Changes in the haemolymph protein and copper in the crab *Ocypoda macrocera* Milne Edwards (Crustacea: Brachyura)*

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Abstract. Wide fluctuations in the concentration of the haemolymph protein and its copper were observed during the moult cycle of the crab *Ocypoda macrocera*. Blood protein and copper concentrations were more in the intermoult (C1) and premoult crabs (C1-C2) but decreased in the postmoult crabs (A1-B3). A direct relationship between the blood protein and copper concentration is observed during the moult cycle. More copper in the hepatopancreas is seen during the premoult stage (D1-D2) and its level increased further in the postmoult stages (A1-B3). Increase of hepatic copper with a decrease in haemolymph copper content during the post moult stages shows the transfer of copper liberated from the haemolymph to the hepatopancreas due to the breakdown of haemocyanin, the major blood protein, during starvation period of moult cycle.

Keywords. *Ocypoda macrocera*; moult cycle; haemolymph; hepatopancreas; blood protein; copper.

1. Introduction

The moult and reproductive cycles of the crustaceans are accompanied by physiological changes including variations in haemolymph proteins. Extensive studies on the physiological changes occurring in the crustacean blood associated with reproduction, moulting, starvation etc have been reported by Barlow and Ridgway (1969), Glynn (1968), Busselen (1970), Djangmah (1970), Djangmah and Grove (1970), Dall (1974) and Hepper (1977). Haemocyanin, the copper containing protein of the crustacea is reported to occur freely in the haemolymph and it has also been reported that it is the major blood protein fraction (Goodwin 1960). In addition to haemolymph, hepatopancreas of crustaceans has been found to be the only tissue which contains considerable amounts of copper (Kerkut *et al* 1961).

In the present investigation, the total blood protein and copper levels of haemolymph of the commonly occurring ghost crab *Ocypoda macrocera* during its moult cycle has been studied to understand the changes in protein and copper concentration of blood during moult cycle. The copper concentration of hepatopancreas during the moult cycle has also been studied to assess the haemolymph and hepatopancreas copper relationship.

2. Materials and methods

Specimens of *O. macrocera* were collected from the Lawson’s Bay and palm beach areas of Visakhapatnam coast. Crabs were maintained separately in glass jars
containing sea water (for submersion of the branchial region) and were fed with fish muscle and the sea water was changed daily. Crabs were found to be quite active for longer time (45–60 days) under laboratory conditions and several crabs moulted. Many of the crabs were seen attaining the C₄ stage after their moult in the laboratory. Male crabs measuring 1.8–2.2 cm carapace width, which have shown high moulting activity were taken in the present experiment. The moult stages were distinguished after Drach (1939) and Nagabushanam and Ranga Rao (1960). Blood samples from the intermoult and other moult stages were collected through a hypodermic syringe from the pericardial space and also from the joints of chelate legs. The total blood protein and blood copper estimations were made using whole blood. Hepatopancreas samples collected from different moult stages were dried, powdered and the same were taken for hepatic copper estimation. Total blood protein was determined by the method of Lowry et al (1951). Blood and hepatopancreas copper quantities were estimated following the oxalylidihydrazide method of Rice (1960) and Ravindranath (1981). Samples of haemolymph and hepatopancreas for each moult stage were taken from several crabs and the values presented in the results for each reading is the mean of 6–8 observations.

3. Results and observations

The mean total blood protein at different moult stages of *O. macrocera* is shown in table 1. Normal intermoult crabs (C₄) collected from the same locality showed considerable variations in their blood protein concentration and it ranged between 64.05–93.63 mg/ml, and a mean value of 81.58 mg/ml was noted. In the premoult crabs (D₁–D₂) blood protein ranged between 94–132 mg/ml and a mean value of 112.50 mg/ml was recorded. A falling blood protein quantity is noticed in the A₁ stage with 69.73 mg/ml (51.75–81.32). Further decline in the total blood protein quantity is noticed in the B₂ stage with a value of 31.12 mg/ml (21.74–46.41). The revival in the blood protein concentration is noticed in the C₂ stage with 43.96 mg/ml (30.93–62.03). Increase in the blood protein level continued and in C₃ stage 66.44 mg/ml of protein (50.76–78.04) was noted.

The blood copper concentration also showed wide variations among different crabs within the same population. The results on the blood copper concentration among different moult stages are shown in table 1. Haemolymph copper concen-

| Table 1. Total haemolymph protein, haemolymph copper and hepatopancreas copper quantities in different moult stages of *O. macrocera*. |
|-----------------------------------------------|--|---|---|
| Moult stage | Haemolymph protein (mg/ml) ± SD | Haemolymph copper (µg/ml) ± SD | Hepatopancreas copper (µg/g dry weight) ± SD |
| C₄ | 81.58 ± 12.07 | 48.78 ± 11.31 | 299.44 ± 70.34 |
| D₁–D₂ | 112.50 ± 16.36 | 79.55 ± 16.48 | 385.00 ± 68.44 |
| A₁ | 69.73 ± 11.08 | 30.01 ± 9.92 | 475.33 ± 61.33 |
| B₂ | 31.12 ± 8.26 | 18.65 ± 6.27 | 501.47 ± 40.37 |
| C₂ | 43.96 ± 15.77 | 28.03 ± 10.24 | 411.18 ± 59.16 |
| C₃ | 66.44 ± 13.08 | 37.60 ± 8.19 | 406.52 ± 51.91 |

SD = Standard deviation.
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Concentration values showed a similar pattern in its variations during the moult cycle as those seen in the case of blood protein.

Intermoult stage (C₄) crabs showed haemolymph copper of 48.78 µg/ml (27.75–63.32) and an increase of haemolymph copper is found in the premoult (D₁–D₂) stages with blood copper concentrations of 79.55 µg/ml (54.55–101.76). Decrease in haemolymph copper concentration is noted in all post moult stages. It is observed that with an increase in blood protein there is a corresponding increase in blood copper concentration, thus showing a direct relationship between haemolymph protein and its copper during moult cycle (figure 1).

Quantity of copper in the hepatopancreas also showed wide variation among crabs of the same population and of similar moult stages. The quantities of hepatic copper found in different moult stages of *O. macrocera* is shown in table 1. The changes in the hepatic copper shows that hepatic copper values increased from premoult (D₁–D₂) stage to early post moult (B₂) and a sharp decrease is found in C₂ and further decrease in C₃ stage is noticed thus reaching to normal level by C₄ stage.

4. Discussion

Busselen (1970) reported a 4-fold decrease in haemocyanin concentration after moult in *Carcinus maenas* and he observed that copper concentration of the blood serves as a measure of the haemocyanin concentration. Negligible amounts of blood copper was reported in C₁ stages of *Maia squinado* (Zuckerkandl 1960). Decrease in blood copper was noticed in late post moult stages of *C. maenas* and *Crangon vulgaris* by Kerkut *et al* (1961) and Djangmah (1970) respectively.

The fall of blood protein in post moult stages is attributed to the absorption of large quantities of water and protein utilization during the moult cycle. Similar to the present observation, a direct relationship of blood protein and copper quantities during moult cycle has also been reported in *C. vulgaris* by Djangmah (1970). The fluctuations of hepatic copper during the moult cycle of *O. macrocera* has shown an

![Figure 1. Haemolymph protein, copper and hepatopancreas copper concentrations during moult cycle.](image-url)
inverse relationship with that of blood copper i.e. with a decrease of blood copper an increase in hepatic copper was observed (figure 1). Zuckerkandl (1960) reported an increase in hepatic copper in the early post moult stages and its decrease in the late post moult stages of *Maia squinado* and he indicated re-entry of hepatic copper into blood in the late post moult stages. Djangmah (1970) reported an increase in hepatic copper and decrease in blood copper during starvation in *C. vulgaris* and he observed that some amount of the liberated copper from haemolymph is absorbed into the hepatopancreas. However he indicated that all the absorbed copper of hepatopancreas is not retransferred for haemocyanin reconstitution.

Djangmah and Grove (1970) did not report any correlation between the blood copper and of hepatopancreas copper during the moult cycle in *C. vulgaris*. Contrastingly Kerkut et al (1961) reported an increase in hepatic copper with simultaneous increase of blood copper.

In the present investigation decrease in blood copper in the early post moult stages with a simultaneous increase in hepatic copper indicates transfer of copper from blood to hepatopancreas. Further the increase in haemolymph copper during the late post moult stages evidently shows some copper re-entering blood in the post moult stage as it is found that the blood copper level increased in the early C2 stage, even before the crab is supplied with food material.

There is no reported evidence on the re-entry of hepatic copper into the haemolymph for the reconstitution of haemocyanin during late post moult stages; while there are reports of copper loss through faeces in the form of copper bodies in *Procambarus clarkii* by Ogura (1959). Similarly Bryan (1964, 1968) also indicated the loss of zinc through faeces and urine in *Homarus vulgaris* and *Austropotomius pallipes*. The finding in the present study shows that certain amount of the copper absorbed from the haemolymph into the hepatopancreas during the early post moult stages is reabsorbed into the haemolymph of the late post moult stage crabs.

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