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Vanadium toxicology – an assessment of general health, haematological aspects and energy response in an Indian catfish *Clarias batrachus* (Linn)

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The pervasive occurrence of vanadium in nature and its use in various industrial processes has increased its inputs in the environment. This has prompted us to elucidate the impact of vanadium on aquatic environment, the primary body for industrial effluent discharge. The energy response of the fish, *Clarias batrachus*, its haematological status including haemoglobin (Hb), haematocrit (Ht), leutocrit (Lt), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) etc. And overall general health conditions have been observed to be significantly hampered leading to deleterious alterations in the general metabolism of the fish following long term exposure to vanadate. The increase in muscle and tissue lactic acid (2–12 fold) in association with decrease in pyruvic acid (72% in mucle; 26% in liver) reflect a shift towards an anaerobic metabolism of the fish. We conclude that vanadium could be toxic for the fish in question under long term exposure at the doses under observation (2–10 mg L^{-1}).

Keywords: *Clarius batrachus*, haematology, lactic acid, pyruvic acid, vanadium

Introduction

Vanadium has gained increased interest during the last few decades because of its toxicological effects in man and animals (WHO 1988), diverse sources of pollution and its dramatic role in constituting a major risk to the global environment in facing silent epidemic of metal poisoning (Nriagu & Pacyna 1988). Although extensive studies have been made of toxicological significance of vanadium in birds, rodents (Eyal & Moran 1984; Sharma *et al*. 1986) and despite the sensitivity of the aquatic life to heavy metals (Johnson 1988) that are frequently introduced in water bodies, insufficient attention, with the exception of a few (Jagadeesh *et al*. 1989; Ray *et al*. 1990) has been paid to elucidate the effect of vanadium on the biochemical changes at cellular and subcellular levels in fish after long term sublethal exposure. Haematology has been used as an index of health status over a number of fish species (Tort *et al*. 1982) and haematological changes have been detected following different types of stressing conditions (Duthie & Tort 1985). In addition, it is also well known that heavy metal exposure can generate tissue hypoxia (Tort *et al*. 1982) thus inducing metabolic alterations via affecting energy response which may lead even to death (Hilmy *et al*. 1987).

To test these hypotheses, changes in lactic acid and pyruvic acid content in skeletal muscles and liver tissues, changes in body weight and total protein content along with the effects on blood parameters were studied in an Indian catfish *Clarias batrachus* (L) exposed to different concentrations of vanadate for a month. The present work was undertaken with an aim to obtain information concerning

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the biochemical mechanism of toxic actions of vanadium in a defined dose range, much lower than considered in the previous literatures, to assess its effects on the above mentioned parameters that are directly related to the health status of the fish from economic and commercial point of view.

Materials and methods

Mature *Clarias batrachus* (16–18 cm in length and 70–80 g in weight) were obtained from a local pond and acclimatized in the laboratory for two weeks before they were exposed to various concentrations of ammonium monovanadate salt (NH_4VO_3) (2, 4, 6, 8 and 10 mg L^{-1}). The fish were held in glass aquaria ($70 \times 30 \times 30$ cm^3) containing 30 L of pond water (pH 7.4–7.6, temp. 22–24 °C, DO content 8–10 p.p.m.) and were kept in a room with 12 h light and 12 h darkness each day. The fish were divided into six groups with six specimens in each group. Group 1 – normal fish receiving no treatment; Group 2 – fish exposed to 2 mg of ammonium monovanadate L^{-1} for 30 days; Group 3 – fish exposed to 4 mg ammonium monovanadate L^{-1} for 30 days; Group 4 – fish exposed to 6 mg of ammonium monovanadate L^{-1} for 30 days; Group 5 – fish exposed to 8 mg of ammonium monovanadate L^{-1} for 30 days and Group 6 – fish exposed to 10 mg of ammonium monovanadate L^{-1} for 30 days.

The working concentrations were selected on the basis of a previous report (Chakraborty *et al.* 1995). Three sets of each experiment were done. The concentrations of vanadium salt in the aquaria of the five experimental groups were maintained at the requisite level by renewing the aquaria volume with new solution every 24 h. The control group of fish was maintained by fresh water renewed daily.

At the end of the exposure period, tissues (liver and muscle) were taken for lactic acid and pyruvic acid analyses by the method of Burton & Speher (1971). Tissue glycogen was estimated by the Anthrone method of Sigal *et al.* (1964). In haematological procedure blood samples were collected from caudal peduncle with sterile plastic syringe using heparin as an anticoagulant. Red blood cell (RBC) count was done by the standard clinical method using Dacie's fluid (Dacie & Lewis 1968) and Neubaur haemocytometer. Haemoglobin (Hb) content (g 100 ml^{-1}) of blood was estimated by the alkali haematin method (Oser 1979). Haematocrit (Ht) and Leutocrit (Lt) were determined by centrifugating the blood sample in heparinized capillary tubes at 11000 *g* for 6 min and using micrometric ocular. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from previous measurements. All haematological studies were performed within 5 h of blood collection. Protein measurement was done following the method of Lowry *et al.* (1951). Unpaired Student's *t*-test was used for statistical analyses.

Results

The results of the present study show an increase in lactic acid concentration in both the tissues (liver and muscle) in a dose-responsive manner as depicted by the sharp and steep slope of the curve (Figures 1 and 2). Approximately 2–fold increase in muscle lactic acid content was obtained at 6 mg L^{-1} dose. Though the trend of increase in liver was similar to that in the muscle, an insignificant 2–fold increase in lactic acid content in liver was observed at a much lower dose (2 mg L^{-1}). Excepting the 2 mg L^{-1} vanadate all other doses caused significant increase in values in the case of liver and muscle, on the other hand, all the doses starting from 2 mg L^{-1} to 10 mg L^{-1} showed significant ($P < 0.001$) increase when comparison was made with untreated control fish. With 10 mg L^{-1} salt exposure, 12–fold increase in hepatic lactic acid content indicates maximum response to toxicity.

The pattern of change in pyruvic acid content depicts a regular decrease in both liver and muscle with the increase in vanadium concentration (Figure 3). In the muscle, unlike liver, the pyruvic acid level first increased slightly (insignificant) with

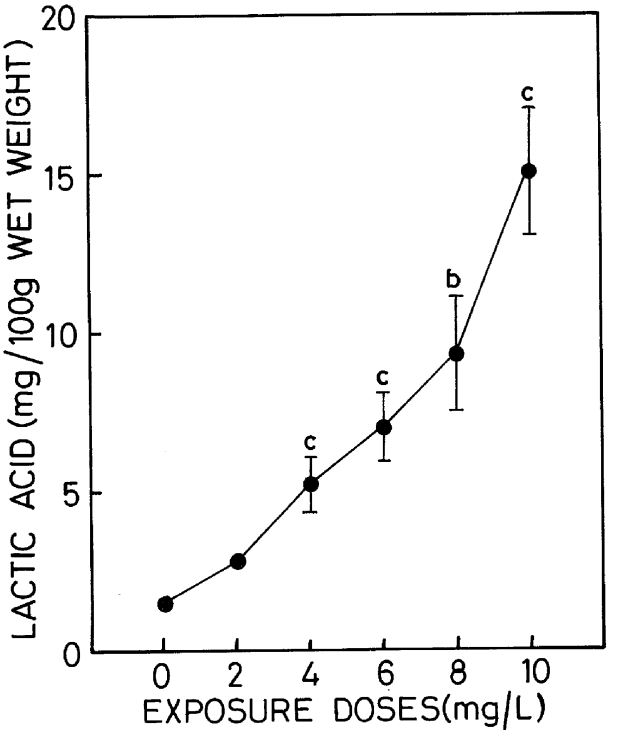

Figure 1. Effect of different doses of vanadium on lactic acid content in liver of cat fish, *Clarias batrachus*. Student's *t*-test was done between control ('0' dose) and treated series. b = $P < 0.01$; c = $P < 0.001$.

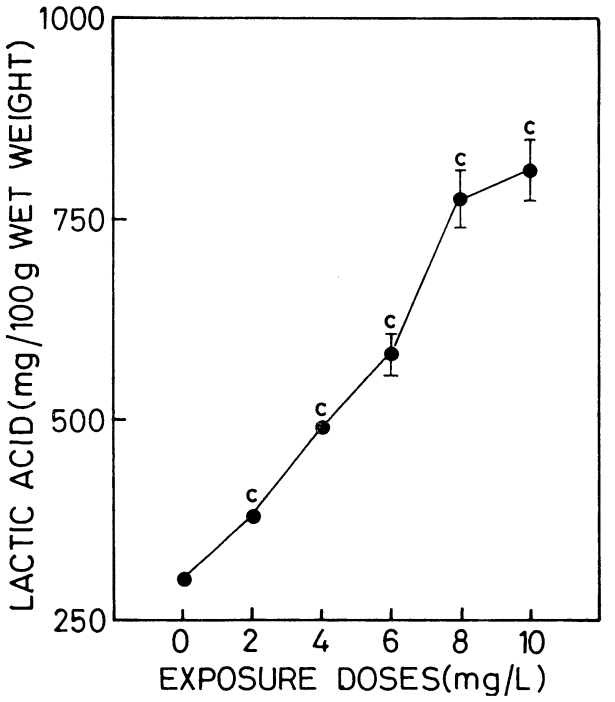

Figure 2. Effect of different doses of vanadium on lactic acid content in muscle of cat fish, *Clarias batrachus*. Student's *t*-test was done between control ('0' dose) and treated series. c = $P < 0.001$.

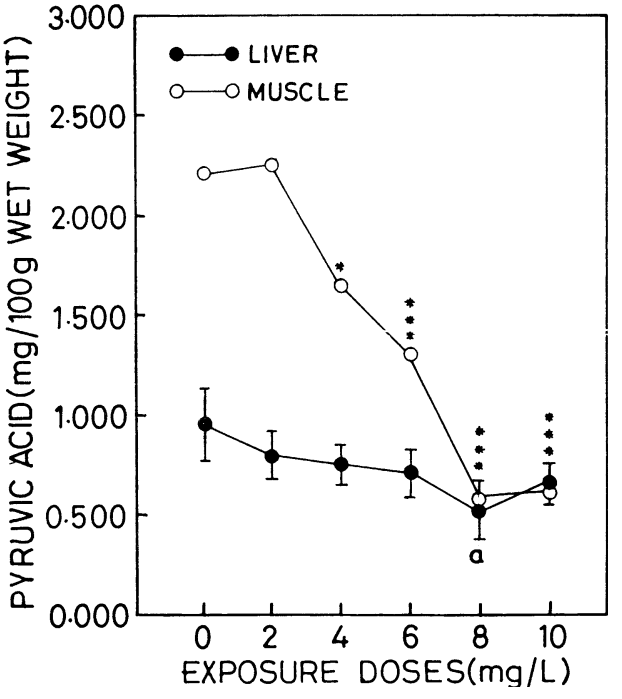

Figure 3. Effect of different doses of vandium on pyruvic acid content in liver and muscle of cat fish, *Clarias batrachus*. Student's *t*-test was done between control ('0' dose) and treated series of liver (a = $P < 0.05$) and muscle (* = $P < 0.05$; *** = $P < 0.001$).

2 mg L^{-1} treatment and then started decreasing. The pyruvic acid content was maximally decreased in both the tissues (72% in muscle, 26% in liver) with 8 mg L^{-1} vanadate administration ($P < 0.001$). In most cases the liver exhibited insignificant decrement in pyruvic acid value with the doses except for the treatment at 8 mg L^{-1} dose.

Long term vanadate exposure caused insignificant decrease in glycogen content (Figure 4) with increase in vanadate concentration. Acute toxic effect of vanadium on the general health of the fish is reflected by the general loss in body weight and total protein content with the gradual increase in vanadium concentration (Figure 5) and the dose 4 mg vanadate L^{-1} onwards elicited highly significant depletion in both the parameters.

The haematological status of the fish under vanadate treatment is demonstrated in Figures 6–8. The Ht percentage was found to decrease sharply with vanadate exposure along with the Lt (Figure 6). A regular pattern of decrease in a dose-response manner in the MCHC was noted with vanadate treatment but two lower doses (2 mg L^{-1} and 4 mg L^{-1}) could not significantly lower the value (Figure 7). With the first two doses (2 mg L^{-1} and 4 mg L^{-1}) of vanadate an increase was elicited in

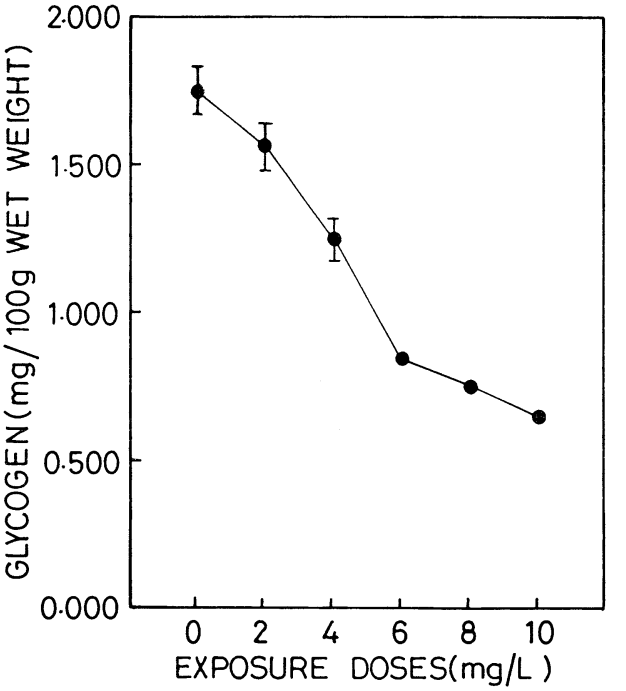

Figure 4. Effect of different doses of vanadium on hepatic glycogen content cat fish, *Clarias batrachus*. Student's *t*-test was done between control ('0' dose) and treated series.

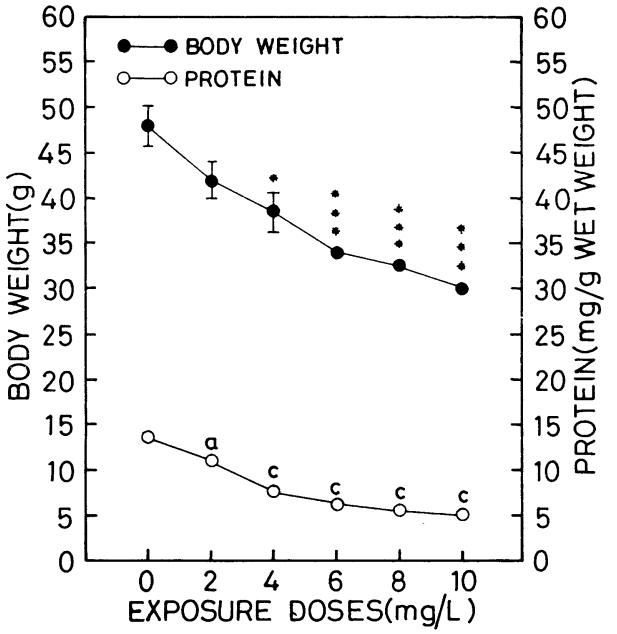

Figure 5. Effect of different doses of vanadium on body weight and protein content of cat fish, *Clarias batrachus*. Student's *t*-test was done between control ('0' dose) and treated series of body weight (* = $P < 0.05$; *** = $P < 0.001$) and protein content (a = $P < 0.05$; c = $P < 0.001$).

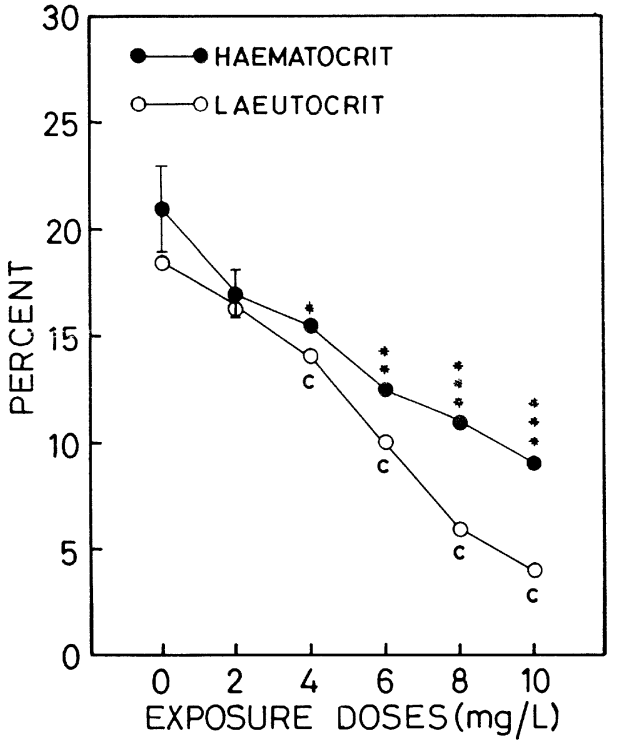

Figure 6. Effect of different doses of vanadium on haematocrit and leutocrit of cat fish, *Clarias batrachus*. Student's *t*-test was done between control ('0' dose) and treated series of haematocrit (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$) and leutocrit (c = $P < 0.001$).

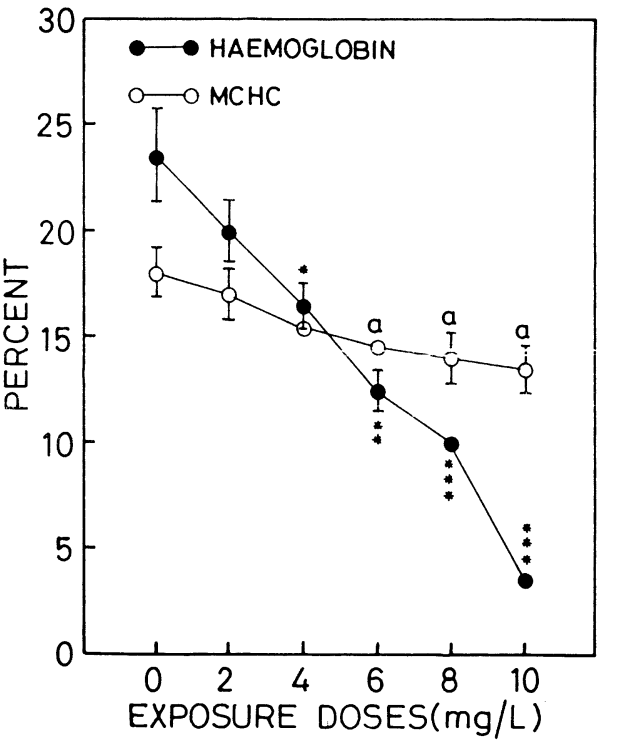

Figure 7. Effect of different doses of vanadium on haemoglobin content and mean corpuscular haemoglobin concentration (MCHC) of cat fish, *Clarias batrachus*. Student's *t*-test was done between control ('0' dose) and treated series of haemoglobin (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$ and MCHC (a = $P < 0.05$).

the MCV which was then gradually lowered with sequential increase in the dose of vanadium. A similar pattern of change was noted in the MCH of *C. batrachus* exposed to vanadate. While MCV did not elicit any significant alteration at any treatment schedule MCH and RBC, on the other hand, showed more or less a similar pattern of decrement after vanadate administration (Figure 8). A steep depletion in Hb concentration was recorded herewith (about 88%) in the highest dose treated group ($P < 0.001$) (Figure 7).

Discussion

The results reported in the present study indicate that long term exposure of vanadate has potential toxic effects on the teleost in terms of its general health and overall metabolism. Changes in haematological status (Figures 6–8) are found to be similar in those following exposure to other metals or different stresses *viz*. hypoxia (Duthie & Tort 1985)

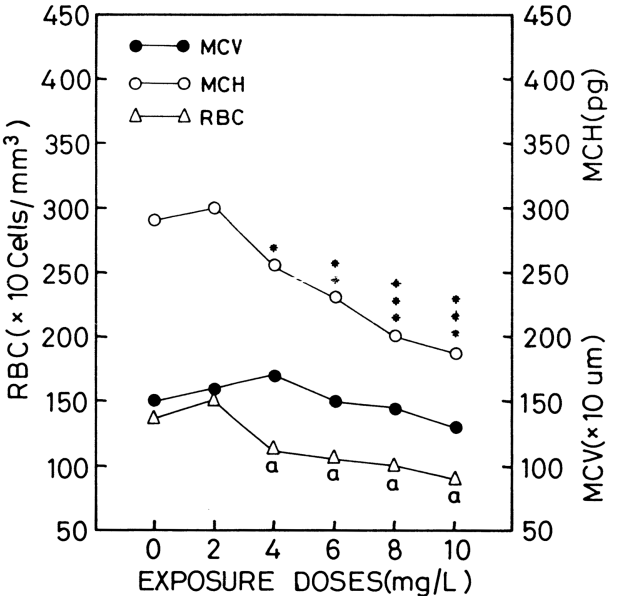

Figure 8. Effect of different doses of vanadium on red blood cell (RBC) count, mean corpuscular haemoglobin concentration (MCH) and mean corpuscular volume (MCV) content of catfish, *Clarias batrachus*. Student's *t*-test was done between control ('0' dose) and treated series of MCV, MCH (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$) and RBC (a = $P < 0.05$).

and suggest a process of swelling as shown by MCV value. Parallel observations are documented by Mosior *et al.* (1992) where orthovanadate increases the critical cell volume of bovine erythrocytes via the interaction of membrane skeleton with the lipid–protein matrix. Decreases in Ht and RBC count may be a result of haemolysis (Zaporowska & Wasilewski 1992). Decrease in Lt value could be correlated with the general trend of the decrease in lymphocyte count with heavy metal toxicity (Torres *et al.* 1986). These changes may be a consequence of a progressive inhibition of cellular respiration. The changes observed in lactic acid level (Figures 1 and 2) are similar to those known to occur in fish subjected to environmental hypoxia (Lal *et al.* 1984). Similar observations have also been reported from *in vitro* studies where vanadium has been shown to be capable of decreasing the rate of respiration and oxidative phosphorylation (Paschoa *et al.* 1987). Thus, a shift towards anaerobic metabolism is noted as lactic acid is the primary anaerobic end product. This finds relevance with the studies of Row *et al.* (1983) where a decrease in partial pressure of oxygen (pO_2) and increase in blood lactate level in the circulatory system of flagfish was observed upon exposure to treated liquid effluent from petroleum refinery. A significant decrease in muscle pyruvic acid may reflect an equilibrium between muscle produced lactic acid and serum level of the same or combination of both. This indicates inhibition of glycolysis thereby limiting the available pool of ATP molecules (Simons, 1979).

Dose-dependent depletion of glycogen, though insignificant (Figure 4) indicates subsequent stress on the host providing evidence that glycogen is metabolized to lactic acid which accumulates in the liver and muscle and thereby a decrease in pyruvic acid content is noted. The rapid depletion of glycogen suggests that there may be an impairment of general energy level through anaerobic glycogenolysis. All these have direct correlation with the general loss in body weight and total protein content after long term exposure of vanadate.

Thus, it can be presumed that sublethal amounts of vanadium could alter the energy requirements and interfere with important bodily functions. This may be extrapolated to have adverse effects on the bioenergetics of the teleosts as has been seen in case of dog fish exposed to copper (Heath, 1984). Such effects leading to alterations in haematological picture and general health status of the fish under observation warrants further study to understand the molecular basis of the toxicity of vanadium.

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