Mobilisation of Organic Reserves during Moult Cycle in the Prawn *Penaeus indicus* (H. Milne Edwards)

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Organic reserves like lipid, cholesterol, protein, glycogen and glucose in muscle, hepatopancreas and haemolymph of *P. indicus* during different stages of moult cycle were estimated. High values of these organic reserves obtained at early premoult stage (D1) followed by a significant decrease at late premoult stage (D4) indicated marked storage and subsequent utilization during stages D1 to D4. Low values were observed at stage A (early post-moult) confirming depletion in the reserves just after moulting. High percentage of water found in the muscle during post-moult stage A and low values during premoult stage (D1), in contrast to the pattern observed for organic reserves during this period substantiated the concept of tissue growth by replacement of water during the periods between the moults.

The organic reserves like carbohydrates, lipids and proteins are important as a source of material not only for the formation of new exoskeleton but also for the energy required during the process of moulting. Proper utilization of these organic reserves ensures the success of the moult as well as good growth at each moult in the crustaceans[1,2]. In the present study quantitative variations in lipid, cholesterol, protein and glycogen contents of muscle, hepatopancreas and haemolymph (glucose and not glycogen) and variation in the water content of the muscle in the prawn *Penaeus indicus* (H. Milne Edwards) at 5 different stages of the moult cycle are measured.

Materials and Methods

Periodic collections were made of farm reared *P. indicus* (80-100 mm in size) from a hatchery. The live animals, transported in seawater, were transferred to perspex tanks (75 l capacity) filled with filtered and aerated seawater and maintained in the same conditions until used.

Moult stage identification was done by the method described earlier[3]. The prawns used for biochemical analysis were selected from 5 moult stages, viz., early postmoult (A), late postmoult (B), intermoult (C), early premoult (D1) and late premoult (D4).

Haemolymph from individual prawn was collected through the pericardial cavity using 1 ml hypodermic glass syringe fitted with No. 22 needle. Trisodium citrate (3% solution) was used as an anticoagulant. The syringe was rinsed with anticoagulant after each collection. Prior to the puncture made through the membrane between the carapace and the first abdominal segment, the carapace and adjacent areas were thoroughly dried with absorbant paper to remove any external water present. As much haemolymph as possible was withdrawn from each individual prawn but care was taken not to extract tissue particles. Haemolymph samples were kept frozen until used. Haemolymph from animals in the same stage of moult was pooled when sufficient amount was not obtained from a single specimen.

After the extraction of haemolymph, the prawns were dissected quickly and the hepatopancreas and muscle tissue excised. Lipid was estimated gravimetrically using modified Bligh and Dyer's method[4]. Cholesterol was determined by Hestrin's method[5] using cholesterol extra pure as the standard. Protein was estimated by the modified biuret method using gelatin as the standard for muscle and hepatopancreas and bovine serum albumin crystals as standard for haemolymph[6]. Glycogen and glucose were estimated[7] using D-glucose as the standard. Water content of the muscle was determined by drying the tissue in a desiccator, the method proposed by Mullainadhan[8].

Results and Discussion

Changes in various organic reserves estimated are presented in Table 1. In the present study, lipid content of muscle, hepatopancreas and haemolymph increased gradually from stage A to reach a maximum at stage D1 and then decreased during the late premoult stage D4. Similar increase in the lipid content of the whole animal in *Palaemon paucidens* during stage D2 and then decrease at late premoult, D3-D4 was reported[9]. During the moulting cycle of *P. japonicus* the most notable variation in the lipid concentration occurred in hepatopancreas[3], which showed a decrease between stages B and C1-C2 and reached a maximum at stage DO i.e. beginning of premoult period and then

Table 1—Changes in Lipid, Cholesterol, Protein, Glycogen, Glucose and Water Content during Moulting Cycle of *P. indicus*

Tissues	Moult stages				
	A	B	C	D1	D4
			Lipid		
Muscle (mg. 100 g^{-1})	$970.5^{a} \pm 2.01$	$989.4^{a} \pm 4.88$	$991.0^{a} \pm 69.75$	$1223.1^{c} \pm 19.06$	$914.3^{b} \pm 5.54$
Hepatopancreas (mg. 100 g^{-1})	$1502.5^{a} \pm 2.09$	$1840.7^{b} \pm 6.79$	$1931.7^{c} \pm 157.50$	$2781.9^{d} \pm 12.27$	$1945.7^{c} \pm 17.11$
Haemolymph (μg. 100 ml^{-1})	$143.5^{a} \pm 2.34$	$154.4^{b} \pm 2.03$	$162.0^{b} \pm 15.94$	$298.8^{c} \pm 8.28$	$170.3^{b} \pm 2.20$
			Cholesterol		
Muscle (mg. 100 mg^{-1})	$1.03^{a} \pm 0.09$	$1.20^{b} \pm 0.05$	$1.45^{d} \pm 0.07$	$1.77^{e} \pm 0.07$	$1.37^{c} \pm 0.07$
Hepatopancreas (mg. 100 mg^{-1})	$3.74^{a} \pm 0.09$	$4.06^{b} \pm 0.11$	$5.52^{d} \pm 0.23$	$6.14^{e} \pm 0.16$	$4.44^{c} \pm 0.21$
Haemolymph (mg. 100 ml^{-1})	$4.80^{a} \pm 0.06$	$5.21^{b} \pm 0.08$	$6.34^{c} \pm 0.15$	$12.22^{d} \pm 0.25$	$9.95^{e} \pm 0.33$
			Protein		
Muscle (mg. 100 mg^{-1})	$35.88^{a} \pm 0.53$	$38.86^{b} \pm 0.64$	$39.96^{c} \pm 1.74$	$43.38^{d} \pm 2.07$	$38.95^{b} \pm 0.22$
Hepatopancreas (mg. 100 mg^{-1})	$28.42^{a} \pm 0.56$	$30.66^{b} \pm 0.60$	$33.21^{b} \pm 3.5$	$35.93^{c} \pm 0.65$	$32.26^{d} \pm 0.10$
Haemolymph (mg. 100 ml^{-1})	$25.45^{a} \pm 0.13$	$28.82^{b} \pm 0.08$	$30.58^{c} \pm 1.09$	$41.20^{d} \pm 1.40$	$36.53^{e} \pm 0.71$
			Glycogen		
Muscle (mg. 100 mg^{-1})	$0.98^{a} \pm 0.01$	$1.11^{b} \pm 0.01$	$1.21^{c} \pm 0.05$	$1.85^{d} \pm 0.06$	$1.30^{e} \pm 0.006$
Hepatopancreas (mg. 100 mg^{-1})	$1.35^{a} \pm 0.04$	$1.44^{a} \pm 0.04$	$1.61^{b} \pm 0.22$	$3.71^{d} \pm 0.08$	$3.29^{c} \pm 0.006$
			Glucose		
Haemolymph (mg. 100 ml^{-1})	$35.00^{a} \pm 1.42$	$38.41^{b} \pm 0.46$	$47.06^{c} \pm 6.44$	$94.84^{e} \pm 0.74$	$67.56^{d} \pm 1.45$
			Water		
Muscle (%)	$76.04^{c} \pm 0.48$	$73.82^{b} \pm 1.17$	$73.14^{b} \pm 1.51$	$71.21^{a} \pm 0.72$	$74.12^{b} \pm 0.74$

Values in mean with the same superscripts do not differ significantly ($P < 0.05$).

decreased to low levels at stages D1″-D1‴. O'Connor and Gilbert[10] observed that in *Orconectes virilis* decrease in synthesis and accumulation of hepatopancreatic lipids at late premoult period is coupled with increase in the rate of lipid release from hepatopancrease into haemolymph. But increase in the haemolymph lipid during stage D1 and decrease during D4, as in present study, could help to substantiate the role played by haemolymph in the storage of reserves. Although hepatopancreas is the primary organ for the storage of reserves, the haemolymph has been suggested to play a secondary role[11]. Further in the mechanism of lipid transport in crustaceans, lipids of the haemolymph are shown to have an important role[12].

Cholesterol content in the muscle, hepatopancreas and haemolymph of *P. indicus* showed a similar trend to that of lipids. There was a gradual increase from stage A to stage D1 and then again it decreased at stage D4. It was observed that during the moult cycle of *P. japonicus*, cholesterol content in the eyestalk and hepatopancreas increased at intermoult and decreased at premoult[13] and only 3 stages of moult cycle viz. AB as postmoult, C intermoult and D premoult were considered. In the present study since the premoult was considered under two sub-divisions early premoult (D1) and late premoult (D4), a more detailed investigation was possible. It is suggested that in prawns cholesterol may be circulated in the body as an unesterified sterol, perhaps as a lipoprotein complex, implicated in the transport of metabolites[14]. The high cholesterol content, observed in the haemolymph during stage D1 of *P. indicus* in the present study, may be for its utilization during the post-moult stages, in the improvement of new exoskeleton[15].

The protein content of the muscle, hepatopancreas and haemolymph during the moulting cycle of *P. indicus* also exhibited a trend similar to that of lipids. In the hepatopancreas, a gradual increase of protein content at stage D1 followed by a decrease at stage D4, is in concordance with observations reported in

P. indicus wherein it is stated that the protein synthesis is not restricted to any particular period of the moult cycle but it commences as soon as the exoskeleton hardens and prawn starts feeding[16]. Dall[17] observed that in the leg muscle of *Panulirus longipes* ninhydrin positive substances showed a maximum at stage C4 and there was a decrease during the stage D4. But in the present study, high value of muscle protein was obtained at D1 which decreased at stage D4. The discrepancy observed between the 2 studies could be attributed to the differences in the moult staging and also the gradual changes of the moult stages in which case the marking of the end of intermoult stage C marks the beginning of premoult stage D.

Travis[18] while working on the blood protein in *Panulirus argus* reported increased value preceding moult and declined level following moult. In *Penaeus duorarum* haemolymph protein was lowest during the post moult stage and the value steadily increased to a maximum during premoult stage and again declined just before moult[19]. Similar trend was seen in the present investigation with regard to protein behaviour. The rise in haemolymph protein preceding moult in the present study is attributed to organic material present due to resorption from the chitino-protein complex of the old exoskeleton as observed earlier[20]. Further, as mentioned some of the amino acids resorpted during the premoult period could be used in the synthesis of a protein when the animal ceases to feed, and the decreased protein concentration in the postmoult stages was due to dilution of haemolymph by water that enters at and following the moult stage[18].

Analysis of glycogen content of muscles during 3 different stages of moult cycle in *Panulirus japonicus* indicated maximum amount of glycogen in the intermoult stage whereas in the premoult condition the level was low[21]. This is in contrast to the results of the present study in *P. indicus*, where in, the maximum glycogen content was observed in early premoult stage. The importance of glycogen in crustaceans in the synthesis of chitin and as the energy sources in intermediary metabolism has been reported[18,22]. Hence depletion in the concentration of glycogen during stage D4 in the present study indicates that it is mobilised for growth of post exuvial layers of the new integument during stages A and B of the moult cycle.

The main function of the haemolymph in the proecdysis is to transport materials between the integument and the hepatopancreas and it may also act as a storage reservoir and as the site of certain enzymatically controlled reactions[20]. Travis[23] observed that during the formation of principal layers of the integument of *Panulirus argus* glycogen accumulates in the epidermis and that the contributing sugars may be traced to the sub-epidermal tissues and blood and ultimately to the hepatopancreas. So the changes in hepatopancreatic and integument glycogen are expected to cause the necessary changes in blood glucose level. In agreement to the postulation of Renaud[24] that conversion of glucose to glucosamine takes place in the haemolymph, the present observation also supports the view that the stored glycogen may give rise to precursors of chitin in the haemolymph. Since there is a increase in haemolymph glucose corresponding to the increase in hepatopancreatic and muscle glycogen, in addition to chitin synthesis the importance of the sugar in the haemolymph as a possible energy source in tissue metabolism is also suggested.

The body volume increase during ecdysis in Crustacea has been understood to be due to water uptake, and considerable variation in the water content of the tissues takes place during the moulting cycle, due to intake of water and consequent hydration of tissues[20]. Rise in the water level during the moult stage A and subsequent decrease to reach the minimum level at stage D1 in the present study is in concordance with the pattern observed by Read and Caulton[16] in the whole animal of *P. indicus*. It is evident from the present investigation that in *P. indicus* also, changes in water content of the muscle tissue, showed a cyclic pattern in association with moult cycle as in most other crustaceans. While there was a depletion in the organic reserves a corresponding increase in the water content was observed. Conversely while the accumulation of organic reserves showed a maximum at stage D1 a corresponding decrease in the water content was noticed. This clearly indicates the replacement of water by tissue growth during the periods between moults which in turn is considered as the actual growth in the animal, when there is an increase in the dry weight of the body.

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