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Erythrocyte Alterations Induced by Malathion in *Channa punctatus* (Bloch)

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The blood of fishes like other vertebrates consists of cellular components suspended in plasma. The cellular components of blood of fishes are red blood cells (RBC), white blood cells (WBC) and thrombocytes designated as formed elements (Ross & Reith, 1985). Erythrocytes contain abundant quantity of haemoglobin (Dube & Munshi, 1973) and exhibit pink colour when stained with Giemsa staining solution. The nucleus is centrally placed and may be round or oblong in shape.

Several species of fish are susceptible to the deleterious effects when exposed to heavy metals and other effluents as reflected by the haematological changes (Johansson - Sjobeck & Larsson, 1978; 1979), eosinophilia (Dawson, 1935) lymphocytosis (Gardner & Yevich, 1970), and alterations in erythrocyte morphology (Gill & Pant, 1985; 1986). The present study has been undertaken to describe haematological and pathological sequelae to malathion poisoning in *C. punctatus*, a freshwater snakehead fish, using RBC.

MATERIALS AND METHODS

The live specimens of *Channa punctatus* were brought from local fish market and acclimatized for 7 days under laboratory conditions and were fed either with minced goat liver or mosquito fish *Gambusia affinis patruelis* (Baird and Girard). 24 hours prior to commencement of toxicity tests, feeding was stopped and fish were not fed during the experimental period. The water temperature calculated during the experimentation was found to be 25 ± 3°C

Malathion (EC 50%) manufactured by Rajhans Chemicals, Faridabad, India, was used during the present course of study. The commercial grade preparation is a yellow coloured liquid containing 50% active ingredients (w/w) and the rest is constituted by inactive ingredients. A stock solution of 1g/L was prepared in water and measured aliquots of this are added to the required levels i.e. 0.05, 0.10, 0.15, 0.20 and 0.25 mg/L.

Toxicity tests were conducted in 10 L water capacity plastic tanks. A minimum

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of 8 fish were exposed to each pesticide concentration and each experiment was conducted in triplicate. Water of each tank containing toxicant was changed daily to remove faecal matter, and waste metabolite of fish and to maintain the required concentration of pesticide. Appropriate controls were run simultaneously in pesticide - free water.

A 23 guage needle was used to draw blood from the ventral aorta. 1 or 2 drops were immediately fixed in 1% buffered glutaraldehyde (1% in 0.2M phosphate buffer, pH 7.2) for 10- 15 min. Fixed blood was centrifuged at 1500g for 5-10 min. Extra fixative was removed and the erythrocyte pellet was completely washed thrice with 0.1 M phosphate buffer (mixed equal volumes of 0.2M phosphate buffer and distilled water, pH 7.2) or till the odour of glutaraldehyde disappeared completely. The pellet was then gently suspended in a small volume of triple distilled water and a small drop of the suspended erythrocytes was placed over silver tape attached to the aluminium stub. Air-dried samples were sputter coated with gold (100A°) and finally viewed using JEOL, JSM-6100 scanning electron microscope.

RESULTS AND DISCUSSION

Each erythrocyte appears elliptical with an oblong nucleus under ordinary and scanning electron microscope (Figs.1a&b). Erythrocytes were found to be swollen (spherical) when the fish was exposed to 0.05 mg/L for 5d (Fig.1c). Number of such spherical erythrocytes increased (Fig.2b&3c) significantly upon exposure for 15 and 30d. Increase in the concentration of pesticide for the exposure period registered an increase in the number of such cells. The spherical erythrocytes may be referred to as “spherocytes”.

Increased anisocytosis was a predominant feature, whereas a significant variation in shape and size of cells was noticed. Lobopodial projections were seen upon exposure to different levels of pesticide for different exposure periods (Figs. 1f;2d;3a;3d). 0.10 mg/L malathion caused clubbing of irregularly shaped erythrocytes (Fig. 1d) and at an exposure of 0.15 mg/L for same time duration, erythrocytic chains were observed (Fig.1e). Cytoplasmic content was also found to ooze out resulting in formation of crenated cells with numerous projections (Fig.2a). These may be designated as “echinocytes”. The frequency of occurrence of such cells increased with an increase in exposure duration. The overlying plasma membrane of these cells was found to be disrupted.

The chronic exposure (15d) of the experimental fish to pesticide (0.10 mg/L) led to contraction of elliptical erythrocytes from one side (Fig.2c). Increased pesticidal concentration (0.20 mg/L) caused cytoplasmic blebbing and ultimately the oozing out of the cytoplasmic content in a thread-like form (Fig.2e). These cells due to their appearance may be labelled as “acanthocytes”. Further increase in pesticidal concentration to 0.25 mg/L had such a profound effect on erythrocytes that the cell membrane of these cells was disrupted badly (Fig.2f).

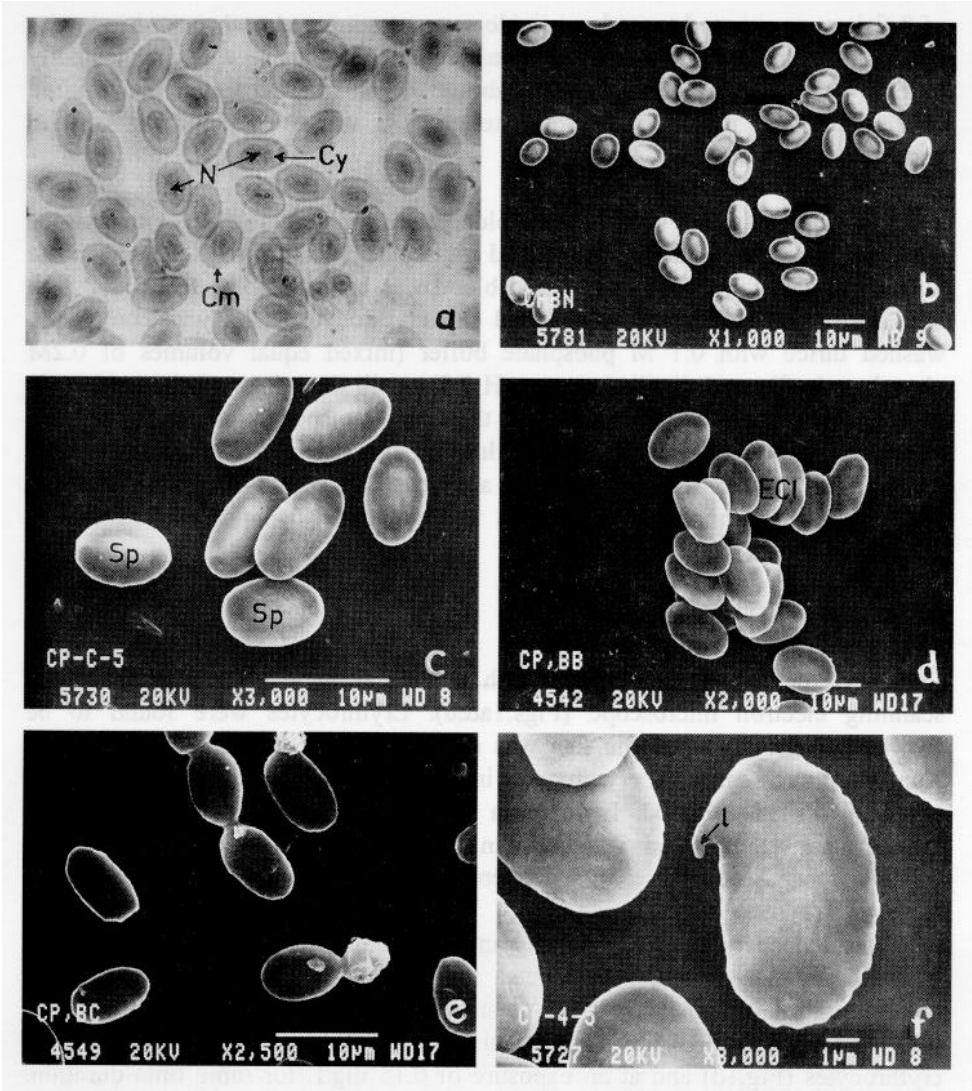

Figure 1. Red blood corpuscles of *Channa punctatus* showing : a) control cells - elliptical with oblong nucleus ; b) scanning electron micrograph of control ; c) swollen cells or spherocytes of 0.05 mg/L treated fish (5d) ; d) clubbing of irregularly shaped cells upon 0.10 mg/L exposure (5d) ; e) formation of chains (0.15 mg/L, 5d) ; f) lobopodial projections (0.20 mg/L, 5d).

Cm - cell membrane, Cy - cytoplasm, EC1 - erythrocyte clubbing, N- nucleus, Sp - spherocyte, 1 - lobopodial projection.

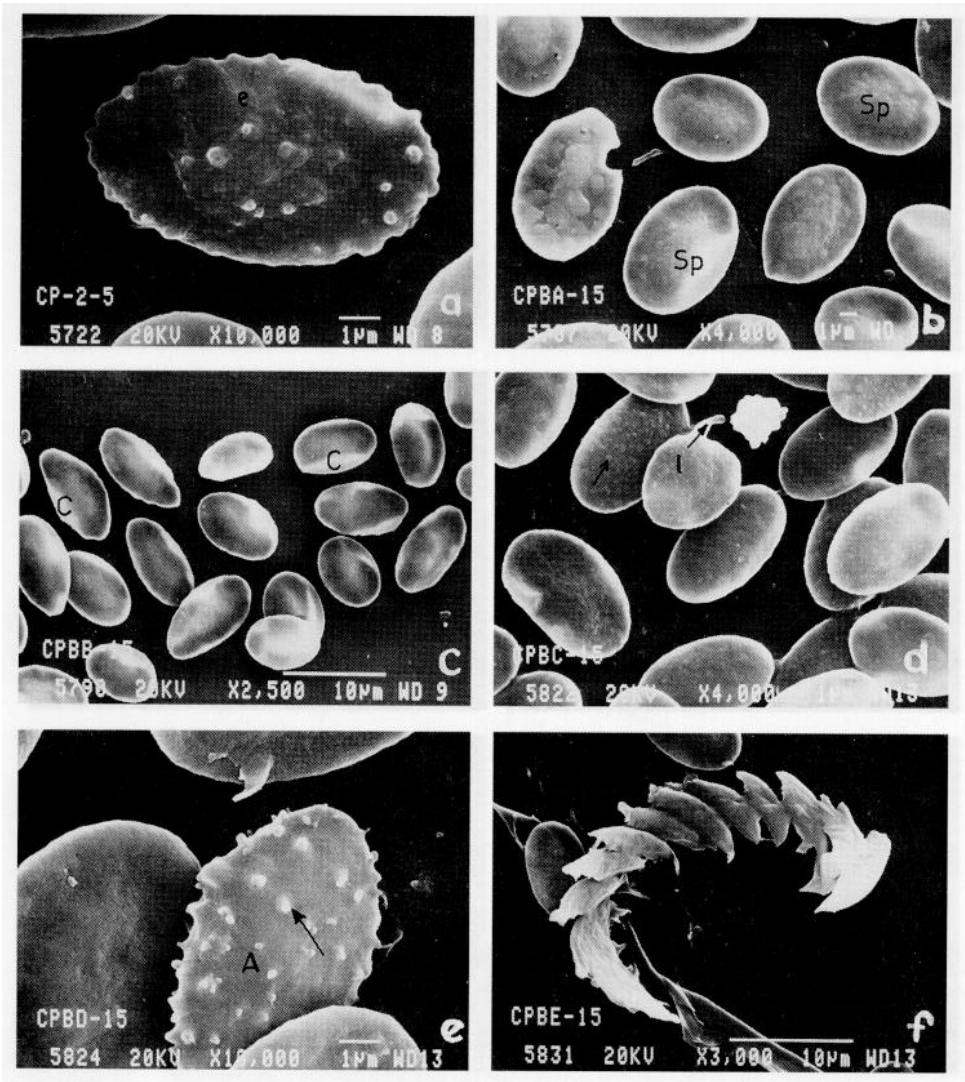

Figure 2. Scanning electron micrographs of erythrocytes showing : a) irregularly crenated cells or echinocytes (0.15 mg/L, 15d) ; b) increased number of spherocytes (0.05 mg/L, 15d) ; c) contraction of elliptical erythrocytes from one side ; d) lobopodial projection (0.15 mg/L,15d) ; e) acanthocytes with cytoplasmic blebbing and oozed out cytoplasmic content (arrow) (0.20 mg/L,15d) ; f) disruption of cell membrane (0.25 mg/L,15d)

A-acanthocytes, c-contraction, e- echinocytes, 1 - lobopodial projection, Sp - spherocyte.

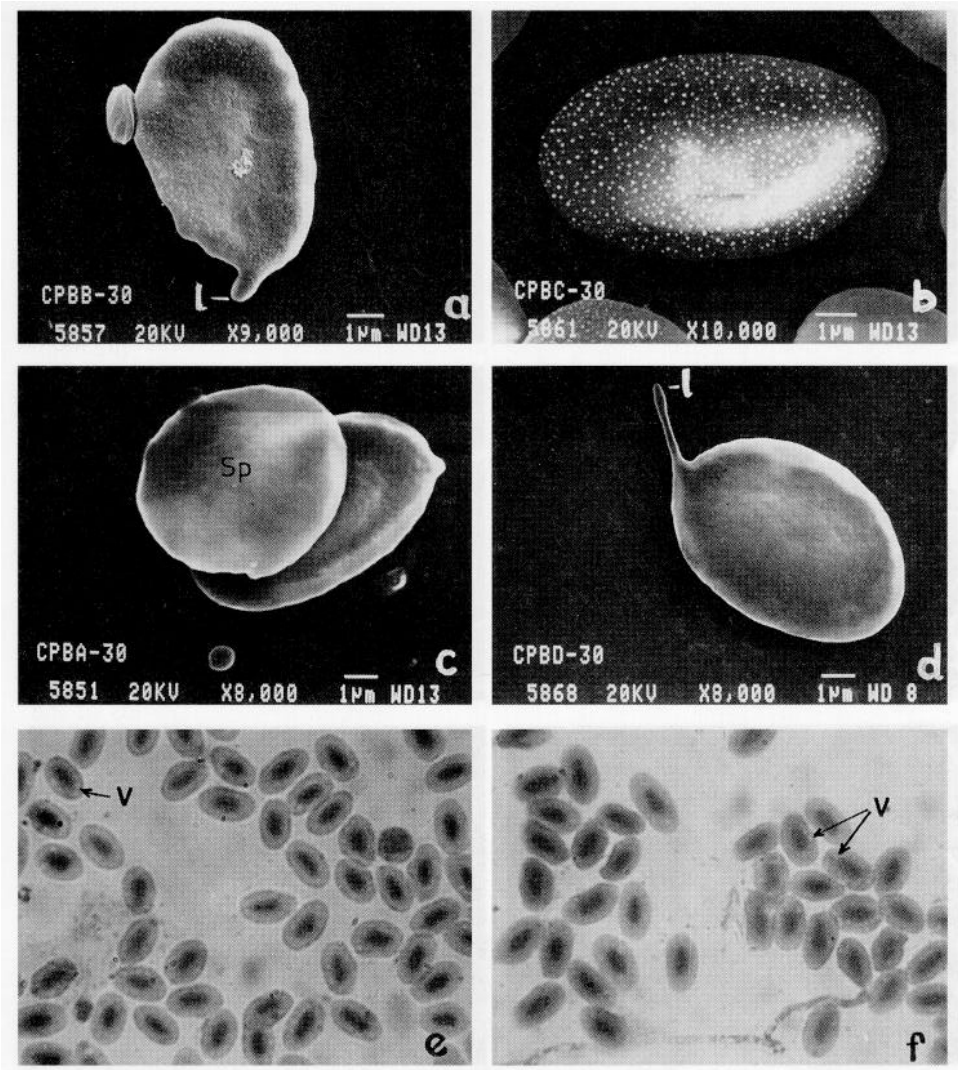

Figure 3. Erythrocytes of *Channa punctatus* showing : a) lobopodial projection (0.05 mg/L, 30d) ; b) speckled pattern over their surface (0.15 mg/L ; 30d) ; c) spherocytes (0.20 mg/L ; 30d) ; d) lobopodial projection (0.20 mg/L, 30d) ; e & f) vacuolization (0.15 mg/L, 30d).

1 - lobopodial projection, Sp - spherocyte, V- vacuole.

In addition to these alterations, with different levels of malathion exposed for 30d, the erythrocytes showed "speckled" pattern over their surface (Fig.3b). Vacuolations in the cytoplasmic zone of the erythrocytes were observed when the cells were stained with Giemsa and viewed at 1000X (light microscopically). The extent of vacuolizations was found to be directly proportional to the pesticide dose and exposure period (Fig.3e&f).

Malathion has been reported to be more toxic to insects and fish than to mammals due to lack of hydrolytic enzymes in the former (Krueger *et al.,* 1960). The hydrolysis of the oxygen analogue of this pesticide (malaoxon) proceeds very slowly in fish (Areechon & Plumb, 1990). Hence malathion, once inside the body of the fish, inhibits the hydrolysis of acetyl-choline and also/or deposits as such or as its oxygen analogue (malaoxon). This complicates the cytoplasmic nature of these cells which, in order to overcome the 'stress' induced by this chemical, shows vacuolizations. The vacuolizations may further aggravate the toxic effect leading to death of the cells. The severe attack of this pesticide leads to membrane disruptions and cytoplasmic blebbing. The erythrocyte membrane seem to be most affected depicting increased porosity. These changes might have resulted due to disturbed lipid microenvironment of the membrane and more so, due to increased lipid peroxidation induced by this chemical, hence, resulting in increased membrane fluidity and porosity. The results of these modifications in membrane of the infected cell is manifested by the rheologic properties of the cells whereby they cannot traverse the microvasculature that leads to accelerated pitting and clearance within the spleen.

Brecher & Bessis (1972) commented that the biconcave red cells (discocytes) of man can be transformed into crenated red cells (echinocytes) by extrinsic factors (plasma incubated at 37°C for 24h, lysolecithin, high levels of fatty acid or physiological levels of fatty acid in the presence of lysolecithin and many others); or by intrinsic factors, such as aging of red cells, which are probably related to depressed ATP. The red cells may even be transformed into sphero-echinocytes and spherocytes due to higher concentrations of echinocytogenic agents. Bessis & Prenant (1972) and Weed & Bessis (1973) further laid possibility of transformation of normal red cells to abnormal ones, due to the superimposed or the underlying structural pathology of the cells. Recently, Agrawal *et al* (1990) reported the presence of abnormal red blood cells (echinocytes and spherocytes) due to the exposure of the test rats to single or repeated exposures of methyl isocynate. The appearance of various abnormal forms of red cells in C. *punctatus,* hence, seems to be a direct effect of this pesticide. The transformed cells may also be termed as 'aged cells', due to depressed ATP as depicted by the fish experiencing hypoxic condition induced by malathion.

Erythrocytes are fundamentally capable of few stereotypic responses to a variety of environmental perturbations, which are sometimes considered to be of vital physiological significance. Furthermore, it is implicated that modifications of the shape and size of the erythrocytes represent the most common morphologic

abnormalities that occur in pathologic conditions (Barnhart *et al., 1983).*

Arutjunov *et al.* (1981) while studying toxic anaemias in mammals caused by exposures to occupational toxic agents, concluded that most of the RBCs transformed are either spherocytes or schizocytes. According to them the altered red cell shape stems from such reasons like abnormal erythropoiesis, inadequate haemoglobin formation, damage to red cells after they leave bone marrow or increased erythropoiesis by bone marrow to compensate for anaemic conditions (Bessis, 1972). Gill *et* al (1991) on the other hand suggested that the fish experience respiratory difficulty when they confront toxic environment and try to compensate for the reduced oxygen uptake at the gill surface by increasing the level of blood constituents concerned with oxygen uptake and delivery. However, a prolonged exposure exhaust the haematopoietic potential revealed by lowered RBC count and haemoglobin.

Various types of alternations when the fish is subjected to malathion, using SEM have been described for the first time. There is every possibility that upon prolonged exposure and with higher dosages, the percentage occurrence of these abnormal erythrocytes may increase manifold. Further investigations for the quantitative and qualitative parameters are required to mark the erythrocytes as direct pollution indicators.

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