is the conditions prevailing at the time of moulting.

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