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# 2,3',4-Triaminoazobenzene-Induced Hematobiochemical Anomalies in Fish (*Channa punctatus*)

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Fish blood is increasingly studied for toxicological research, for environmental monitoring using fish, and as a possible indicator of physiological or pathological change in fishery management and disease investigations. Despite many publications in the field (HOWKINS & MAWDESLEY THOMAS 1972), fish hematology in health and disease is still in a fragmented and developing state and as yet general indicative principles have not emerged.

The scattered literature on blood change in fish pathology is varied in content and objective. The fish hematology under chemical stress (pesticidial and other) has been studied by many workers (SCHIFFMANN & FROMN 1959, GLAGOLOVA & NALIKOVA 1968, SAAD et al. 1973, CAIMS & CHRISTIAN 1978, MAHAJAN et al. 1979). The hazardous contamination of the fish habitat by dyes (both azo and nonazo) in industrial wastes from industries (like textile etc.) is of great concern. The runoff of these dyes in the inland fresh water reservoirs creates a critical chemico-azo stress and sometimes causes a huge mortality among nutritious fishes.

The present paper deals with the hematobiochemical alterations in Channa induced by the chronic exposure to 2,4-diamino, 3-aminoazobenzene (DAAB).

## MATERIAL & METHODS

Living fishes (Channa punctatus) were procured from local fresh water resources and kept in a big glass aquarium to acclimatize to laboratory conditions for one week, before the experiment was conducted. About 40 fishes (40 to 60 g) of the experimental group were treated by a sublethal dosage of 0.0025% dye (DAAB) [LC(50) being 0.005% for 96 hr] by bath in a separate aquarium. The forty fishes of control group (40 to 60 g) were kept in ordinary tap water having no dye. Both the aquaria of fishes of control and experimental group were seal-covered with black paper to avoid any possible photo-oxidation of the dye. The absorbency of the azo group of dye molecule was determined before and after the experiment. There was no detectable change in the wave-length of absorption maxima throughout the experiment. It showed no structural change in the dye molecule and hence in the concentration of dye.

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The fishes of both experimental and control groups were sacrificed after one month and the blood from the cut caudal vein was collected in a vial having EDTA anticoagulant. The red cell count per cmm of blood was determined by Newbauer double haemocytometer (Germany) using Hayem's solution as the diluting fluid. The haemoglobin concentration per dl of blood was determined by Sahli's haemometer (Germany). The haematocrit value (Packed Cell Volume, PCV) was determined with Wintrobe's haematocrit pipette and micro-haematocrit pipette after VAN ALLEN (USA). The tubes were centrifuged for 30 min at 4000 rpm. From these data Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated by standard methods (DACIE & LEWIS 1977). Surface area of red cells and their nuclei was calculated by measuring their largest diameters in length and width with a micro-oculometer (Germany).

For biochemical analysis of the blood of fish of control and experimental groups, the methods, as given by OSER (1965), have been followed. Serum amylase has been determined according to the method of STREET & CLOSE (1956). The experiment was conducted in the month of October at room temperature of 27°C. The 't' test was applied to study the significance of the differences between the control and the treatment means.

## RESULTS

The comparative data of the hematological and biochemical analysis of both control and experimental fishes have been summarized in Table 1. Each value represents an average of measurements from 40 fishes.

The results indicate that there was a marked fall in the red cell count, haematocrit value (PCV) and haemoglobin content. While the increase in red cell number was approximately 50%, the white cell count only increased by about 14%. Thus, the long term exposure to DAAB induced anemia though Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH) increased. However, the ratio of surface area of red cell and their nuclei was least affected.

Biochemical analysis of the plasma revealed a significant decrease of total proteins and sugar associated with an increase in cholesterol, urea nitrogen, creatinine, calcium and magnesium in the blood of treated fishes. The changes in chlorides and phosphorus are insignificant. Enzymological observations indicate suppressed activity of phosphatases and amylase associated with enhanced reaction for transaminases (GOT & GPT) and lipase in plasma of treatment group in comparison to

TABLE 1

Haematobiochemical analyses of DAAB treated and control group of Channa

| | Control | Treated | |
|---|---|---|---|
| Red cell x $10^6$/cmm | 1.9±0.016 | 0.94±0.007 | b |
| White cell x $10^3$/cmm | 8.4±0.005 | 9.50±0.020 | b |
| Haemoglobin/dl | 10.0±0.16 | 6.2±0.12 | b |
| PCV/dl | 22.5±1.05 | 15.5±1.8 | a |
| MCV/µmsq | 118.5±2.08 | 156.5±2.4 | b |
| MCHC g/dl | 33.3±1.04 | 40.0±1.95 | a |
| MCH pg | 52.6±1.90 | 65.9±2.01 | a |
| Red cell:white cell | 237.5±3.70 | 99.1±1.05 | b |
| Nuclear surface area of red cell (n) µmsq | 15.08±1.3 | 14.8±2.9 | |
| Surface area of red cell (c) µmsq | 74.9±3.2 | 70.05±2.69 | |
| n/c | 0.20 | 0.21 | |
| Icteric index | 15.6±0.25 | 40.0±4.0 | b |
| Proteins mg/ml | 10.0±0.25 | 4.0±0.8 | b |
| Reducing sugar mg/ml | 0.7±0.00 | 0.32±0.001 | b |
| Cholesterol mg/ml | 1.95±0.0025 | 2.25±0.004 | b |
| Iron mg/ml | 22.1±2.80 | 20.4±3.10 | |
| Calcium mg/ml | 19.4±0.013 | 32.0±6.3 | |
| Magnesium mg/ml | 0.25±0.16 | 0.4 ±0.00 | |
| Inorg. phosphorus mg/ml | 1.0±0.00 | 0.66±0.00 | |
| Chlorides mg/ml | 11.3±0.0016 | 13.5±0.05 | b |
| Urea N mg/dl | 36.2±1.2 | 49.9±0.8 | b |
| Creatinine mg/dl | 4.09±0.04 | 5.8±0.2 | b |
| Phosphatase alkaline* | 0.76±0.022 | 0.49±0.002 | b |
| Phosphatase acid* | 0.53±0.028 | 0.34±0.006 | b |
| 5-nucleotidase* | 0.34±0.05 | 0.18±0.00 | a |
| Glucose-6-phosphatase* | 0.31±0.16 | 0.14±0.001 | |
| Lipase units/ml | 0.161±0.003 | 0.4±0.001 | b |
| Amylase units/ml | 0.45±0.16 | 0.28±0.00 | |
| GOT units/dl | 7.5±0.5 | 9.7±0.93 | |
| GPT units/dl | 5.8±0.08 | 11.02±0.88 | a |

All values are mean ± S.E.
*Activity expressed in terms of mg of phosphorus liberated per hour per ml blood.
a, $P < 0.05$; b, $P < 0.01$.

control fishes. Observations on icteric index indicate a possible increase of bile pigments in the blood of experimental fishes.

## DISCUSSION

The present study demonstrates that long term exposure to DAAB induces an acute anemia: the red cell count, Hb content and PCV decreased significantly. Similar altered blood parameters have been reported in fishes by CHRISTENSSEN et al. (1972), PANIGRAHI (1977), PANDEY et al. (1976) and GOEL et al. (1979) under different chemical stresses. Erythropenia has been reported by JOSHI et al. (1979) in Heteropneustes following its exposure to folidol. AGRAWAL et al.(1978) have also reported for the macrocytic anemia in Clarias treated with alloxan. However, SHAMMI & QAYYUM (1978), QAYYUM & SHAMMI (1979) and VERMA et al. (1978) demonstrated an increase in red and white cell count and haematocrit value in Saccobranchus and Clarias, respectively, after exposure to chlordant, gammexene and lindane. Similarly SINGH et al. (1979) have shown that endrin poisoning is associated with polycythemia (an increased in red cell count followed by an increase in Hb content and PCV). Our results on total white cell count are similar to the findings of PANDEY et al. (1976), VERMA et al. (1978) and AGRAWAL & GOEL (1978). Erythropenia is considered to be the result of suppressing effect of toxin on erythropoiesis. The exact mechanism of increase in total white cell count, not well understood, is thought to be an adaptive response to dye treatment. The red:white cell ratio is reduced in the fishes of treatment group, which reflects more a drop in red cell count than a rise in the number of white cells. These findings are similar to reports of MULCAHY (1963). Lowering of red cell count, haematocrit and Hb have also been found in Esox lucicus by MULCAHY (1963) during lymphosarcoma state.

Biochemical measurements revealed a marked decrease in plasma proteins and sugar associated with an increase of calcium, magnesium, urea, creatinine and cholesterol. Changes in iron and chloride were slight. Marked alterations in plasma proteins, glucose and total chlorides levels have been reported by CHRISTENSSEN et al. (1972) in fishes exposed to copper. These changes, specially fall in plasma proteins and sugar, are deemed secondary effects due to the impaired function of kidney induced by DAAB treatment (GOEL & GARG 1980, in press). These findings are consonant with the reports of MULCAHY (1969).

Increase of serum urea nitrogen and creatinine reflects the malfunction of kidney under chemico-azo

stress of DAAB. GOEL & GARG (1980, in press) have shown dysfunction of renal tissue, it being functionally insignificant because of cellular damage after DAAB exposure.

The increase in icteric index of plasma of treated fishes indicated a higher concentration of bile pigments in plasma which is correlated with hepato-dysfunction (GOEL & GARG 1980, in press) during dye intoxication. Hepatomal dysfunction also leads to an elevation of cholesterol level in serum of treated fishes. Elevated serum cholesterol associated with impaired hepatic function has been reported by EISLER (1972).

Enzymological results show the suppressed phosphatases' activity in the serum under DAAB chemical stress. However, transaminases analyses show increased reaction which is about 30% in case of GOT (glutamic-oxaloacetate transaminase) and about 90% in case of GPT (glutamic-pyruvate transaminase). It is revealed that the ratio GOT/GPT in the serum of the treated fishes is less than 1.0 and this value is more than 1.0 in normal fishes. These results are almost similar to the findings of SCHNEIDERBAUER & RETTENBACHER (1959). GOEL & GARG (1980 in press) have found the hepatomal dysfunction associated with cellular dysarchitecture of the liver in fishes exposed to DAAB. The injured liver cells under chemico-azo stress of DAAB are considered to release more of GOT and GPT, elevating serum GOT and GPT levels, the release of GPT being comparatively greater than GOT. In fact, the enzyme pattern in the serum reflects physiological state of the organs. This pattern is characteristically changed according to a distorted pattern of the enzyme resulting from cellular injury of an organ caused by toxins or diseases. Moreover, the distortion of the enzyme pattern of an organ, with reference to the passage of enzymes into the serum, depends on the severity of the cellular damage. SHAY et al. (1957) and WALMAN & BORMAN (1959), as well, hold that the changes in the serum enzymatic activity are secondary to the liver dysfunction. Increased serum transaminases' activity has also been shown by $CCl_4$ poisoning by PRISE-DAVIES & WILKINSON (1965) and MOLANDER et al. (1955).

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