Bull. Environ. Contam. Toxicol. (1988) 40:198–203

Bulletin of Environmental Contamination and Toxicology

Acid-Base Balance and Blood Gas Changes in the Fresh Water Field Crab, *Barytelphusa guerini*, on Exposure to Organic and Inorganic Lead

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The acid-base status of crustacean haemolymph depends on various environmental and physiological factors. Acid base status of the haemolymph is known to be influenced by temperature, salinity, strenuous activity and moulting (Truchot 1978; 1981; Philips et al 1977; McDonald et al 1979; Wood and Randall 1981; Dejours and Beekenkamp 1978). Studies dealing with the acid-base disturbance in the terrestrial crabs include those on Gecarcinus lateralis and Cardisoma carnifex during post excerise and excerise period (Smatresk et al 1979; Wood and Randall 1981). The studies on the acid-base regulation of the fresh water crabs are meagre (Truchot 1983).

The acid-base changes in fishes during environmental stress conditions like acid stress and zinc toxicity had been reported (Eddy 1976; Packer 1979; Spry and Wood 1984). But the effect of environmental pollutants like the heavy metals on the acid-base regulation of the fresh water crabs have not been previously reported. The haemolymph of the fresh water crab was found to accumulate high amounts of lead on exposure to organic and inorganic lead (Tulasi et al 1987). Hence the present investigation has been undertaken to study the haemolymph acid-base status on exposure to subtoxic levels of organic and inorganic lead.

MATERIALS AND METHODS

Fresh water crabs were collected from the local paddy fields and transported to the laboratory where they were maintained in round plastic tubs. Crabs were fed with fish meat every day and feed was withdrawn one day prior to experimentation. Uninjured males of uniform size, in the intermoult stages were used for the experiment. After two weeks of acclimation to laboratory conditions, the crabs were exposed to different concentrations of lead nitrate and lead acetate and LC50 values were determined according to Finney (1964). The LC50 values for 96 hours was found to be 20 ppm for lead acetate and 26 ppm for lead nitrate (Tulasi et al 1985). Later they were exposed to a sublethal concentration of 0.5 ppm for a period of 30 days and changes in blood pH, p02, pC02, bicarbonates and lactic acid was studied on different days of exposure

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(1, 4, 7, 15 and 30 days). Blood pH, p02, pC02 and bicarbonates were determined on a blood gas analyser (Model 165/2 pH-Blood gas system) and lactic acid levels were estimated according to Hollanders et al (1968).

RESULTS AND DISCUSSION

Responses of the fresh water crab exposed to lead nitrate and lead acetate are given in Figure 1. On 1st and 4th days of exposure the blood pH levels were found to increase when compared to controls, the increase being maximum on 1st day. On 1st day the values were 7.49 ± 0.03 in controls and 7.63 ± 0.02 in lead nitrate and 7.66 ± 0.01 in lead acetate exposed crabs. On 4th day of exposure the blood pH levels were 7.48 ± 0.03 in controls, 7.59 ± 0.01 in lead nitrate and 7.57 ± 0.01 in lead acetate exposed crabs. But after 4th day the blood pH levels were found to gradually decrease. On 30th day the blood pH values in controls were 7.43 ± 0.01, 6.9 ± 0.02 in lead nitrate and 6.72 ± 0.02 in lead acetate exposed crabs, indicating development of acidosis.

p02 levels in the haemolymph decreased gradually from a mean rate of 53.82 ± 5.88 mm Hg in controls to 22.75 ± 2.31 mm Hg in lead nitrate and 22.12 ± 1.61 in lead acetate exposed crabs. pC02 levels were found to be decreased slightly during the initial stages but on prolonged exposure the pC02 levels were increased from 14.22 ± 0.46mm Hg in controls to 24.25 ± 1.35mm Hg in lead nitrate and 24.41 ± 0.58mm Hg in lead acetate exposed crabs. Bicarbonate levels were found to be elevated on 1st and 4th days of exposure (Figure I). From 7th day onwards there was a gradual decrease in the bicarbonate levels. Bicarbonate levels decreased from a control rate of 5.95 ± 0.20 Millimoles/litre to 2.27 ± 0.22 Millimoles/litre in lead nitrate and 2.76 ± 0.55 Millimoles/litre in lead acetate exposed crabs at the end of 30th day. Haemolymph lactate levels were found to be decreased during initial stages of exposure as did haemolymph pC02 but later there was gradual increase of lactate levels from 0.49 ± 0.04mg lactate/100ml haemolymph to 1.16 ± 0.02 in lead nitrate exposed and 1.04 ± 0.08 in lead acetate exposed crabs (Figure I).

Exposure of the fresh water crab to sublethal concentrations of organic and inorganic lead showed a severe disturbance in the acid-base parameters.

Lead exposure caused a decrease in blood p02 content. Reduction of p02 is usually indicative of reduced oxygen transfer at the gill surface. Reduction in whole animal oxygen consumption of the fresh water crab on exposure to organic and inorganic lead (Tulasi and Ramana Rao In Press) imply that the gill's ability to transfer oxygen is impaired. Hence a reduction in p02 value is observed.

An alternative explanation is that the blood's ability to transport oxygen was reduced due to a decrease in blood oxygen capacity resulting from Bohr and Root effects caused by a decrease in blood pH. Reduction in blood p02 values during stress conditions has been reported by Smart (1978) in Salmo gairdneri and during zinc toxicity by Spry and Wood (1984) in rainbow trout. Thus a decrease in blood oxygen content may be due to the hypoxic conditions prevailing during stress and can be directly related to a decrease in oxygen consumption.

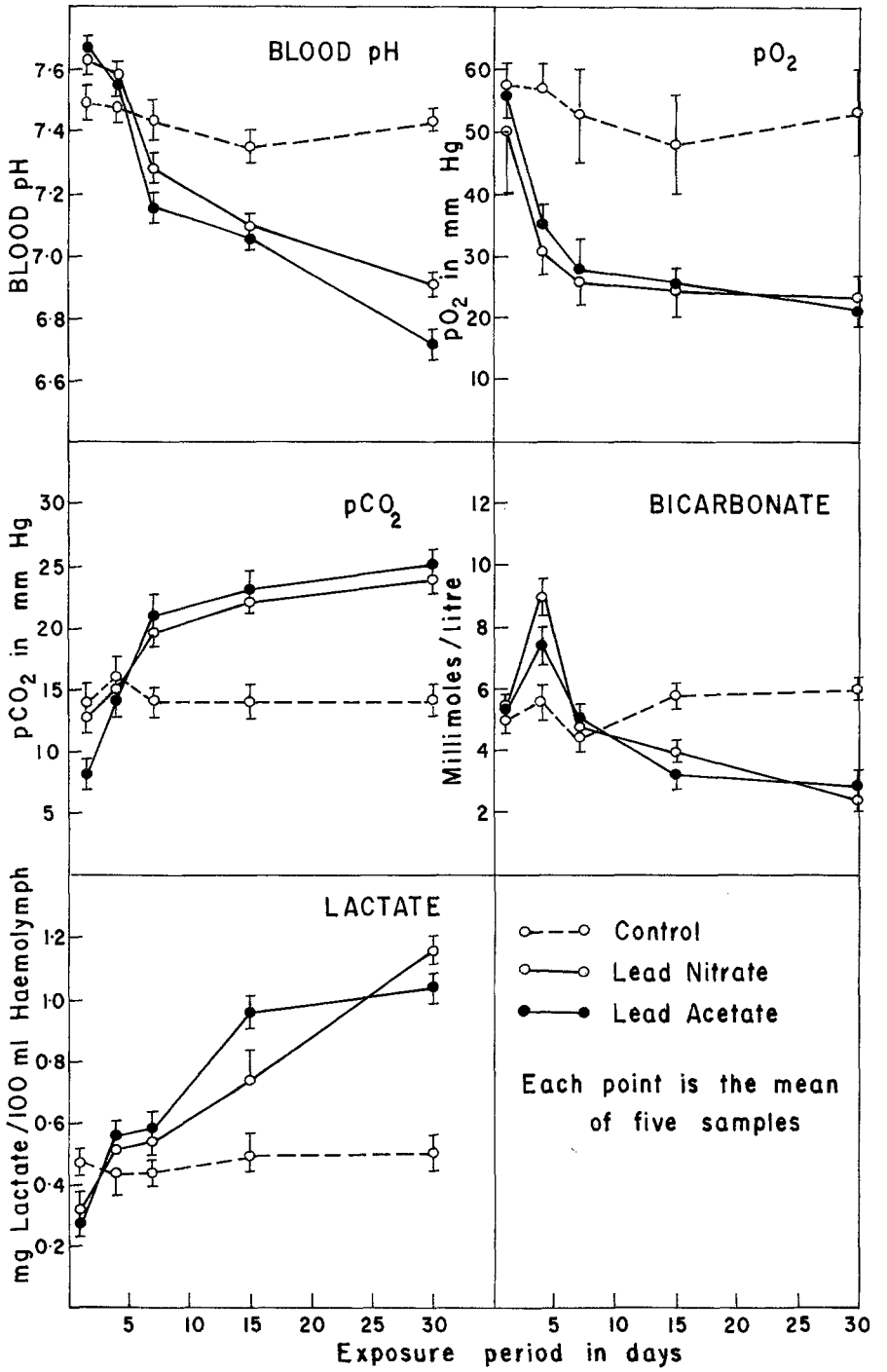

Figure.1. ACID-BASE AND BLOOD GAS LEVELS IN THE HAEMOLYMPH OF THE FRESH WATER CRAB ON EXPOSURE TO ORGANIC AND INORGANIC LEAD

Blood pC02 is the respiratory parameter and is the partial pressure of the Carbondioxide of the blood taken in anaerobically and is expressed in mm Hg. Exposure to lead elicited two different types of responses. Initially, on 1st and 4th days of exposure there was a slight increase in blood pH and bicarbonate levels and a slight decrease in pC02 and lactate levels. This new acid base status was maintained for 4 days, but later there was a transient shift from alkalosis to acidosis. The transient shift from alkolosis to acidosis has been observed in other studies (Truchot 1981).

On prolonged exposure lead had pronounced affects causing a drop in blood pH and a rise in pC02 values. Root and Bohr effects would also impede oxygen uptake. The ensuing hypoxemia necessitated a dependence upon glycolysis as demonstrated by a rise in blood lactate concentration. Increase in blood lactate is a common consequence of environmental hypoxia (Holetan and Randall 1967; Burggren and Cameron 1980). Increase in blood lactate values have suggested an increase in pC02 content which would reflect increased resistance to diffusion of gases by the damaged branchial epithelium which is probably a major cause of decreased p02.

Strenuous activity caused marked decrease of haemolymph pH in decapode crustaceans (Johansen et al 1970; Philips et al 1977; Wood and Randall 1981). In the aquatic crab, Cancer magister as well as in terrestrial crabs Gecarcinus lateralis and Cardisoma carnifex this acidosis was shown to have both a metabolic and a respiratory component (McDonald et al 1979; Smatresk et al 1979; Wood and Randall 1981). The respiratory component a rise of pC02 in haemolymph developed first. By contrast the metabolic component mainly due to lactate appeared more slowly but persisted for longer time due to the low rate of disappearance of lactate from Crustacean haemolymph (Truchot 1983). The nature of acid-base disturbance provoked by lead toxicity has not been previously studied in Crustaceans and thus no direct comparisons are possible. Although acidosis seen in lead exposed crabs was due to respiratory, metabolic or a combination of two.

Another reason for the elevation of pC02 may be due to the inhibition of the enzyme carbonic anhydrase which catalyses carbonic acid formation during respiration. Carbonic anhydrase is present in gill and is thought to be necssary for carbondioxide excretion (Maetz 1971). Spry and Wood (1984) have suggested that an elevation of carbondioxide may be due to the inhibition of carbonic anhydrase enzyme by the heavy metal zinc during zinc toxicity in the rainbow trout, perhaps a similar situation may occur during lead toxicity also. Since lead is a divalent cation and carbonic anhydrase is a zinc dependent metalloprotein, it is possible that the lead may replace zinc thus leading to the inhibition of enzyme activity.

Bicarbonate content in the blood is another parameter which is actively involved in the regulation of acid-base status of the blood. A primary change in the bicarbonate arises mainly due to the disturbances of the non-volatile or fixed acids and bases. The decrease in the bicarbonate content is attributable to either a decrease in pH or an increase in carbondioxide content during lead exposure. Similar results have been reported by a number of investigators during toxicity studies (Smart 1978; Spry and Wood 1984).

Hence it may be concluded that the lead induced disturbance during prolonged exposure is caused by three factors. Drop in pH, Rise in pC02 and lactic acid formation due to tissue hypoxia.

Acknowledgments. We thank the Council of Scientific and Industrial Research (CSIR) New Delhi, India for providing the Fellowship.

REFERENCES

Burggren WW, Cameron JN (1980) Anerobic metabolism, gas exchange and acid-base balance during hypoxic exposure in the channel catfish, Ictalurus punctatus. J Exp Zool 213 : 405 - 416.

Dejours P, Beekenkamp, H (1978) Acid-base balance during moulting in the crayfish, C.R.Hebd. Seances. Acad Sci 286:1895-1898.

Eddy FB (1976) Acid-base balance in rainbow trout, Salmo gairdneri subjected to acid stress. J Exp Biol 64 : 159 - 171.

Finney DJ (1964) Probit Analysis. Cambridge University Press, London.

Hollanders FD (1968) The Production of lactic acid by the perfused rat diaphragm. Comp Biochem Physiol 26(3) : 907 - 916.

Holeton GF, Randall DJ (1967) The effects of hypoxia upon the partial prssure of gases in the blood and water afferent and efferent to the gills of rainbow trout. J Exp Biol 46:317 - 327.

Johansen K, Lenfant C, Meckie burg TA (1970) Respiration in the crab, Cancer magister. Z Vergl Physiol 70 : 1 - 19.

Mc Donald DG, McMahon BR, Wood CM (1979) An analysis of acid-base disturbance in the haemolymph following strenuous activity in the dungeness crab, Cancer magister. J Exp Biol 79:47-58.

Maetz J (1971) Fish gills : Mechanisms of salt transfer in fresh water and sea water. Proc R Soc London (Biol) 262:209-250.

Packer RK (1979) Acid-base balance and gas exchange in brook trout, Salvelinus fontinalis, exposed to acidic environments. J Exp Biol 79 : 127 - 134.

Philips JW, Mckinney RJW, Hird FJR, Macmillan DL (1977) Lactic acid formation in crustaceans and the liver function of the midgut gland questioned. Comp Biochem Physiol B 56B:427-434.

Smart GR (1978). Investigation of the toxic mechanism of ammonia to fish. Gas exchange in rainbow trout exposed to acutely lethal levels. J Fish Biol 12(1) 93 - 104.

Smartresk NJ, Presiar AJ, Cameron JN (1979) Post exercise acid-base disturbance in Gecarcinus lateralis, a terrestrial crab. J Exp Zool 210 : 205 - 210.

Spry DJ, Wood CM (1984) Acid - base, plasma ion and blood gas changes in rainbow trout during short term zinc exposure. J Comp PhysiolB 154 : 149 - 158.

Truchot JP (1978) Mechanisms of extra cellular acid base regulation as temperature changes in decapod crustaceans. Resp Physiol 33 : 161 - 176.

Truchot JP (1981) The effect of water salinity and acid-base state on the blood acid-base balance in the euryhaline crab, Carcinus maenas, Comp Biochem Physiol A 68A : 555 - 561.

Truchot JP (1983) Regulation of acid-base balance. In : The Biology of Crustacea, Vol 5. Internal Anatomy and Physiological regulation (Ed) Linda H Mantel, Academic Press, New York. 431 - 452.

Tulasi SJ, Rafath Yasmeen, Ramana Rao JV (1985) Toxicity of organic and inorganic lead to the fresh water field crab, Barytelphusa guerini. J Aqu Biol 3 (1 & 2) : 38 - 41.
Tulasi SJ, Rafath Yasmeen, Padmaja Reddy C, Ramana Rao JV (1987) Lead uptake and lead loss in the fresh water field crab, Barytelphusa guerini on exposure to organic and inorganic lead. Bull Environ Contam Toxicol. 39(1) : 63 - 68.
Tulasi SJ, Ramana Rao JV. Effect of organic and inorganic lead on the oxygen consumption of the fresh water crab, Barytelphusa guerini during toxicity and recovery. Ind J Phy Nat Sci (In Press).
Wood CM, Randall DJ (1981) Oxygen and Carbon dioxide exchange during exercise in the land crab, Cardisoma carnifex. J Exp Zool 218 (1) : 7 - 22.
Received August 18, 1987; accepted September 28, 1987.