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Some Physiological Consequences to Fresh Water Fish, *Channa punctatus*, after Exposure to Lindane

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Alterations in the physiology and biochemistry of aquatic organisms are being investigated as potential diagnostic tools in assessing environmental effects of the chemicals. The role of Na^+/K^+ ATPase (Sargent and Thomson 1974) and Cl^-/K^+ ATPase in the active transport of electrolytes (Cl^-/HCO_3^-) across the gills of teleost (Bornancin et al. 1980) has been reported. Regulation of ionic fluxes is intimately related to maintenance of ionic and osmotic homeostasis (Eddy 1982). It is well established that Na^+/K^+ ATPase is localized in the chloride cells of gill epithelium (Towle 1981). Alterations in Na^+/K^+ ATPase activity has been shown in adaptation to a wide variety of environmental contaminants (McKeown et al. 1985).

Lindane, a stable and persistent insecticide, is known to cross the gill epithelium of fish and found to produce biochemical and histological changes (Gopal et al. 1988). The present study elucidates the effect of lindane on the gill ATPase activity, lactic acid level and tissue water content of a fresh water fish Channa punctatus.

MATERIALS AND METHODS

Healthy specimens of fresh water fish Channa punctatus were collected from local resources and acclimatized to standard laboratory conditions. Experiments were conducted in wide mouthed jar containing 20 L of tapwater with following characteristics: temperature 26±2°C, pH 6.9-7.2, alkalinity 95-100mg/L as $CaCO_3$ and hardness 118-122 mg/L as $CaCO_3$. Technical grade (95%) lindane in acetone (Swaroop Chemicals, Lucknow, India) was added to water containing tests (6) specimens to attain the desired concentrations (0.03 and 0.006 mg/L) for 96 hr. Parallel group of acetone control fish was also maintained under identical conditions. For determination of water content in gill, liver, brain and muscle, the tissues were removed from the fish after decapitation weighed wet and then dried for 48-96 hr and reweighed (Heath 1984). To assay

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the activity of ATPase the gill tissues were homogenized immediately with chilled 0.32M sucrose solution (1:4), homogenate was centrifuged for 10 min at 800xg at 4°C and supernatant was removed. The supernatant (0.2 mL) was taken for assaying the enzyme activity. The assay system contained Tris-HCl buffer (50 mM, pH 7.8), sodium chloride (100 mM), potassium chloride (20 mM) and magnesium chloride (4 mM). The incubation was done for 1 hr at 27°C and the reaction was stopped by adding 1 ml chilled trichloroacetic acid (TCA, 10%). The inorganic phosphate (Pi) liberated was estimated by the method of Fiske and Subbarow (1925). The unit of enzyme activity was expressed as µmol Pi liberated/mL homogenate/hr. Ouabain (1 mM) was added to the reaction mixture to inhibit the Na^+/K^+ ATPase activity. Statistical comparisons between mean values were performed by using Student's 't' test. Homogenate of brain, liver, muscle and gill were mixed with equal amount of 10% trichloroacetic acid (TCA) and centrifuged. To 1 mL of supernatant 0.5 mL 20% $CuSO_4.5H_2O$ was added and made upto 5 mL by adding distilled water. Calcium hydroxide $CA(OH)_2$ 0.5 gm was added and centrifuged again after occasional shaking upto 30 minutes. The lactic acid content was estimated in the supernatant of the fresh tissue homogenate according to the procedure described (Barker and Summerson 1941).

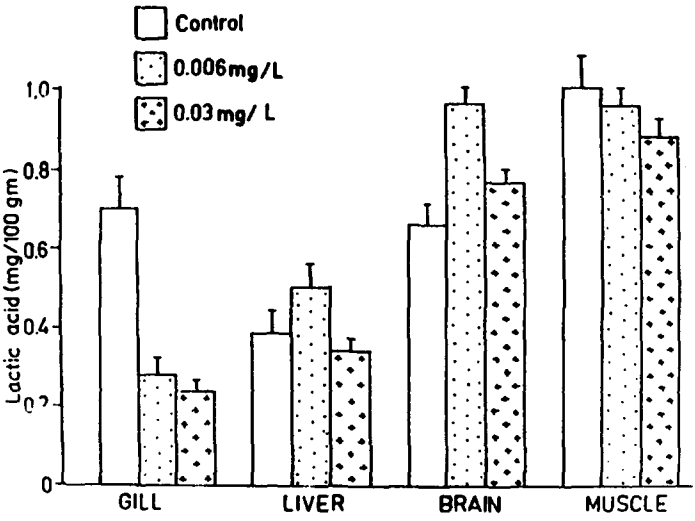
RESULTS AND DISCUSSION

At lower concentration of lindane treatment (0.006 mg/L, a decrease in the lactic acid content was noticed in gill and muscle whereas a significant increase ($P<0.0010$ was observed in liver and brain (Fig. 1). The fish exposed to higher concentration of lindane (0.03 mg/L) for 96 hr showed significant inhibition ($P<0.001$) of Na^+/K^+ and Mg^{++} ATPase in gill tissues (Table 1). The exposure of fish at this concentration

Table 1. ATPase activity in gill tissues of fresh water fish C. punctatus exposed to lindane for 96 hr.

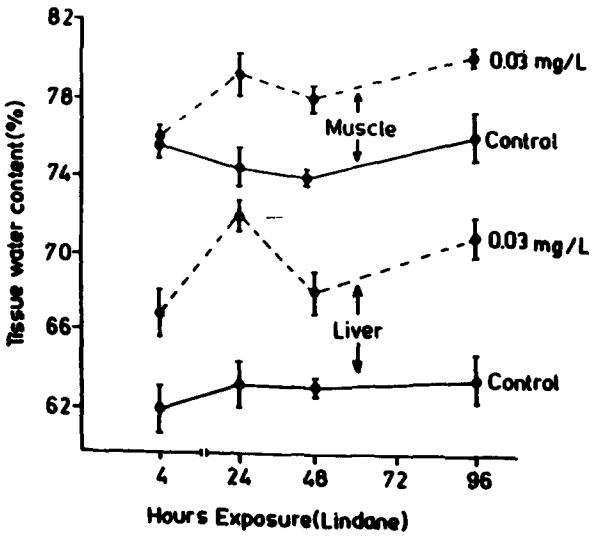
Tissue	Treatment	Total ATPase activity	MG^{++} ATPase activity	Na^+K^+ ATPase activity
Gill	Control	2688.1±15	1673.06±53.1	1015.06±18
	(0.006 mg/L) Lindane	2544.8±13	1623.33±64.1	921.46±15
	(0.03 mg/L)	355.8±26***	210.2±16***	145.6±15***

Values are mean ± S.E. (3 observations)
Values are significant at ***$P<0.001$ (Student's 't' test)
Units of enzyme activity expressed as µmol Pi liberated/h/g. tissue



Lactic acid level after lindane exposure(96hr) to fresh water fish Channa punctatus

·**Figure 1.**



·**Figure 2.** Effect of lindane on tissue water content of fish Channa punctatus.

of lindane (0.03 mg/L) showed marked decrease in the level of lactic acid in liver, muscle and gill; however, a significant increase was observed in brain. Water content of muscle and liver of the fish exposed to lindane (0.03 mg/L) increased significantly at two test durations (24 hr and 96 hr) followed by a marked effect at 24 hr of exposure (Fig. 2). The observed decrease in the activity of Na^+/K^+ and Mg^{++} ATPase in gill of the pesticide treated fish could be the result of direct action of the toxicants on the enzymes. This finding supports the idea that the pesticides can directly interfere with the membrane enzymes. The inhibition in the ATPase activity in gill may also be due to primary lethal lesions in gills. Inhibition or stimulation of ATPase activity could be expected to have metabolic or ionic effect in fishes in relation to osmoregulation (Verma et al. 1988). Lindane exposure of C. punctatus resulted in an increase in water content of liver and muscle. A decrease in osmolarity and/or Na^+ and Cl^- ion concentrations in blood of Cu^{++} ion stressed fresh water fish have been reported (Lewis and Lewis 1972) reflecting thereby an elevated water content in the tissues. Fresh water fish regulate their blood and tissue osmolality by excreting excess water via kidney and recovering salts by an active transport of Na^+/K^+ and Cl^- from the water into the blood by chloride cells in the gills (Gordon 1982). This process involves the enzyme Na^+/K^+ activated ATPase. Such an inhibition of enzymes could cause reduction of blood osmolality and presumably interstitial fluid osmolality was well. An osmotic gradient thus developed into the cells resulted in an increase of water content. The level of lactic acid in the tissues of C. punctatus is increased after exposure to lindane (Heath 1984). It is evident that these alterations are not due to hypoxemia. Lindane acts on tissues bringing changes in enzyme function. The increase in water content in the tissues of C. punctatus can be attributed to the dilution of cellular metabolites contributing significant decrease in activity of ATPase under stress condition. Similar observations have been reported in Cu^{2+} exposed Lepomis macrochirus (Heath 1984).

It is evident from our data (Fig. 1) that the brain of the fish is most sensitive organ as the level of lactic acid drastically increased even at very low concentration of lindane (0.006 mg/L). At this concentration the fish is under stress to experience hypoxial condition leading to increase in anaerobiasis resulting in metabolism of lactic acid from gills and muscles into the blood circulatory system and from there it reaches liver and brain. After the exposure of fish with 5-times greater concentration of lindane, the mobilisation/depletion of lactic acid is enhanced resulting in decrease in the level of lactic acid in liver, gills and muscles whereas it is still higher in brain in comparison to that of control. The reason may be due to higher rate of accumulation of lactic acid in brain.

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REFERENCES

Bakthavathsalam, R (1988) Toxicity of lindane to some air breathing fishes. Environ Ecol 6:222-224

Barker SB, Summerson WH (1941) The colorimetric determination of lactic acid in biological material. J Biol Chem 138: 535-554

Bornancin M, deRonzis G, Naon RA (1980) Cl^- HCO_3^- ATPase in gills of rainbow trout: evidence for its microsomal localization. Am J Physiol 238: 251-259

Eddy FB (1982) Osmotic and ionic regulation in captive fish with particular reference to salmonids. Comp Biochem Physiol 73B:125-141

Fiske CH, Subbarow Y (1925) The colorimetric determination of phosphorus. J Biol Chem 66:375-400

Gopal K, Ram MD, Gupta GSD, Anand M (1988) Lindane induced histological changes in the gills of Channa punctatus (Bloch) Ind J Bio Res 8:25-29

Gordon MS (1982) Animal physiology: Principles and adaptation. 4th ed. MacMillan, New York

Haya K, Waiwood VA, Burridge LE, Vaneeckhaute L (1985) Liver energy metabolism and gill ATPase activity of Salmosalar Juveniles after exposure to sublethal levels of phenols. In: Vernberg FJ, Thurarg FP(ed).Marine Pollution and Physiology Recent Advances. Univ. of South Carolina Press, Columbia, pp 521-535

Heath AG (1984) Changes in tissue adenylates and water content of bluegill, Lepomis macrochirus, exposed to copper. J Fish Biol 24:299-309

Lewis SD, Lewis WM (1972) The effects of zinc and copper on the osmolality of blood serum of the channel catfish Ictalurus punctatus, rat and golden shiner, Notemigonus Crysoleucos Mitchell. Tran Am Fish Soc 100:639-643

McKeown BA, Green GH, Watson TA, Powell JF, Parker DB (1985) The effects of pH on plasma electrolytes, carbonic anhydrase and ATPase activities in rainbow trout Salmogairdneri (Richardson). Comp Biochem Physiol 68C:151-159

Sargent JR, Thomson AJ (1974) The nature and properties of inducible Na^+/K^+ ions dependent ATPase in the gills of eels (Anguilla anguilla) adapted to the fresh water and sea water. Biochem J 144:69-75

Towle DW (1981) Role of Na^+/K^+ ATPase in ionic regulation by marine and estuarine animals. Mar Biol Lett 2:107-122

Verma SR, Bansal SK, Gupta AK, Dalela RC (1988) In vivo effect of ATPase in certain tissues of Labeo rohita and Saccobranchus fossilis following chronic chlordane intoxication. Bull Environ Contam Toxicol 20:769-777

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