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Hemoglobin and Hematocrit Values in the Fish *Oreochromis mossambicus* (Peters) after Short Term Exposure to Copper and Mercury

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Haematology is used as an index of health status in a number of fish species (Blaxhall 1972). Haematological changes have been detected following different types of stress conditions like exposure to pollutants, diseases, hypoxia, etc. (Duthie and Tort 1985). Copper and mercury are two known aquatic pollutants. Though copper is an essential micro-nutrient, it is highly toxic at high concentrations (Tort et al 1987; El-Domiaty 1987). Mercury has no biological function to serve and causes serious impairment in the metabolic and physiological functions of the body (Calabrese et al 1975). In this paper haematocrit and haemoglobin (Hb) values in the fish Oreochromis mossambicus separately exposed to two different sublethal concentrations of copper and mercury for a period of 168 h are reported.

MATERIALS AND METHODS

Specimens of O. mossambicus were collected from Cochin area ($9^{o}58'$N, $76^{o}14'$E). They were acclimatized in the laboratory for a month. Fishes of length 9 to 11 cm and weighing 30 to 40 g were selected for the experiment. Later, they were transferred to large experimental tanks (250 L) containing 200 L dechlorinated tap water (pH 6.8; dissolved oxygen 4.8 mL/L; hardness 22 mg/L as $CaCO_3$). Calculated volumes of 1000 mg/L solutions of copper sulphate (BDH) and mercuric chloride (Merck) were separately added to the tanks to give concentrations of 100 ug/L and 200 ug/L for copper and 100 ug/L and 150 ug/L, for mercury. The last tank without any toxicant served as the control. The test medium was renewed every 24 h. The temperature in the tanks was maintained at $28 \pm 1^{o}C$. Fishes were not fed during the experiment. Fish blood samples were collected at 24, 72, 120 and 168 h by severing the caudal peduncle. Haemoglobin was determined by the cyanmethaemoglobin method (Drabkin 1946). Blood-filled heparinized microhaematocrit capillary tubes were centrifuged at 12000 rpm for 5 min and the haematocrit values were read directly. The results were analyzed statistically. The significant difference ($P < 0.05$) between experimental groups and control groups were determined using student's t-test.

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RESULTS AND DISCUSSION

The results are presented in Tables 1 and 2. The Hb values of copper dosed animals were significantly lower than the control at 24 h. But mercury-dosed fishes did not show any significant difference in the Hb content. At 72 h the copper-exposed fishes showed a different trend in Hb values. A highly significant increase in the Hb content of copper-treated fishes was seen at 72 h. But there was no statistical difference in the Hb values of mercury-dosed fishes at 24 h and 72 h. The Hb content increased significantly in the copper and mercury-dosed fishes at 120 and 168 h.

Haematocrit values did not alter significantly in all metal-treated fishes at 24 h. But at 72 h the copper-treated fishes and fishes exposed to the higher concentration (150 ug/L) of mercury showed a statistical increase in haematocrit values. At 120 and 168 h the haematocrit increased in all metal-treated fishes compared to the controls.

In the present study there is a distinct difference in Hb values between copper-dosed and mercury-dosed fishes in the initial stage (24 h) of the experiment. Unlike mercury, the copper-dosed fishes showed a significant decrease in Hb content at 24 h, but there was no change in the haematocrit values of these fishes. This shows a haemodilution and the resultant swelling of the erythrocytes combined with the release of erythrocytes from the erythropoietic organs. Haemodilution has been interpreted as a mechanism which reduces the concentration of an irritating factor in the circulatory system (Smit et al 1979). Haemodilution has been observed in <u>Colisa fasciatus</u> exposed to zinc by Mishra and Srivasthava (1979). Tort et al (1987) observed a process of erythrocyte swelling in the dog fish, <u>Scyliorhinus canicula</u> exposed to copper. Such an increase of erythrocyte size is generally considered as a response against stress. The swelling would be a consequence of factors like high PCO_2, high lactate concentration or low PO_2 in the blood, leading to a low ATP concentration, which would increase the oxygen affinity of blood (Soivio and Nikinmaa 1981). Since metals produce changes on blood gases and lactate, the swelling of red blood cells could be involved in the response of fish against heavy metal pollution (Tort et al 1987). A decrease in Hb content and an increased RBC count were observed by Tort and Torres (1988) in the dog fish <u>Scyliorhinus canicula</u> exposed to cadmium. Abrahamsson and Nilsson (1975) observed that the contraction of spleen of cod exposed to a stress would release blood cells into the blood stream. A similar pattern has been detected in <u>Cyprinus carpio</u> after Cd exposure (Koyama and Ozaki 1984). These findings also support the hypothesis that haemodilution is a probable cause for decrease in Hb content in copper-dosed fishes. Such a decrease in Hb value was also observed by van Vuren (1986) in <u>Labeo umbratus</u> exposed to various pollutants. Juveniles of <u>Clarias lazera</u> exposed to copper for 96 h showed a decrease in Hb values (El-Domiaty 1987). Tort et al (1987) observed this phenomenon in the dog fish exposed to copper.

Table 1. Haemoglobin values in *Oreochromis mossambicus* exposed to copper and mercury

Concentration ug/L	Hb g/100 mL blood, $\bar{x} \pm$ S.D. (N = 22) 24 hr	72 hr	120 hr	168 hr
Cu 100	8.59* ± 0.55	9.25** ± 0.69	9.92** ± 0.69	9.94** ± 0.63
200	8.28** ± 0.69	9.43** ± 0.45	10.19** ± 0.79	10.27** ± 0.71
Hg 100	8.88 ± 0.67	8.57 ± 0.88	9.15** ± 0.58	9.56** ± 0.67
150	8.76 ± 0.51	8.51 ± 0.73	9.34** ± 0.62	9.70** ± 0.57
Control	9.01 ± 0.74	8.52 ± 0.84	8.19 ± 0.76	8.05 ± 0.53

Table 2. Haematocrit values in *Oreochromis mossambicus* exposed to copper and mercury

Concentration ug/L	Haematocrit percentage, $\bar{x} \pm$ S.D. (N = 12) 24 hr	72 hr	120 hr	168 hr
Cu 100	34 ± 2.6	34* ± 2.9	35** ± 2.6	35** ± 3.1
200	34 ± 2.6	34* ± 3.4	38** ± 2.3	39** ± 2.7
Hg 100	34 ± 2.2	33 ± 2.9	34* ± 2.8	35** ± 2.9
150	33 ± 2.4	34* ± 2.8	36** ± 2.4	37** ± 2.3
Control	33 ± 2.8	31 ± 2.7	31 ± 2.9	30 ± 2.4

*P 0.05 $\leq$ **P$\leq$0.01

At 72 h the copper-dosed _O. mossambicus_ showed a reverse trend. There was a significant increase in Hb content and a corresponding increase in haematocrit values. The body of the copper-exposed fish might have adapted to the metal stress by this time. Haemodilution could be an initial reaction of the body to the stress. Afterwards the living system rectified the imbalance by removing water from the blood. This could result in haemoconcentration.

The Hb content in both the copper and mercury-exposed fishes increased at 120 and 168 h. There was a corresponding increase in the haematocrit values as well. The effect of mercury in fish was felt only at 120 h and after. This could have been due to an increased production by the erythropoietic organs. McKim et al (1970) found a statistically significant increase in RBC count, haematocrit, and Hb in the brook trout _Salvelinus fontinalis_ after short term (6 d) and long term (21 d and 337 d) exposure to copper. Svobodova (1982) obtained a highly significant increase in haematocrit, RBC count, and Hb in _C. carpio_ exposed to copper for 48 h. The mean cell volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) in this experiment remained without change. This indicates that the increase in Hb is due to an increase in RBC number. He has also explained the changes in the haematological parameters in the intoxicated carp as disorders in the oxidation process in the fish. Haematocrit and Hb values were elevated at 6 and 30 d in _Ictalurus nebulosus_ exposed to copper. But the total RBC count was constant. This indicated an increase in average cell size, due to either cellular swelling or mortality of small immature cells and replacement by larger cells from the spleen (Christensen et al 1972).

Buckley (1976) has shown that there is an increase in the number of circulating immature erythrocytes, when fishes were exposed to different pollutants. This may be due to a stimulation of erythropoiesis by elevated demands for O_2 or CO_2 transport as a result of increased metabolic activity or by destruction of gill membranes causing faulty gaseous exchange. Davis (1973) found an increase in the oxygen uptake, ventilatory water flow, cough, and buccal pressure in sockeye salmon (_Oncorhynchus nerka_) exposed to Bleached Kraft Pulp Mill Effluent (BKME). He also found that arterial oxygen tension decreases rapidly when exposed to BKME. On the average, this decline represented a 20% decrease in oxygen saturation of the blood. Increase in Hb content could be a mechanism by which the body tries to absorb more O_2 from the surrounding medium to meet the increased demand.

It has been widely reported that many pollutants enter the RBC and either oxidize or denature the Hb by inhibiting the glycolysis or metabolism of the hexose monophosphate shunt (HMPS). Buckley (1976) found degenerative changes including formation of Heinz bodies in the erythrocytes in fingerlings of coho salmon (_Oncorhynchus kisutch_) exposed to chlorinated waste water containing Total Residual Chlorine ($TRCl_2$). Fairbanks (1967) showed that copper penetrate the intact erythrocyte, inhibiting glycolysis, denaturing Hb and oxidising glutathione. The increased Hb content in metal exposed fishes could be explained as a process where the body produces an increased amount of Hb to replace the oxidized or denatured Hb formed as a result of metal exposure. In a study on the mechanism of acute toxicity of monochloramine to fathead minnows (Pimephales promelas), Grothe and Eaton (1975) found a methemoglobin (Mhb) level of 30% of total Hb. Therefore, methemoglobinemia and the resulting anoxia were considered as the basis for toxicity of monochloramine under test conditions (Buckley 1976). So, formation of methemoglobin reduces the oxygen carrying capacity of the blood. Reaction of the body to this situation would be by stimulating the erythropoietic tissue and increasing the Hb content of the blood.

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