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Purified haemocyanin in the blood of fresh water amphibious snail, *Pila globosa* (Swainson) in relation to aestivation

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Abstract. The copper and protein content in the blood and in the purified haemocyanin of active and aestivated snails are determined. There is a marked increase in the copper content and also in the haemocyanin content in the blood upon aestivation which is discussed in the light of the available literature. The per cent copper/protein ratio was 0·255 in the purified haemocyanin of the active and aestivated snails, suggesting similar nature of haemocyanin molecule whose minimal molecular weight was calculated to be 24,910.

Keywords. Haemocyanin; minimal molecular weight; *Pila globosa*; aestivation.

1. Introduction

Haemocyanin, a copper containing respiratory pigment, is next to haemoglobin in occurrence. It is found dissolved in the haemolymph (Redfield 1934, 1950) of some molluscs and arthropods (Manwell 1960). The copper content in the molluscan haemocyanins ranged from 0·245 to 0·260% when compared to the low level of copper (0·166 to 0·180%) in arthropods (Lontie and Witters 1973). The copper content is taken as a measure of the haemocyanin (Montgomery 1930). In the present study an attempt has been made to calculate copper/protein ratios and the minimal molecular weight of the purified haemocyanin, and the haemocyanin concentration in the blood of the snail, *Pila globosa* (Swainson) with reference to aestivation.

2. Materials and methods

The snails were collected from the fresh water ponds around Kavali town (Nellore District of A.P.) 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 placed at the bottom of the glass troughs under a ceiling fan, so that when they sprawl about, the water present in the mantle cavity was absorbed by the filter papers before they retreat into their shells. These were represented as active snails. Some of the well fed snails were made to aestivate for 4 months as described earlier (Sreenivasa Reddy and Swami 1976). Four months aestivated as well as active snails were used in the present investigation.

The blood was collected from the snails as described earlier (Murali Mohan and Sasira Babu 1976). The purified haemocyanin was obtained by following the procedure given by Konings *et al* (1969). The haemocyanin was dialysed against 0·1 M tris buffer of pH 8·0, the normal pH of the blood, instead of against pH 7·0 buffer. The final preparation was blue in colour and is not denatured, since the blue colour is lost upon the addition of a pinch of sodium hydrosulphite. The copper was estimated with sodium diethyl-dithiocarbamate (Barnes and Rothschild 1950) after wet ashing with concentrated sulphuric acid using Kjeldahl flasks. The proteins were estimated by the method of Lowry *et al* (1951).

The haemocyanin content of the blood was calculated using per cent copper of the purified haemocyanin and copper content of the blood. The minimal molecular weight was calculated by dividing the atomic weight of copper (63·54) by the fraction of protein due to this element in the purified haemocyanin (Montgomery 1930).

3. Results and discussion

The data on the copper and protein contents in the blood of active and aestivated snails are presented in table 1. The copper content in the blood of the active snails was 46·97 μg/ml. There was a significant increase of about 23% in the copper content (57·79 μg/ml) upon aestivation and with a similar increase in the haemocyanin content (table 1), since the copper content in the blood gives a measure of haemocyanin concentration (Montgomery 1930). The increase in the copper content in the blood upon aestivation may have been due to the release of copper from the hepatopancreas (Djangmah and Grove 1970). The copper and the protein contents

Table 1. Copper and protein contents and per cent copper in the blood and in the purified haemocyanin of active and aestivated snail, *Pila globosa*.
(The figures in the parantheses denote the number of observations)

	Active	Aestivated
Blood:		
Copper (μg/ml)	46·97 ± 15·4 (21)	57·79 ± 17·0* (21)
Protein (mg/ml)	36·99 ± 10·1 (21)	29·54 ± 7·6* (21)
% Copper	0·1269	0·1956
Haemocyanin content (mg/ml)	18·4	22·7
% of protein as haemocyanin	49·9	76·7
% of proteins other than haemocyanin	50·1	23·3
Purified haemocyanin:		
Copper (μg/ml)	37·98 (5)	45·20 (5)
Protein (mg/ml)	14·89 (5)	17·72 (5)
% Copper	0·255	0·255

*$P < 0.001$

in the purified haemocyanin also increased upon aestivation (table 1). As a result the per cent copper in the purified haemocyanin of both active and aestivated snails was 0·255, a value which falls within the range reported for other molluscs (Lontie and Witters 1973). It suggests that the purified haemocyanins of both active and aestivated snails may have similar physico-chemical properties.

The total protein content in blood has decreased by about 20·1% upon aestivation (table 1), suggesting the utilization of proteins. In the active snails only 49·9% of the total proteins are represented as haemocyanin as against 90% of the total blood proteins representing as haemocyanin in a large number of animals containing haemocyanin in the blood (Prosser 1973). The low percentage of proteins represented as haemocyanin in the active animals reflects the passive mode of life of snails. The remaining percentage of proteins (50·1) in the active animals may be other proteins of low molecular weight (Ghiretti 1966). Upon aestivation 76·7% of the total proteins are represented as haemocyanin. This increase may be due to the utilization of low molecular weight proteins which are decreased to 23·3% (table 1). The increase in the copper content and decrease in the low molecular weight proteins in the blood upon aestivation accounts for the increase in the haemocyanin concentration. This gives us a clue that the synthesis of haemocyanin may occur in the blood cells as shown in *Limulus* (Fahrenbach 1968).

The increase of haemocyanin concentration upon aestivation might be in response to hypoxic conditions prevailing during aestivation (Von Brand 1946). Similar increase in the haemoglobin concentration under hypoxia has been reported (Prosser *et al* 1957). While oxygen storage is basically the function of oxygen—combining pigments located in the tissues, the significant aspect of this is the ability of the haemocyanin in the blood in the aestivated condition to accept into chemical combination most of the oxygen molecules present, thereby maintaining a steep gradient, resulting in a rapid net movement of oxygen into the tissues. This may be an adoptation correlated with the scarcity (or complete lack) of active transport mechanisms for molecular oxygen (Manwell 1960).

The minimal molecular weight of haemocyanin of *Pila globosa* is about 24,910. This value is readily comparable to the values already reported for other gastropods (table 2). Since a molecule of oxygen combines stoichiometrically with two copper atoms (Redfield *et al* 1926; Rawlinson 1940), the molecular weight of the functional unit of oxyhaemocyanin is about 49,820. The molecular weights of the functional units of some of the gastropods are also presented in table 2 for comparison. This

Table 2. Copper content and the minimal molecular weight of the haemocyanin of *Pila globosa* in comparison with other gastropods

Species	% Cu	M/Cu	M/2Cu	Ref
Busycon Canaliculatum	0·245	25,930	51,860	Hernler and Philippi (1933)
Helix pomatia	0·250	25,420	50,840	Roche (1936)
*Pila globosa**	0·255	24,910	49,820	—
Murex trunculus	0·257	24,730	49,460	Ghiretti-Magaldi *et al* (1966)

*Present investigation

suggests that the functional units of all the gastropod haemocyanins have more or less the same physiochemical properties.

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