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Copper Toxicity in the Crab, *Scylla serrata*, Copper Levels in Tissues and Regulation After Exposure to a Copper-Rich Medium

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In the decapod crustaceans copper is distributed in various tissues (Bryan, 1968; Arumugam & Ravindranath, 1983). In these animals the tissue copper generally exists in four forms; ionic, bound to proteins, lipids and membrane (Wieser and Klima 1969; Arumugam & Ravindranath 1983). In the estuarine crab Scylla serrata, the haemolymph copper exists only in association with proteins, whereas in the hepatopancreas it exists in all the four forms and in gills it exists in all the forms except in combination with lipids (Arumugam & Ravindranath 1983, 1986).

Although food is the major source of copper in decapod crustaceans (Zuckerkandl 1960; Wieser 1968) evidence indicate that copper may be directly obtained from the environment (Kerkut, Moritz & Munday 1961; Bryan 1971; Djangmah & Grove 1970). It was postulated earlier that in Scylla serrata the haemolymph and hepatopancreas may be involved in copper regulation (Arumugam & Ravindranath 1983).

In the present work we have studied the nature and levels of copper in different tissues after exposing the crabs to copper-rich medium. The results indicate the relative importance of various tissues in accumulation and the possible mechanisms of regulation of the environmental copper. Besides, as a pre-requisite for studies of this kind, the toxic levels for different forms of copper were estimated since the form of toxicant is known to influence the toxicity to the decapod crustaceans (Bryan 1971; Saliba & Kryz 1976).

MATERIALS AND METHODS

For the purpose of assessing survival of the estuarine crab, Scylla serrata (Forskal) in different concentrations of copper salts in different forms, five sets were maintained in each of the experimental media prepared by dissolving copper carbonate, copper chloride and copper sulphate in 50% artificial sea water, each with the concentrations of 0.1, 1.0, 10.0, 50.0, and 100 mg/L copper ions. The salts used are in cupric form. Five sets of control animals were maintained in 50% artificial sea water for 14 days. Only male, intermoult and uninjured crabs of 250-300 gms of body weight were used.

Each set (5-6 crabs) was placed individually in tanks (size 60 x 30x 21.5 cms) containing 2 litres of medium, and the medium was changed once in 2 days. The time of death of each crab was noted down and

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the percentage survival was calculated. After assesing the survival for 14 days, a set of 5-6 crabs was placed in a medium containing copper chloride (50mg/litre) for 48 hours. The tissues were removed and fractioned into TCA soluble and insoluble, chloroform : methanol soluble and insoluble as mentioned elsewhere (Arumugam & Ravindranath 1983). The nature and concentration of copper in haemolymph, hepatopancreas and gills after 24, 36 and 48 hours of exposure to the medium were analysed. The copper concentration in each fraction was determined using 2,2' Biquinoline method following suggestions of (Arumugam & Ravindranath 1980) and the absolute quantity of copper was calculated taking the mass/volume into consideration. The haemolymph protein concentration was estimated using Biuret method (Gornall et al. 1949). The copper/protein ratio was calculated following the suggestions of Horn & Kerr (1963). The only problem encountered is with reference to the gills of animals exposed to copper rich medium. In these animals the mucous covering the gills was dense and could not be removed completely from the individual rachis.

The blood volume was determined following the dye dilution technique using Congo red following suggestions of Mullainadhan & Ravindranath (1981).The statistical treatments include paired sample 't' test and mean difference 't' test following Bailey (1969).

RESULTS AND DISCUSSION

The crab Scylla serrata survived in media containing lower concentrations (0.1mg/L) of all the three forms of copper salts for 14 days. In copper carbonate, the crab survived in all the concentrations (0.1,1.0,10.0,50 and 100mg/L) during 14 days, whereas the percentages of mortality in the medium containing copper sulphate were 40, 80 and 100% at the level of 10, 50 and 100mg Cu/L respectively. On the otherhand, the percentage mortality were 60, 100 and 100% in the above said concentrations of copper chloride after 14 days of exposure. To study the regulation of environmental copper by the crab, 50mg copper/L concentration of copper chloride was selected, since it was observed that there was 100% mortality during 14 days of exposure and all the crabs survived well up to 48 hours.

The exposure of the crabs to 50 mg copper/L medium up to 48 hours, increased the concentration of copper in all the three tissues namely haemolymph, hepatopancreas and gills suggesting entry of copper into the animal.

In the haemolymph, it was observed that the copper concentration increased by 22% during the initial 24 hours period. However, the increase after 36 and 48 hours declined to 10 and 8% respectively (Table 1). The haemolymph protein concentration did not vary up to 36 hours of exposure and it did not show any change at 48 hours. The total quantity of haemolymph protein also followed the same pattern since there was no change in the blood volume (Table 1).

A concomitant rise in total quantity of copper was observed in hepatopancreas after 24, 36 and 48 hours of exposure. The increase was 48% after 24 hours of exposure and it declined to 24% after 36

hours. However, it was noticed to have increased to 50% after 48 hours of exposure (Table 2). In gills, the percentage increase in the total quantity of copper at 24, 36 and 48 hours after exposure was observed to be 207, 199 and 410% respectively (Table 3).

The analysis of different copper fractions reveal that the increase in copper content in hepatopancreas and gills was predominantly associated with the TCA-soluble fraction and only a slight increase in TCA-insoluble fraction. In gills, the chloroform-methanol insoluble fraction also increased.

The results indicate that one of the factors influencing copper toxicity is certainly the form of the metal itself. The copper carbonate is the least toxic form, may be due to the fact that it readily precipitates with the sea water (Saliba & Kryz 1976). Therefore, it is not suitable for this kind of study. Of the other two salts, copper sulphate seems to be less toxic than copper chloride, the results are in conformity with the findings of Saliba & Kryz (1976) in Artemia salina.

The increase in haemolmph and hepatopancreas copper content during the initial hours reveals that the copper would have gained entry into these tissues through gills. The percentage increase in the copper content of haemolymph declined after 36 and 48 hours, which may be due to the fact that the copper, which enters into the haemolymph would have been taken in by some other tissues or excreted through gills (Bryan 1968).

The haemolymph protein concentration and content did not vary despite the increase in haemolymph copper level during 24 and 36 hours of exposure, indicating that the existing proteins in haemolymph, the copper free protein and/or apohaemocyanin would have accommodated the excess copper entering into the haemolymph (Johnston & Barber 1969).After 48 hours, the haemolymph protein concentration increased probably to accommodate the excess copper. The origin of these proteins need elucidation.

After 24 hours the hepatopancreas accumulated the copper that has entered into the haemolymph from the environment (Kerkut et al.1961). The amount of copper accumulated in this tissue is about 48% and is prominent in the TCA-soluble fraction, which suggests that detoxification may be brought about by the low molecular weight substances present in this fraction. Probably, the copper may be accommodated as free ions or salts or with free aminoacids, dipeptides or nucleic acids or metallothioneins in this fraction; as suggested by Arumugam & Ravindranath (1983) and Overnell (1982) and needs further elucidation.

The possible pathway of elimination of this copper may be either via gut and excretion through faeces (Miyawaki et al 1961; Gibson & Barker 1979), or via haemolymph through excretory organs or gills (Bryan 1971). The continuous increase of copper concentration in gills supports the latter possibility. However, with the absence of any marked variation in haemolymph copper concentration after 36 hours, it is difficult to envisage how the copper from haemolymph

Table 1 Changes in the haemolymph copper (ug/ml), protein (mg/ml), copper/protein ratio (x 100) and blood volume (ml/animal) of crab Scylla serrata during different hours of exposure in the medium containing 50 mg copper/L as copper chloride

Constituents Analysed	Control	Hours of exposure		
		24	36	48
Copper	68.8 ± 16.6 85.1 ± 13.8 61.1 ± 15.1	84.0 ± 20.0*	94.1 ± 13.6**	65.9 ± 16.1*
Protein	56.6 ± 12.0 70.7 ± 15.7 49.5 ± 11.1	59.7 ± 12.3	73.8 ± 13.9	54.2 ± 11.8*
Copper/Protein ratio	0.126 ± 0.006 0.129 ± 0.017 0.135 ± 0.012	0.140 ± 0.019	0.135 ± 0.015	0.131 ± 0.012
Blood Volume #	23.4 ± 1.5	25.3 ± 2.4	23.3 ± 0.7	23.4 ± 1.8

Values expressed as mean ± S.E.; Sample size = 5.

* Indicates $P > 0.05$; ** indicates $P > 0.01$

Values obtained from 4 different groups of animals

Table 2 Changes in total mass, total copper and copper concentration in different fractions of hepatopancreas of crabs S. serrata during different hours of exposure to the medium containing 50 mg copper/L as copper chloride

Constituents analysed	Control	Hours of exposure		
		24	36	48
Total mass (gm/animal)	12.86 ± 0.85	10.94 ± 1.01	12.09 ± 1.80	10.62 ± 2.25
Copper concentration (ug/gm)				
TCA-soluble	96.1 ± 13.8	173.9 ± 11.9	154.3 ± 16.9*	242.4 ± 50.9
TCA-insoluble	35.6 ± 3.4	44.6 ± 0.6	29.5 ± 3.4	34.8 ± 5.60
Chloroform: Methanol soluble	13.2 ± 2.2	5.4 ± 0.9	5.7 ± 1.5	8.7 ± 2.8
Chloroform:Methanol insoluble	13.7 ± 1.2	13.4 ± 0.9	18.4 ± 3.9	24.6 ± 6.9
Total Concentration (ug/gm)	160.9 ± 14.7	274.2 ± 25.6	208.0 ± 20.5	298.0 ± 53.6*
Quantity (ug/animal)	2025.1 ±181.9	2998.4 ±280.0*	2516.2 ±247.5	3164.2 ±569.7

Value expressed as mean ± S.E.

Sample size : 6; * indicates $P > 0.05$

Table 3 Changes in total mass, total copper and copper concentration in different fractions of gills of S. serrata during different hours of exposure to the medium containing 50 mg copper/L as copper chloride

Constituents analysed	Control	Hours of exposure		
		24	36	48
Total mass (gm/animal)	7.82 ± 0.42	8.54 ± 0.43	8.65 ± 0.49	8.31 ± 0.63
Copper concentration (ug/gm)				
TCA-soluble	26.4 ± 4.3	72.2 ± 3.4*	103.1 ± 8.6	166.1 ± 27.2**
TCA-insoluble	25.3 ± 5.8	56.1 ± 3.5	15.6 ± 2.1	50.4 ± 9.8**
Chloroform:Methanol soluble	N.D.	N.D.	N.D.	N.D.
Chloroform:Methanol insoluble	6.5 ± 1.1	35.4 ± 6.4**	38.1 ± 4.5	144.6 ± 36.1**
Total concentration (ug/mg)	58.2 ± 9.3	163.7 ± 0.6**	150.8 ± 14.0	327.2 ± 58.2**
Quantity (ug/animal)	454.9 ± 73.3	1397.0 ± 4.7**	1359.9 ±121.6	2320.2 ±346.7*

Values expressed mean ± S.E.; Sample size = 6

* indicates $P > 0.05$; ** indicates $P > 0.01$

N.D. : not detectable

would have been eliminated by gills against concentration gradient. An observation that deserves consideration is the production of excess mucous by gills of the animals exposed to the toxic environment for a longer duration. Mucous is produced as a result of irritation caused by the toxicant (Bryan 1964). The observation of the excess accumulation of mucous in the animals which died upon exposure to higher copper concentrations reveals that the death would have occurred possibly due to excess mucous covering the gills and thus the consequent asphyxiation and hypoxia.

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