Bull. Environm. Contam. Toxicol. 23,784-787 (1979)

Effect of Mercury on the Morphology of Erythrocytes in *Anabas scandens*

A. K. Panigrahi and B. N. Misra

Laboratory of Environmental Toxicology, Department of Botany, Berhampur University, Berhampur-760007, Orissa, India

Relatively little is known about the effects of mercurial compounds on the morphology of erythrocytes in fresh water teleosts. Until recently studies mostly pertaining to the changes in haematological parameters were carried out by FANGE & JOHANSSON-SJOBECK 1975, HOUSTON et al. 1976, CALABRESE et al. 1975, LEVANDER et al. 1977, WESTMAN et al. 1975, JOHANSSON-SJOBECK et al. 1975, O'CONNOR & FROMM 1975 and PANIGRAHI & MISRA 1978. Previous reports from this laboratory have shown that mercury caused hemolytic anemia.

The purpose of this investigation was to study the effect of inorganic mercury on the morphology of erythrocytes in a freshwater fish, Anabas scandens Cuv. and Val.

MATERIALS AND METHODS

A. scandens of medium size (10-15 cm in length and 8-15 g weight) were collected from the Nursery, Berhampur (Ganjam), Orissa and were acclimatised in the laboratory. Fish were fed with mercury free chopped goat liver daily during holding and throughout the exposure period. Twenty fish were exposed in 100-L glass tanks filled to 50 L. The test solutions were changed daily to maintain the constancy of the concentration of the medium at 0.5 mg/L of mercury as mercuric nitrate. Air was bubbled through the tank water for 8-10 h a day and kept under 10 h of illumination/day at 1500-1600 Lux and at 28 ± 2° C. Dissolved oxygen of water was maintained at 95% or more air saturation. The following is the water quality of both control and treated media: pH = 7.5 ± 0.3; Specific conductivity = 3.4 x 100 μmho; Total hardness = 78 ± 4 mg/L.

Blood was collected directly from the ventral aorta with a heparinised syringe and a drop of blood was spread on a clean slide following the usual procedure described by DACIE & LEWIS (1970). The blood smear was prepared, stained with Leishman's stain and examined.

RESULTS

An enlargement of red blood cells (RBC) in exposed fish A. scandens was observed, when compared to RBCs of control fishes within 7 days of exposure (Fig. 2 & 3).

A striking difference in RBC morphology was noticed in exposed fishes when compared to control fishes. After 7 days, finger shaped proliferations were marked in the neutrophilic granulocytes of exposed fishes (Fig. 2). Vacuoles were seen in the RBCs (Fig. 3). Beak-

0007-4861/79/0023-0784 $01.00

like structures appeared on the RBCs (Fig. 3) after 14 days of exposure.

Rupturing of RBC membrane after 28 days exposure was noticed (Fig. 4) and in some cells disintegration of nucleus and cell membrane was apparent. After 35 days exposure complete hemolysis and disappearance of cell membrane was marked (Fig. 4).

DISCUSSION

Results from the previous investigation by PANIGRAHI & MISRA (1979) had shown a remarkable increase in haematocrit value up to an exposure period of 21 days and then a depletion in haematocrit value on further exposure. The rise in haematocrit value was due to swelling of erythrocytes (Fig. 2 & 3). Swelling of the RBC was probably due to change in the osmotic pressure induced by mercury. As reported earlier (SOVIO & NYHOLM 1974) swelling of RBCs used to take place under reduced O_2 tension but the trend was reversible in O_2 equilibrated condition. The decrease in haematocrit value was due to shrinkage of the RBCs as reported by SOVIO & NYHOLM (1974) seemed improbable.

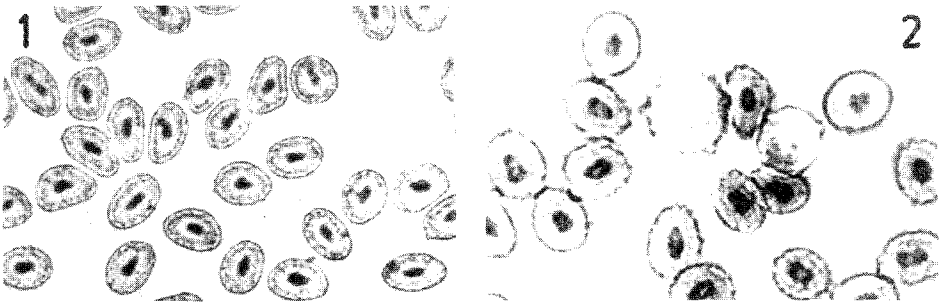

Fig. 1. Photomicrograph of blood cells of control fish x 2400

Fig. 2. Photomicrograph of blood cells of fish exposed for 7 days to mercuric nitrate x 2400

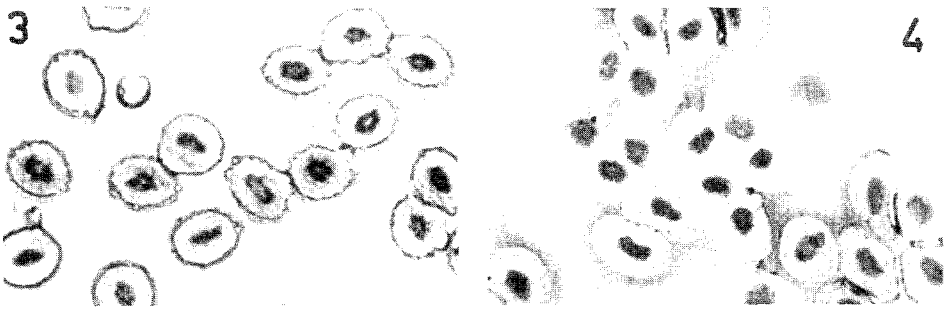

Fig. 3. Photomicrograph of blood cells of fish exposed for 14 days to mercuric nitrate x 2400

Fig. 4. Photomicrograph of blood cells of fish exposed for 28 days to mercuric nitrate x 2400

Instead of shrinkage of the RBCs to their normal size, it has undergone hemolysis. BORG et al. (1969) reported finger like projections in the RBCs of terrestrial Swedish wild life. Fig. 2 shows small finger like projections and irregular shaped RBCs. LEVANDER et al. (1977) reported that the shape of RBCs can be changed by incubating the RBCs with both cationic and anionic compounds. Earlier reports showed that mercury affected the osmotic resistance and mechanical fragility of the RBCs (DE BRUIN 1976) which confirmed the findings of LEVANDER et al. (1977) and also the present findings relating to freshwater teleost, A. scandens. However, reports pertaining to molecular level studies on these RBC shape changes and small finger like proliferations are scanty. SHEETZ & SINGER (1976) suggested that the asymmetrically lipid composition of the RBC membrane bilayer could account for the stomatocytic and echinocytic transformations by concentrations of cations in the inner half of the bilayer and of anions in the outer half, respectively. OLSON (1972) reported vacuolization of RBCs after exposure for periods of 4 and 8 wk to 0.03 µg/L Hg administered as methyl mercuric chloride. Our observations of vacuolization after 2 weeks' exposure to 0.5 µg/L administered as mercuric nitrate confirms the results shown by OLSON (1972).

As shown in Fig. 4 the cell membranes disintegrated completely. Later disintegration of nucleus and complete disappearance of cell membrane exposing the cellular material to blood plasma was marked. WEBB (1966) pointed out that mercurial compounds caused hemolysis of RBCs. A drastic depletion in hematocrit value after 21 days and a depletion in RBC count and Hb % reported by PANIGRAHI & MISRA (1978, 1979), confirms the view that mercury is the causal factor of hemolysis. Anemias associated with chronic or acute poisoning by certain heavy metals are by no means entirely accountable to direct detrimental actions upon the RBC membrane.

ACKNOWLEDGEMENT

We express our gratitude to H. Patnaik for providing laboratory facilities. Financial support by the CSIR (New Delhi) is sincerely acknowledged.

REFERENCES

BORG, K., H. WANNTORP, K. ERNE & E. HANKO: Viltry 6, 301 (1969).

CALABRESE, A. L., F. P. THURBERG, M. A. DAWSON & D. R. WENZLOFF: Sub-lethal Effects of Toxic Chemicals on Aquatic Animals, Elsevier Scientific Publishing Co., Amsterdam, 15-21 (1975).

DACIE, J. V. and S. M. LEWIS: Practical Haematology. J & A Churchill (1970).

DE BRUIN, A.: Biochemical Toxicology of Environmental Agents. Elsevier/North-Holland Biomedical Press. The Netherlands, 1283-1307 (1976).

FANGE, R. and M-L JOHANSSON-SJOBECK: Comp. Biochem. Physiol. 52 A, 577 (1975).

HOUSTON, A. H., K. M. MEAROW and J. S. SMEDA: Comp. Biochem. Physiol. 54 A, 267 (1976).

JOHANSSON-SJOBECK, M-L., G. DAVE and U. LIDMAN: Sub-lethal Effects of Toxic Chemicals on Aquatic Animals. Elsevier Scientific Publishing Co., Amsterdam, 189 (1975).
LEVANDER, O. A., M. FISHER, V. C. MORRIS and R. J. FERRETTI: J. Nutr. 107, 1828 (1977).
O'CONNOR, D. V. and P. O. FROMM: Bull. Environ. Contam. Toxicol. 13, 406 (1975).
OLSON, K. R.: Ph.D. thesis, Dept. of Physiol., Mich. State Univ., E. Lansing, Mich. (1972).
PANIGRAHI, A. K. and B. N. MISRA: Environ. Pollut. 16, 31 (1978).
PANIGRAHI, A. K. and B. N. MISRA: Arch. Toxicol. (1979) in press.
SHEETZ, M. P. and S. J. SINGER: J. Cell Biol. 70, 247 (1976).
SOIVIO, A. and K. NYHOLM: Aquaculture 3, 395 (1974).
WEBB, J. L.: Enzyme and Metabolic Inhibitors, Vol. 2, Academic Press, New York (1966).
WESTMAN, I., M-L. JOHANSSON-SJOBECK and R. FANGE: Sub-lethal Effects of Toxic Chemicals on Aquatic Animals. Elsevier Scientific Publishing Co., Amsterdam, 111 (1975).