ROLE OF HEPATOPANCREAS IN THE METABOLISM OF COPPER IN THE INDIAN AMPHIBIOUS SNAIL, *PILA GLOBOSA* (SWAINSON), WITH SPECIAL REFERENCE TO AESTIVATION AND STARVATION

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ABSTRACT

The copper and protein contents in the blood and the copper levels in the hepatopancreas of the active, aestivated and one month starved snails were determined. The blood copper increased significantly upon aestivation and decreased upon starvation, while a reverse trend was observed in the hepatopancreatic copper. An inverse relationship between the copper level in the blood and hepatopancreas of both aestivated and starved snails has been shown and the role of hepatopancreas in the metabolism of copper is discussed. The protein content decreases more upon starvation than upon aestivation.

INTRODUCTION

COPPER is an important metal constituent of the respiratory protein, haemocyanin, which is found dissolved in the haemolymph of a majority of Arthropoda and Mollusca[1-3]. The percentage copper of the purified haemocyanin is constant for a given species and is higher in molluscs than in arthropods[4]. The decapod crustaceans, isopods and amphipods are known to concentrate and store considerable quantity of copper in the hepatopancreas[5,6] often as semicrystalline or refractile granules in specific cells[7]. It has been shown that in spider crab, *Maia*, the haemocyanin content in the blood drops preceding the moult with a corresponding rise in the copper level in the hepatopancreas[6]. Since there is not much work on these lines in molluscs, studies have been carried out to elucidate the role of hepatopancreas in the metabolism of copper in the Indian amphibious snail, *Pila globosa*, with reference to aestivation and starvation.

MATERIAL AND METHODS

The snails were collected from the fresh water ponds around Kavali Town (A.P., India) and maintained in aquarium tanks in the laboratory. They were fed with green algae, *Nitella*, for a week. Some of them were kept overnight on filter papers to remove water in the mantle cavity. These were taken as active snails. Some of the well fed snails were made to aestivate as described earlier[8]. A batch of snails were kept in glass troughs containing tap water changed at intervals of 24 hours for a period of one month. These were treated in the same way as that of active snails prior to experimentation and represented the starved snails.

The blood was collected as described earlier[9]. The hepatopancreas was excised, washed thoroughly with distilled water and dried to constant weight at 110° C.

The copper content was estimated in the blood and in the dried hepatopancreas using sodium diethyl dithiocarbamate[10], after wet ashing with concentrated sulphuric acid in microkjeldahl flasks. The haemocyanin content in the blood was calculated based upon per cent copper in the purified haemocyanin[11,12]. The protein content in the blood was estimated by the method of Lowry *et al.*[13].

RESULTS AND DISCUSSION

The data on the copper and protein contents in the blood and the copper content in the hepatopancreas in relation to aestivation and starvation are presented in Table I. The copper content of the blood of the snails increased significantly from 46·97 μg/ml in the active snails to 56·09 and 71·67 μg/ml upon 3 and 6 months aestivation respectively. The increase in copper content, to a small extent, may be due to dehydration (Table I). The copper requirements of the snail is largely met from diet. But during aestivation there is no food intake and hence the increase in the copper content in the blood should be due to the mobilization of copper from within the body. The only part of the body that has considerable quantities of copper is the hepatopancreas[5,14-16]. Accordingly, the copper content in the hepatopancreas decreased significantly upon aestivation (Table I). This shows that the copper has been mobilized into the blood from hepatopancreas upon aestivation.

TABLE I

Hepatopancreatic copper and the blood copper and protein contents in the fresh water amphibious snail, Pila globosa *in relation to aestivation and starvation*

(The number in the paranthesis denotes the number of observations)

Sample	Active snails	1 month starved snails	3 months aestivated snails	6 months aestivated snails
Hepatopancreatic copper µg/gm dry wt.	232·72 ±21·44 (10)	451·484* ±18·461 (10)	199·726* ± 18·456 (10)	134·165* ± 19·800 (10)
Blood copper µg/ml	46·97 ±15·44 (21)	10·12* ±2·64 (21)	56·09*** ±11·0 (21)	71·67* ±10·27 (21)
Blood proteins mg/ml	36·99 ±10·08 (21)	12·61* ± 2·45 (21)	30·75*** ± 7·58 (21)	28·52** ±5·46 (21)
% of proteins as haemocyanin	49·9	31·47	71·52	98·56
% of other proteins	50·1	68·53	28·48	1·44
% of water content in the blood	98·77 ±0·0398	..	96·062* ±0·072	93·838* ±0·217

* $P < 0·001$; ** $P < 0·01$; *** $P < 0·05$

There was a marked decrease of 78·45% of copper in the blood upon 1 month starvation. From this it can be suggested that copper is either excreted or stored elsewhere in the body. The first possibility does not appear to be feasible because there is no known mechanism in the biological system to excrete copper from the body[15]. In concurrence with the second assumption, the copper level in the hepatopancreas increased significantly (Table I). This reveals the fact that the haemocyanin is catabolised during starvation and the freed copper is transported and translocated in the hepatopancreas. Similar accumulation of copper in copper vesicles of hepatopancreas of *Crangon* has been reported during starvation[15]. There is an inverse relationship in the copper content between hepatopancreas and blood upon aestivation and starvation. Such inverse relationships between copper content of blood and hepatopancreas have been established during starvation in the spider crab, *Maia squinado*[5] and *Carcinus maenas*[16].

An increase in the copper level in the blood upon aestivation is associated with corresponding increase in the haemocyanin content[11] (Table I). The increase in the haemocyanin content observed may be in response to hypoxic conditions prevailing during aestivation[17]. Similar increase in the haemoglobin concentration has been reported during hypoxia[18]. Manwell[3] has suggested that an increase in the haemocyanin content helps the animal to maintain a high oxygen gradient between the blood and the tissues.

The decrease in the copper content in the blood of the snail upon starvation is associated with a decrease in the haemocyanin content (Table I). It is known that during starvation the metabolic activities of the animal are lowered. Hence the low haemocyanin content reported may be sufficient to meet the oxygen demands of the animal.

Even though there is a general decrease in the total protein content in the blood upon aestivation and starvation, the decrease in the total proteins upon starvation is significantly greater (Table I). This reveals the greater orientation towards protein metabolism during starvation Further, the reduction in the haemocyanin content upon starvation shows that it is also an energy source during starvation[15].

From the results it is clear that 49·9% of the total proteins of the blood has been represented as haemocyanin in the active snails and the remaining percentage as proteins other than haemocyanin (low molecular weight proteins[19]). The low molecular weight proteins decreased as the haemocyanin content decreased upon starvation, showing an inverse relationship. Such inverse relationship between haemocyanin and proteins other than haemocyanin has been demonstrated in the blood of *Maia* and *Carcinus*[15,16]. The fact that decrease in the low molecular weight proteins is associated with an increase in the concentration of haemocyanin in the blood of the aestivated snails suggests the utilization of these proteins in the synthesis of haemocyanin. However, the experimental evidence for the synthesis of haemocyanin in *Pila globosa* is awaited. It may, therefore, be concluded that the hepatopancreas in the snail, *Pila globosa*, perhaps, serves as a storage organ for copper and also acts as a copper donor, at times of need, for the synthesis of haemocyanin.

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ANAEROBIC FERMENTATION OF PLANT MATERIALS INTO ACIDS AND BIOGAS

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ABSTRACT

Plant materials, *viz.*, berseem, hybrid napier, oat, dhub grass, paddy straw with and without 2·5% nitrogen as ammonium sulphate were anaerobically fermented at 5% solids concentration at 35° C for 20 days. The hybrid napier and berseem yielded the highest plant acid recovery of 30·5% and 32·0% of their dry matter when the plant materials were digested for 10 or 15 days, filtered, charged with water again and digested upto 20 days. However, with other plantt materials, the plant acid recovery ranged from 21·5% to 31·8%. The pH changes during digestion were also observed. The biogas yield from berseem plant acids, in the methanogenic phase digester, ranged from 0·46 to 0·78 litre/g plant acids added as a single charging whereas with triple charging a maximum biogas yield of 0·85 litre/g plant acid was obtained. The biogas comprised of 58% methane, 28% carbon dioxide and 11% of other constituents like carbon monoxide, oxygen, nitrogen, hydrogen, etc.

INTRODUCTION

UTILIZATION of agricultural and animal wastes as an energy source has received wide attention recently. Wolf and Keenan[9] suggested a two stage digestion of dog food. Batty[4] and Sarma[8] emphasized that energy crops could be converted into methane through anaerobic fermentation with reference to conversion of solar energy