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Physiological Dysfunction of the Haemopoietic System in a Fresh Water Teleost, *Labeo rohita*, Following Chronic Chlordane Exposure. Part II — Alterations in Certain Organic Components and Serum Electrolytes

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Clinical chemical analysis used in mammalian studies are highly developed and reliable, but only modest application of the principles and methods have been extended to the realm of aquatic organisms. However, there is accumulating evidence that valid and useful analytical relations can be drawn between biochemical - physiological factors and pathology of an aquatic species. Physiological changes in the blood and tissue of fishes exposed to varying degrees and types of stressors have been measured by several investigators, but a systematic and detailed work on blood biochemical changes on a fresh water major carp Labeo rohita, a commercial food fish,in Indian environmental conditions is not available. Therefore, it is hoped that specific blood analysis will lead to methods for determining the health of aquatic organisms, particularly when the animals are under great stress that is not great enough to cause gross changes and mortality. The absence of severe and mortal effects does not guarantee reproductive success and the maintanence of a healthy population untill a clinical type of examination of representatives of aquatic species may reveal the presence of toxic situations caused by unidentified pollutants or by a variety of stressors operating additively.

Therefore, the present study was undertaken to evaluate the effects of chlordane (an organochlorine pesticide) on the blood chemistry of a fresh water teleost, L. rohita after 60 days treatment at different concentrations.

MATERIALS AND METHODS

All the initial experimental conditions as fish size and weight, pesticide concentrations, solvents used along with the acclimatizing and anesthetizing procedures are the same as in our part-I studies, BANSAL et al. (1979).

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Blood samples were taken immediately after the loss of equilibrium by caudal puncture with a 5.0 cc disposable plastic syringe, previously rinsed with 10% sodium heparin and equiped with a 20- gauge short bevel needle. All equipment that came in contact with the blood was cooled on ice prior to use. Little or no haemolysis was observed. In other vials, blood samples were allowed to clot in an incubator at 37°C, serum was collected and stored in a refrigerator for subsequent analysis.

For determination of glucose, Nelson-Somogi method; for protein, biuret method; for lactate, Barker and Summerson method; for non-protein nitrogen (NPN), Folin Wu method; for phospholipids, modified method of Youngburg, as given by OSER (1965) in 'Hawk's Physiological Chemistry', while for cholesterol, WEBSTER (1962); for free fatty acids (FFA), LAURELL and TIBBLING (1967) and for triglycerides, colorimetric method of CARLSON (1963) were used.

For determination of magnesium (Mg), Titan yellow method; for chloride (Cl), Schales and Schales method and the phosphorus (P) and iron (Fe) were determined by the methods given by OSER (1965) in 'Hawk's Physiological Chemistry', while sodium (Na), potassium (K) and calcium (Ca) were determined by flame photometeric methods.

RESULTS AND DISCUSSION

The marked changes detected after chronic chlordane poisoning in 60 days treated L. rohita elicited some distinct physiological adjustments in certain organic components and serum electrolytes (Table 1). The elevated blood sugar level (hyperglycaemia) indicates a disrupted carbohydrate metabolism, which may be mainly due to the enhanced breakdown of liver glycogen (glycogenolysis), mediated perhaps by adrenocortical hormones and reduced insulin secretory activity. The observed elevation of blood glucose increased with increased exposure concentration but strong hyperglycaemic response ($P<0.05$) was observed at 35 and 70 µg/L of chlordane. GRANT AND MEHRLE (1973) also found a 50% increase in serum glucose level of trout fed highest dietry dose of endrin. SILBERGELD (1974) exposed the johnny darter (Etheostoma nigrum R.) to 2.33 ppb of dieldrin and showed significant increase in blood glucose, to more than 133% of control levels, after 5 days, but after 15 days the level returned to its initial levels. KRAYBILL (1969) and OLIVEREAU (1964) prooved that blood glucose is directly related to organochlorine insecticides in fish and mammals. HART et al. (1971a, 1971b) showed that chlorinated hydrocarbons act as adrenal pituitary gluco-

corticoid mediated stressors, which affect blood glucose levels by this pathway of stress response. LARSSON (1975) and LARSSON et al. (1976) observed hyperglycaemia and decreased blood lactate level, concomitant with increased glycogen in liver and decreased in muscles in flounders exposed for 15 days to various concentrations of cadmium in water and suggested a stimulated liver glycogen synthesis. CHRISTENSEN et al. (1972) observed significant ($P<0.01$) increased glucose level in Ictalurus nebulosus after 6 and 30 days when exposed to 49 and 107 µg/L of Cu (II) but no effect was observed after 600 days when exposed to still lowerer concentrations of copper. They further noted a non-significant increase in protein after 6 days, significant decrease after 30 days but no variation after 600 days. McKIM et al. (1970) also noted increased protein level after 6 days but decreased level after 21 days in brook trout (Salvelinus fontinalis) exposed to various concentrations of copper. Authors, however, here noted a non-significant increase only at two higher concentrations.

Lactate levels after 60 days treatment appeared affected slightly or not at all by chlordane treatment, which may be due to the sustained activity of the fish in experimental tanks. STEVENS AND BLACK (1966) reported that forced swimming (for 5 min) of rainbow trout caused muscle lactate to peak immediately and the level in blood to increase more slowly, concurrent with lactate release from muscles. NPN remained statistically unaffected, but non-significant increase was always observed at all exposures. VERMA et al. (1979) found increased NPN and total phosphorus in S. fossilis exposed to sublethal concentration of chlordane.

Cholesterol level was found significantly decreased at 23 µg/L ($P<0.05$) and 35 and 70 µg/L ($P<0.01$). Phospholipids showed significant decrease ($P<0.05$) at 70 µg/L, while triglycerides showed significant decrease at 17 and 35 µg/L ($P<0.05$). However, FFA were found significantly increased at 17 µg/L ($P<0.05$) and 35 µg/L ($P<0.01$) which may probably be due to the increased breakdown of cholesterol into these fatty acids. Hypocholestaemia due to muscular exhaustion and dissolved oxygen depletion has also been observed by VERMA et al. (1979) in S. fossilis. GRANT AND MEHRLE (1970) observed increased lipid content in proportion to the endrin dosage and showed by TLC that mono, di- and triglycerides were the lipids principally affected. ISHIHARA et al. (1967) observed that pentachlorophenol, dimethylthiophosphate, endrin and phenyl mercuric acetate all caused changes in the component ratio of serum proteins and lipoproteins in the blood of carp.

TABLE 1

Alterations in organic components and serum electrolytes on exposure to chlordane in *L. rohita* after 60 days.

Parameters[a]	Control	17 µg/L	23 µg/L	35 µg/L	70 µg/L
Glucose (m mol/L)	4.7 ± 0.2	5.0 ± 0.3	5.4 ± 0.5	6.1 ± 0.3*	6.6 ± 0.6*
Protein (g/L)	48.0 ± 5.0	42.0 ± 2.2	45.6 ± 4.1	51.2 ± 7.5	49.1 ± 3.7
Cholesterol (m mol/L)	12.1 ± 0.6	11.1 ± 0.7	9.8 ± 0.4*	8.7 ± 0.2**	7.6 ± 0.2**
Phospholipids(m mol/L)	6.8 ± 0.6	6.7 ± 0.4	6.2 ± 0.8	5.2 ± 0.4	4.8 ± 0.3*
Triglycerides(m mol/L)	32.7 ± 2.1	24.5 ± 1.6*	27.3 ± 2.3	22.9 ± 1.5*	-
Lactate (m mol/L)	1.9 ± 0.2	2.0 ± 0.2	1.8 ± 0.1	1.7 ± 0.1	-
NPN (m mol/L)	42.2 ± 2.1	44.3 ± 2.5	48.6 ± 2.0	46.7 ± 3.4	-
FFA (µ mol/L)	810.0 ±15.8	880.2 ±11.0*	850.0 ±11.5	950.0 ±20.1**	-
Na (m mol/L)	143.5 ± 7.5	139.1 ± 8.1	152.2 ± 6.2	169.9 ± 6.0*	-
K (m mol/L)	2.1 ± 0.2	2.2 ± 0.4	2.1 ± 0.3	2.3 ± 0.2	-
Cl (m mol/L)	76.1 ± 1.7	83.7 ± 2.6	82.7 ± 3.4	89.0 ± 2.8*	-
Ca (m mol/L)	5.3 ± 0.4	6.4 ± 0.7	7.8 ± 0.5*	7.2 ± 0.3*	-
Mg (m mol/L)	1.3 ± 0.1	2.3 ± 0.3*	3.3 ± 0.2**	3.1 ± 0.4**	-
Iron (mg/dl)	25.2 ± 2.5	23.5 ± 1.5	30.9 ± 3.1	39.7 ± 2.1*	-

a Values are mean ± S.E. (3 observations).
*P<0.05; **P<0.01; ***P<0.001 (Fisher's 't' test).

None of the electrolyte and certain organic components were determined because of the increased mortality at 70 µg/L after 60 days resulting in the scarcity of blood. The major serum electrolytes Na and Cl were highly correlated and found significantly increased ($P<0.05$) at 35 µg/L of chlordane. For the time being we have no satisfactory explanation for these elevated Na and Cl levels which must have resulted in increased serum osmolality at higher concentrations. Authors infer that osmoregulation in L. rohita has been affected either directly or indirectly by toxicant solution. HOLMES AND McBEAN (1963) and HOUSTON (1959) reported 9.4 and 4.4% increase in serum Na (Natremia) in rainbow trout on adaptation to salt water respectively. GRANT AND MEHRLE (1970) reported that a high dose (430 µg/L body wt) of endrin in goldfish caused Na and Cl loss and complete failure of osmoregulatory process. EISLER AND EDMUNDS (1966) observed an increased concentration of Na in blood and decreased in the liver in northern puffers (Sphaeroides maculatus), a marine fish, exposed for 96 h to near lethal concentration of endrin. Their observations suggest a transfer of Na but not of K from tissues to blood by some means. Authors conclude that the effect may either be due to a stimulated active uptake of Na and Cl ions from the external media or to a loss of these ions from other tissues to the blood.

The effect of chlordane exposure on other electrolytes other than Na and Cl was also pronounced. The potassium content, which is the principal intracellular cation and is intimately involved in nerve and muscle functions, remained almost unaffected. Significant increase ($P<0.05$) in Ca (hypercalcaemia) at 23 and 35 µg/L, which is a general regulator of permeability of cell membranes to water and ions, was observed. In addition, membrane potential and development of action potential in muscles and nerves, for which Ca is essential, must have been affected by chlordane. On the other hand, serum Mg content was remarkably elevated at 17 µg/L ($P<0.05$) and 23 and 35 µg/L ($P<0.01$). The pronounced chlordane induced elevation of serum Mg in L. rohita might be due to an impaired ability of the fish to actively excrete the excess of this ion, hereby showing much renal damage. HARPER (1971) also observed this interesting parallel in man that renal failures are often associated with an elevated serum Mg concentration. Iron was found unaffected at lower concentrations but significant ($P<0.05$) increase was observed at 35 µg/L of chlordane exposure. Therefore, authors assume that chlordane toxicosis is characterised by chronic dysfunction of osmoregulatory and other related mechanisms, manifested by increased levels of the major

electrolytes. Similar trend of increment of certain organic components and serum electrolytes has also been observed by VERMA et al. (1979) in S. fossilis following chronic chlordane exposure. GRANT AND MEHRLE (1970, 1973) also reported other related altered physiological parameters of growth, reproduction, thyroid activity, intermediary metabolism and osmoregulation in goldfish (Carassius auratus) and rainbow trout (Salmo gairdneri).

In conclusion, the complex clinical picture noted indicate that chlordane in water produce dysfunctions of several physiological and biochemical processes in fish. As apparent, osmoregulation is extremely precise, therefore, increased mortality after 60 days at 70 µg/L, may be the failure of fish to regulate the major electrolytes within very narrow ranges. However, the results, encourage further work with still lower concentrations and with longer test durations. Such sublethal chronic studies are important as even small physiological disturbances might reduce the chance of the animal being successful in the environment. It must also be emphasized that elimination of aquatic animals by small, insidious physiological or behavioral changes can be regarded as more serious than a massive fish kill, since it is less likely to be observed.

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REFERENCES

BANSAL, S.K., S.R. VERMA, A.K. GUPTA, and R.C. DALELA: Bull. Environ. Contam. Toxicol. (1979) Communicated.

CARLSON, L.A.: J. Atheroscler. Res. 3, 334 (1963).

CHRISTENSEN, G.M., J.M. McKIM, W.A. BRUNGS, and E.P. HUNT: Toxicol. Appl. Pharmacol. 23, 417 (1972).

EISLER, R., and P.H. EDMUNDS: Trans. Am. Fish. Soc. 95, 153 (1966).

GRANT, B.F., and P.M. MEHRLE: J. Fish. Res. Bd. Can. 27, 2225 (1970).

GRANT, B.F., and P.M. MEHRLE: J. Fish. Res. Bd. Can. 30, 31 (1973).

HARPER, H.A.: Review of physiological chemistry. Lange Med. Pub. Los Altos, California (1971).

HART, M.M., and J.A. STRAW: Steroids, 17, 559 (1971a).

HART, M.M., E.S. SWACKHAMER, and J.A. STRAW: Steroids, 17, 575 (1971b).

HOLMES, W.N., and R.L. McBEAN: J. Exp. Biol. 40, 335 (1963).

HOUSTON, A.H.: Can. J. Zool. 37, 729 (1959).

ISHIHARA, T., M. YASUD, and O. TAMURA: Bull. Fac. Fish. Nagasaki Univ. (Japan) 24, 65 (1967).

KRAYBILL, H. (ed): Biological effects of pesticides in mammalian systems. Annals N.Y. Acad. Sci. 160, 1 (1969).

LARSSON, A.: From sublethal effects of Toxic Chemicals on aquatic animals. ed. J.H. Koeman, and J.J.T.W. A. Strik. Elsevier Sci. Pub. Com. Amsterdam (1975).

LARSSON, A., B.E. BENGTSSON, and O. SVANBERG: From effects of pollutants on Aquatic organisms, ed. A.P.M. Lockwood. Soc. for Exptl. Biol. Seminar Series, Vol. 2, Cambridge Univ. Press (1976).

LAURELL, S., and G. TIBBLING: Clin. Chim. Acta, 16, 15 (1967).

McKIM, J.M., G.M. CHRISTENSEN, and E.P. HUNT: J. Fish. Res. Bd. Canada, 27, 1883 (1970).

OLIVEREAU, M.: Texas Rept. Biol. Med. 22, 1 (1964).

OSER, B.L.: 'Hawk's Physiological Chemistry' 14th ed. Tata Mc-Graw Hill Book Co. New York (1965).

SILBERGELD, E.K.: Bull. Environ. Contam. Toxicol. 11, 20 (1974).

STEVENS, E.D., and E.C. BLACK: J. Fish. Res. Bd. Can. 23, 371 (1966).

VERMA, S.R., S.K. BANSAL, A.K. GUPTA, and R.C. DALELA: Bull. Environ. Contam. Toxicol. 22 (1979) In Press.

WEBSTER, D.: Clin. Chim. Acta, 7, 277 (1962).