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Effects of Water-Borne Copper and Lead on the Peripheral Blood in the Rosy Barb, *Barbus* (*Puntius*) *conchonius* Hamilton

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Hemopathologies causally related to a variety of natural and simulated conditions have been extensively studied in fishes. The blood parameters of diagnostic importance such as erythrocyte and leucocyte counts, Hb, Hct, and leucocyte differential counts usually readily respond to incidental factors such as physical stress (Railo et al. 1985) and environmental stress due to water contaminants (Buckley 1977). Blood changes in fishes resulting in anemia (Johansson-Sjobeck & Larsson 1979), eosinophilia (Gardner & Yevich 1970), increase in circulating immature erythrocyte counts and lymphocytosis (Dawson 1935), and cytopathological changes in erythrocytes and altered leucocyte distribution (Gill & Pant 1987) may be induced by a variety of toxic chemicals. Exposure to low concentrations of Cu is known to cause increased RBC production, greater Hb synthesis, and decrease in available liver Fe stores in the brook trout, *Salvelinus fontinalis* (McFadden 1965). Effects of experimental Cu poisoning on the peripheral blood of other teleosts have also been described (Christensen et al. 1972, Hilmy et al. 1978).

The Pb-induced abnormalities of hematopoiesis are primarily confined to the erythrocytes; the leucocytes and platelets do not appear to be altered during chronic exposure. Besides, an acute hemolytic crisis resulting in anemia and stippling of erythrocytes have been described in Pb-exposed mammals (Albahary 1972). The enzyme, δ-aminolevulinic acid dehydratase (δ-ALAD), which catalyzes an early step in the synthesis of protoporphoryn and heme for Hb, is stated to be inhibited by Pb (Hernberg & Nikkanen 1970), and therefore, provides a useful diagnostic tool to assess the extent of Pb poisoning (Somashekharaih et al. 1990). Limited hematological investigations on the Pb effects in fishes show responses similar to those in mammals. Hodson (1976) showed depressed activity of red blood cell δ-ALAD in the rainbow trout, *Salmo gairdneri*, brook trout, *Salvelinus fontinalis*, goldfish, *Carassius auratus*, and pumpkin seeds, *Lepomis gibbosus*, exposed to Pb. Further, the absence of erythrocyte δ-ALAD inhibition in fish exposed to Cd, Cu, Zn, and Hg indicated that this enzyme is quite specific for Pb. In the Kuwait mullet, *Liza macrolepis*, Hilmy et al. (1978) observed significant polychromasia, +1 anisocytosis, increase in eosinophils, and a decrease in lymphocytes following 96-h exposure to graded doses of 1.15 - 18.36 mg/l Pb in a flow through marine bioassay system. The objective of this work was to examine the effects of chronically sublethal concentrations of Cu and Pb on the peripheral blood parameters in the rosy barb, *Barbus* (*Puntius*) *conchonius*, a widely distributed freshwater bony fish.

MATERIALS AND METHODS

Ninety six rosy barbs, *Barbus (Puntius) conchonius* Hamilton, with a mean body weight of 5±0.03 g (mean±SE) were collected from Naini Tal lake and held in an indoor 400-litre stocking tank. During acclimatization to laboratory conditions for 2 weeks, fish were maintained in tap water with continuous aeration, pH 7.3, at 18±1°C on a 13/11 light/dark regime, and fed commercial fish food ad libitum. Before the experiments began, fish were divided into three groups (n=32 individuals each) of which two, Groups I and II, were kept

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in experimental tanks (50-litre capacity) and one, Group III, served as common control. Groups I and II were exposed to metal concentrations equivalent to 1/3rd of their 96-h median tolerance limit (TLm), being 0.190 mg/l Cu as copper suphate and 0.126 mg/l Pb as lead nitrate, dissolved in the test medium, hardness 359 mg/l and 6 mg/l (as $CaCO_3$), respectively. During the experimental period, the test medium was continuously aerated and completely renewed every week to sustain nominal concentrations.

Blood samples were taken four times during the 8 week exposure period (after 2, 4, 6, and 8 weeks) from unanaesthetized control and experimental fish (n=8 individuals each). Fresh blood collected from ductus cuvieri was immediately used for (i) total erythrocyte counts using Spencer's brightline hematocytometer at 1:200 dilution, (ii) Hb by cyanmethemoglobin method, (iii) Hct by centrifuging blood-filled microhematocrit capillary tubes, and (iv) preparation of blood smears for evaluating percent occurrence of leucocyte subpopulations. For differential counts, leucocytes categorized into large and small lymphocytes, monocytes, neutrophils, thrombocytes, and basophils were scored under an oil immersion objective on blood smears stained with Leishman's or Giemsa's stain (2 slides per individual). All results are given in terms of percent change from controls, and the data were subjected to t-test.

RESULTS AND DISCUSSION

In the present study, prolonged exposure to a sublethal concentration of Cu resulted in a consistent polycythemia accompanied by a marked decrease in the Hb and Hct (Fig. 1). The mean corpuscular hemoglobin concentration (MCHC) was reduced by 23 and12% after 2 and 4 weeks.These findings in the rosy barb are in partial agreement with results obtained in other piscine species. For example, the brook trout, *Salvelinus fontinalis*, also showed an increase in RBC counts during long-term Cu exposure, but unlike the rosy barb, Hb and Hct were raised as well. An increase in Hb and Hct was also noted in the brown bullhead, *Ictalurus nebulosus*, exposed to 3.4-104 mg/l Cu (II) (Christensen et al. 1972). Lanno et al. (1985) conducted feeding trials to determine maximum tolerable and toxicity levels of dietary Cu to rainbow trout, *Salmo gairdneri*, and found that at 730 mg Cu/kg diet, reduction in growth, feed refusal, and a rise in hepatic Cu levels occurred. The dietary Cu, however, had no significant effect on Hb, Hct, plasma Cu, glucose, and liver size. On the other hand, Cu intoxication by bath caused a reduction in erythrocyte count, Hb and Hct in juvenile *Clarias lazera* (El-Domiaty 1987). Although Cu effects on fish erythrocyte counts are, in general, inconsistent, hemolytic anemia seems to be a fairly common finding. This is reasonable because excess dietary Cu is a known antagonist of Fe absorption (El-Shobaki & Rummel 1979). Therefore, the anemic condition observed in the Cu-exposed rosy barbs, despite elevated erythrocyte numbers, could be due to Fe deficiency. Furthermore, since MCHC is the ratio of blood hemoglobin concentration to Hct, it is not dependent on the number of circulating erythrocytes or blood volume, and a reduction in this parameter would indicate release of new Hb-deficient erythrocytes from hematopoietic loci into general circulation. As the Cu intoxication caused an increase in the erythrocyte counts with a concomitant reduction in MCHC, defective erythropoiesis would seem to be the most logical explanation for the observed effects in the rosy barb.

Long-term Pb exposure in the rosy barb induced a progressive erythropenia with parallel reductions in the Hb and Hct (Fig. 2). Johansson-Sjobeck & Larsson (1979) found decreased erythrocyte counts and MCHC in the rainbow trout, *Salmo gairdneri*, exposed to 300 mg Pb/l for 30 days. The Hct remained unchanged in this species related to an increase in the corpuscular volume. In the rosy barb, however, red cell volume was decreased up to 4 weeks, insigificantly though, which suggests microcytic anemia. Anemia in Pb-exposed mammals is stated to be micro- or normo-cytic (Tuschiya 1979), and is the result of inhibition of δ-ALAD which catalyzes the formation of porphobilinogen from δ-aminolevulinic acid (Albahary 1972). In fishes also, the sensitivity of δ-ALAD to Pb

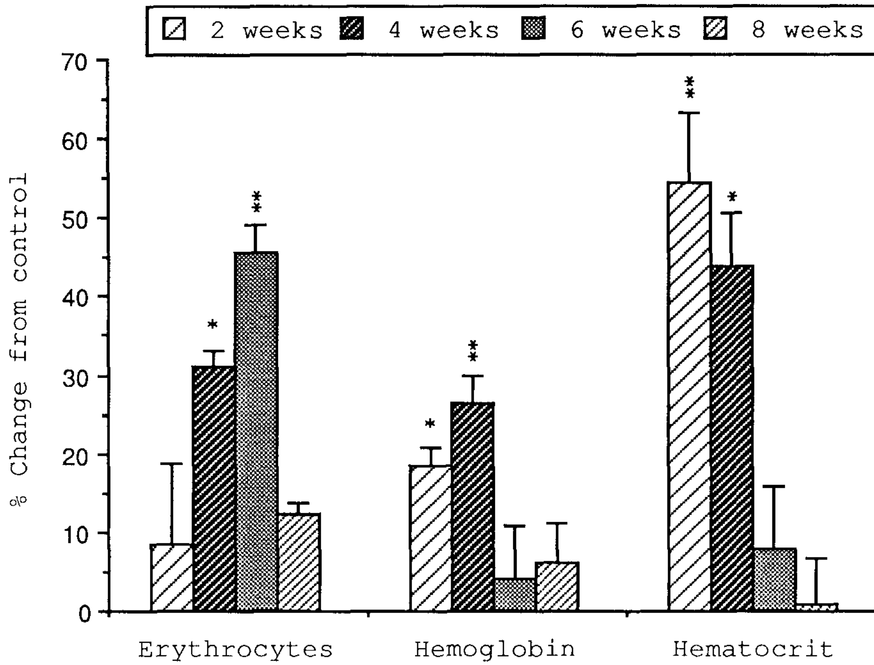

Figure 1. Changes in the erythrocytic indices in Barbus conchonius chronically exposed to Cu (0.190 mg/l). Mean±SE (n=8). *p<0.05, **p<0.01, ***p<0.001.

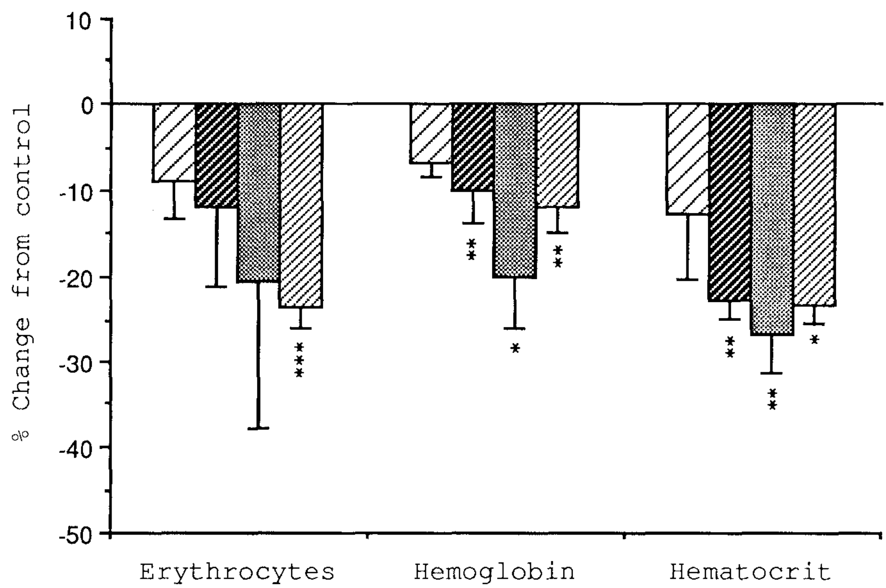

Figure 2. Changes in the erythrocytic indices in Barbus conchonius chronically exposed to Pb (0.126 mg/l). Mean±SE (n=8). *p<0.05, **p<0.01, ***p<0.001.

poisoning has been demonstrated in the liver, kidney, spleen, and erythrocytes of different species (Hodson 1976, Johansson-Sjobeck & Larsson 1979). In the rainbow trout, *Salmo gairdneri*, δ-ALAD activity remained unaltered in the head kidney while the trunk kidney showed only a small decrease in response to Pb exposure (Johansson-Sjobeck & Larsson 1979). The authors opined that this indicated a stimulated erythropoietic activity in the head kidney as a probable compensation to decreased δ-ALAD activity in the erythrocytes and other blood anomalies.

The erythrocyte counts in the Pb-exposed rosy barbs were found to be consistently depressed. It has been suspected for long that Pb damages plasma membranes of young and mature erythrocytes in the erythropoietic sites and peripheral blood, considerably shortening their survival. Also, the hemolytic effect of Pb occurs independently of the effect on different stages in the heme biosynthesis in the erythroid precursors (Albahary 1972). It appears that in the fish under report, Pb-induced erythroclasia surpasses the red blood cell production and release resulting in an overall erythropenic situation. Further, the persistence of toxic cations under prolonged exposure seems to override any compensatory effort by the exposed fish.

Responses of white blood cells to Cu poisoning in the rosy barb included a reduction in both large (2 and 4 weeks) and small (2 weeks) lymphocytes and a consistently significant rise in monocyte subpopulations (Fig. 3). After 4 weeks, however, the small lymphocyte counts registered a marked rise lasting until the end of stipulated 8-week exposure. Like Cu, Pb also induced a statistically significant reduction in large (2, 4, and 8 weeks) and small (4 weeks) lymphocytes, but, unlike in the Cu-exposed fish, consistently severe monocytopenia was noted (Fig. 4). However, significantly elevated large (6 weeks) and small (6 and 8 weeks) lymphocyte counts were also manifested in the Pb-exposed fish. Both Cu and Pb caused, in general, the large lymphocyte numbers to fall below control levels but small lymphocytes exhibited an increase. This suggests a compensatory increase in the number of circulating lymphocytes apparently necessitated by an accelerated peripheral destruction of large lymphocytes. However, as the rate of differentiation of small lymphocytes into large ones does not seem to be fast enough, an overall increase in the small lymphocytes results. Mushiake et al. (1985) studied susceptibility of the Japanese eel, *Anguilla japonica*, to the bacterial pathogens, *Edwardsia tarda* and *Pseudomonas anguilliseptica*, under exposure to 100 or 250 mg Cu/l for 24 and 48 hours, and found a decrease in the number of lymphocytes and granulocytes along with increased corticosteroids in the plasma of Cu-exposed eels. The authors opined that increased susceptibility to bacteria in the Cu-exposed eels was due to lowered phagocytosis.

Studies on leucocyte responses to Cu and Pb in fishes are relatively few and no definite pattern seems to exist with regard to the fluctuations in their subpopulations. For example, Pb poisoning of the catfish, *Ameiurus nebulosus*, induced an increase in small and large lymphocytes, monocytes, large granular eosinophils, basophils, and thrombocytes including 'spindle cells' (Dawson 1935). The rainbow trout, *Salmo gairdneri*, on the other hand, showed no alteration in the number of lymphocytes, thrombocytes, and neutrophilic granulocytes during chronic Pb exposure (Johansson-Sjobeck & Larsson 1979). Interestingly, the authors noted that the number of lymphocytes and thrombocytes doubled in both the Pb-treated and controls after a 40-day recovery in clean water. Results of the present study reveal that thrombocyte counts were both increased and decreased after Cu and Pb exposure (Fig. 5 and 6). The neutrophilic granulocytes were generally depressed and basophils increased in the Cu-exposed fish, but in those exposed to Pb neutrophils were increased (except after 6 weeks) while basophils were marginally reduced in numbers. Changes in thrombocyte counts may depend upon both the rate of thrombopoiesis as well as their peripheral destruction. Similarly, fluctuations in the neutrophils and monocytes apparently reflect the magnitude

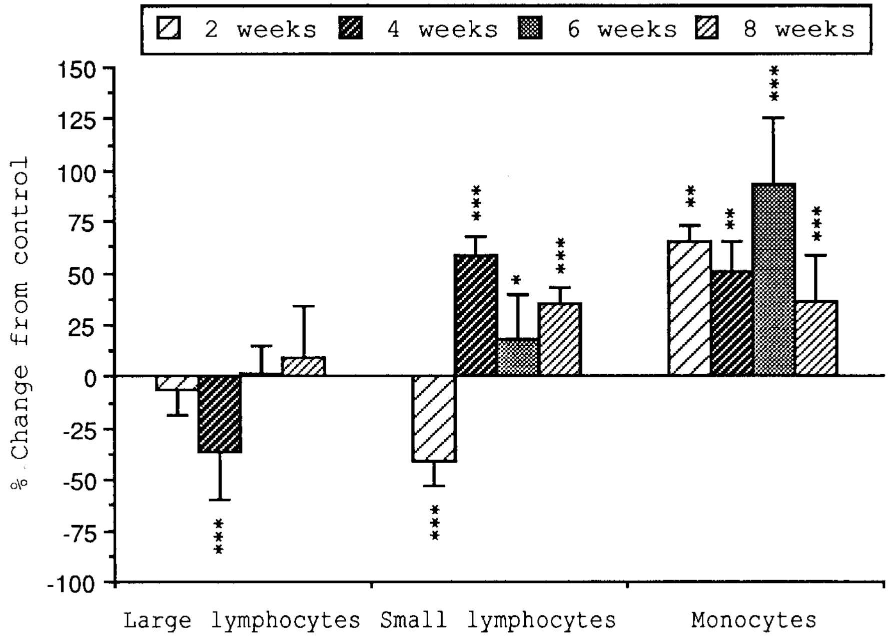

Figure 3. Changes in the leucocyte ratios in Barbus conchonius chronically exposed to Cu (0.190 mg/l). Mean±SE (n=8). *p<0.05, **p<0.01, ***p<0.001.

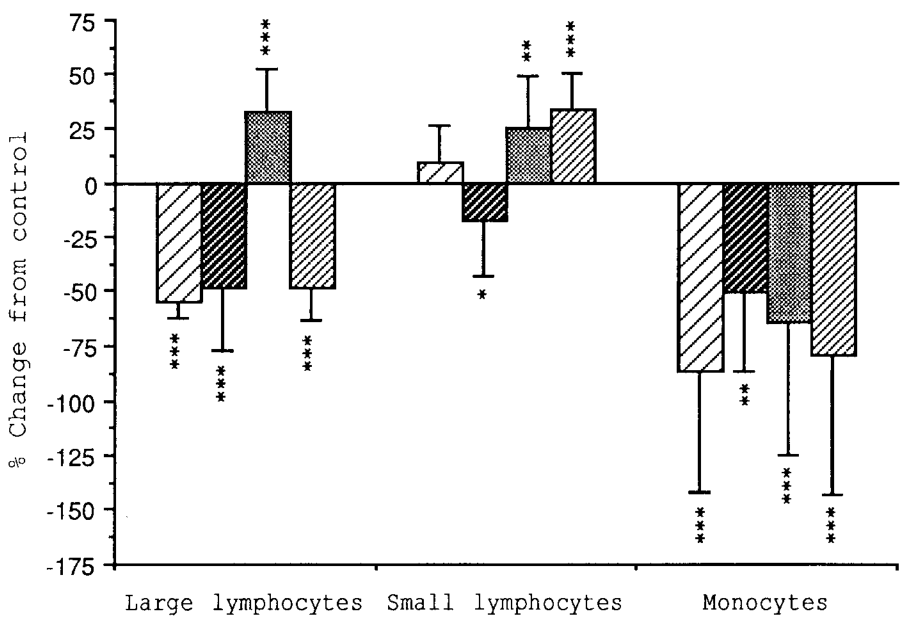

Figure 4. Changes in the leucocyte ratios in Barbus conchonius chronically exposed to Pb (0.126 mg/l). Mean±SE (n=8). *p<0.05, **p<0.01, ***p<0.001.

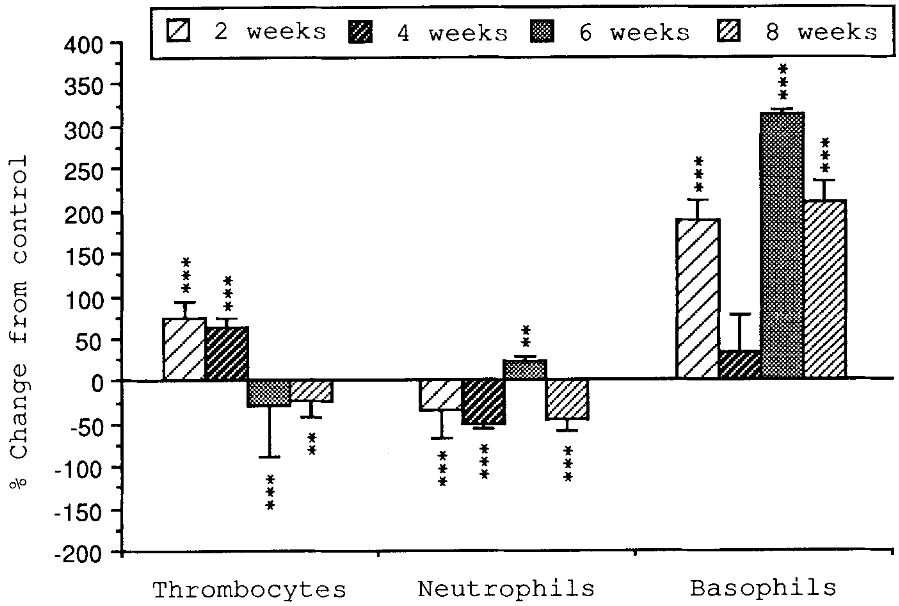

Figure 5. Changes in the leucocyte ratios in Barbus conchonius chronically exposed to Cu (0.190 mg/l). Mean±SE (n=8). *p<0.05, **p<0.01, ***p<0.001.

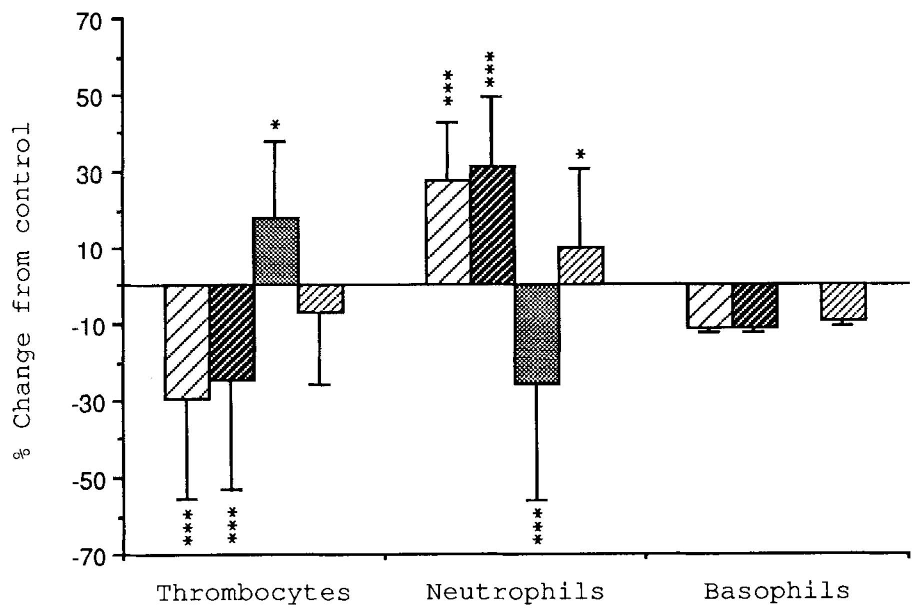

Figure. 6. Changes in the leucocyte ratios in Barbus conchonius chronically exposec to Pb (0.126 mg/l). Mean±SE (n=8). *p<0.05, **p<0.01, ***p<0.001.

of phagocytic activity for these cells, like their counterparts in the mammals, are implicated with phagocytosis (Ellis 1977). Structural damage is also known to occur in response to heavy metal exposure (Gardner & Yevich 1970) and this probably mobilizes phagocytic machinery to remove cell organelle and other debris from the lesioned tissues. Obviously, a reduction in neutrophils and thrombocytes would indicate lowered phagocytic activity, and, therefore, increased susceptibility to infections and potential hemorrhagic risk, respectively.

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