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BIOCHEMICAL CONSTITUENTS AS STRESS INDICATORS IN FISHES

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Certain tissue biochemical constituents serve as good indicators of stressors in animals (Le Resche *et al.*, 1974). Transportation and exercise stress elevated the blood total carbohydrates of *S. mossambicus*. Similarly, toxic stress due to endosulfan caused hyperglycemic and hyperlactemic responses. The changes in the liver and muscle carbohydrate components and proteins during toxic stress revealed their usage in meeting the toxicity mediated energy demand.

INTRODUCTION

Fish farming and aquaculture practices emphasize the importance of edible forms with more breeding potential. In recent years, man made artificial stresses have introduced the problem of selecting more resistant species. Increased concern over the production of resistant edible forms have led to the development of considerable literature on artificial stresses. Stressors on fish include handling, netting and trawling, asphyxiation, transportation, injury, anaesthetization, hyperactivity/exercise (physical), starvation, migration (physiological), and temperature, salinity, pH and toxicity (environmental).

Information about these factors are available on temperate species of fishes (Black *et al.*, 1966; Chavin & Young, 1970; Soivio & Oikari, 1974). Similar reports on tropical species are meagre. In this study, the stressors such as transportation, exercise and toxicity (endosulfan) have been described pertaining to their effects on tissue carbohydrates and proteins. Resistance to transportation is considered as one of the pointers in fish farming as the seeds from an available source have to be transported to distant places. The hyperactivity/exercise is a natural stress associated with the migratory, escape and predatory behaviour of fishes. Toxic stress is man made due to the introduction of several xenobiotic compounds. The present study was attempted to determine the effects of these stress factors on *Sarotherodon mossambicus*.

MATERIAL AND METHODS

S. mossambicus (Tilapia), an exotic euryhaline teleost was used in this study. The fishes were captured from the natural ponds maintained by Tamil Nadu State Inland Fisheries Research Station, Chetpet, Madras and from Ennore. The fishes after capturing by net were transported by bus in plastic containers (100 individuals in 50 litres), to the laboratory. Field water (26 ± 1°C) was used for transport. The time taken for transport was about an hour. The fishes were released in aquarium tanks (110 x 85 x 75 cm) with sufficient volume of water. They were fed on alternate days with cooked rice mixed with dried prawn powder or frog muscles *ad libitum*. They were acclimated to laboratory conditions for 15 days as suggested by Chavin & Young (1970). Water was changed in the stocking tanks on alternate days. The food remnants and wastes were washed out from tanks prior to changing the water. The protocol of study comprised of the following experiments.

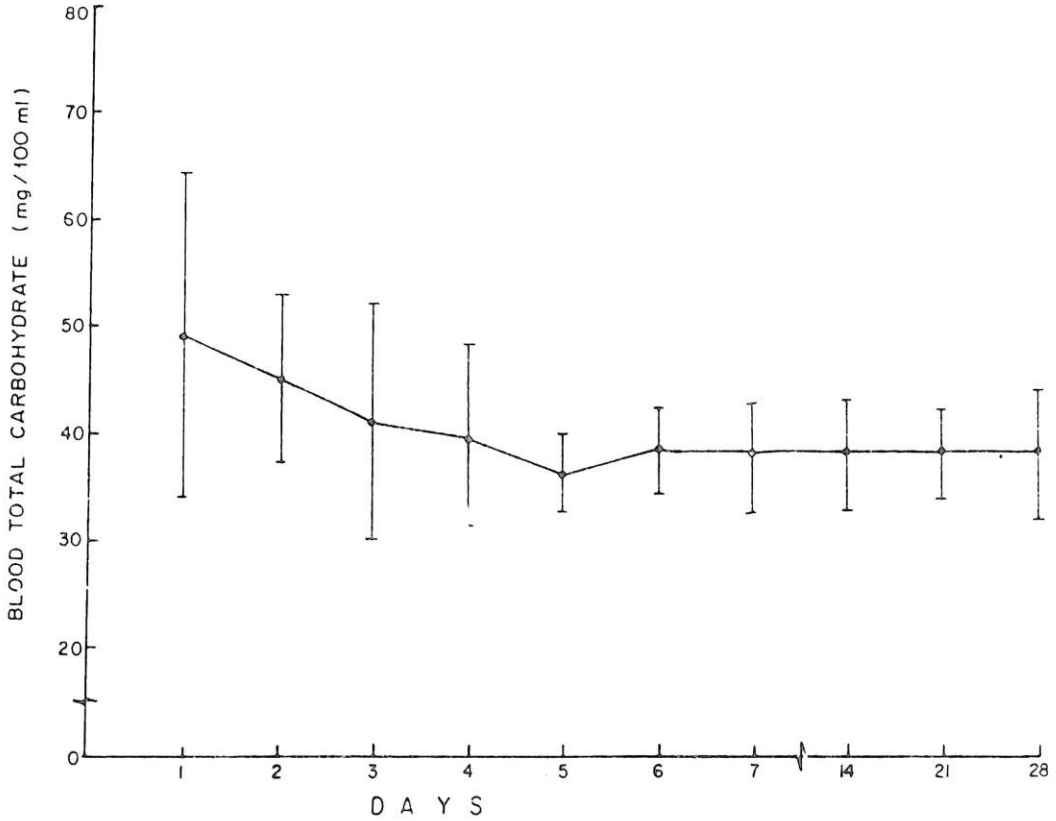

Fig.1. Blood total Carbohydrate level in *S.mossambicus* after transportation

Experiment 1 *Blood carbohydrate analyses after transportation:* The level of blood total carbohydrates in males of the stock population was estimated after release, continuously for one week and after 2nd, 3rd and 4th week.

Experiment 2 *Exercise stress on blood total carbohydrates :* The fishes acclimated to laboratory were subjected to exercise stress in a rotator (electrical motor driven). The plastic container (58 cm diameter; 23 cm height) with 25 litres of water was placed over the stage of the rotator and made to rotate. The speed was regulated to give 25 rpm. The fishes were introduced and subjected to stress of water current for 30 mins. Fishes swam against the water flow and thus become hyperactive. After 30 minutes, they were sampled for blood carbohydrate analyses. The sampling was done from 0-4 hr; 15 to 19 hr; and at 23rd, 24th, 48th and 72nd hr.

Experiment 3 *Toxicity Stress :* 4% commercial grade endosulfan (thiodon) (wettable powder), an organochlorine compound, supplied by Parry & Co., was used in this study. A sublethal concentration of 0.01 ppm of 4% endosulfan was selected for the study. The above dose is one tenth the fraction of its 96 hour LC50 value which is within the range of safe maxima recommended for pesticides to fishes (Mount & Stephen, 1967). The endosulfan was dissolved in experimental water (50 litres) without any solvent. Four groups each consisting of 10 fishes were selected among which two served as control and other two represented the experiment. In the latter, one group was exposed to 7 days and the other for 15 days. Water and the pesticide were renewed after every 24 hours. After the experimental periods, the fishes (control Vs experiment) were sampled for tissue analyses.

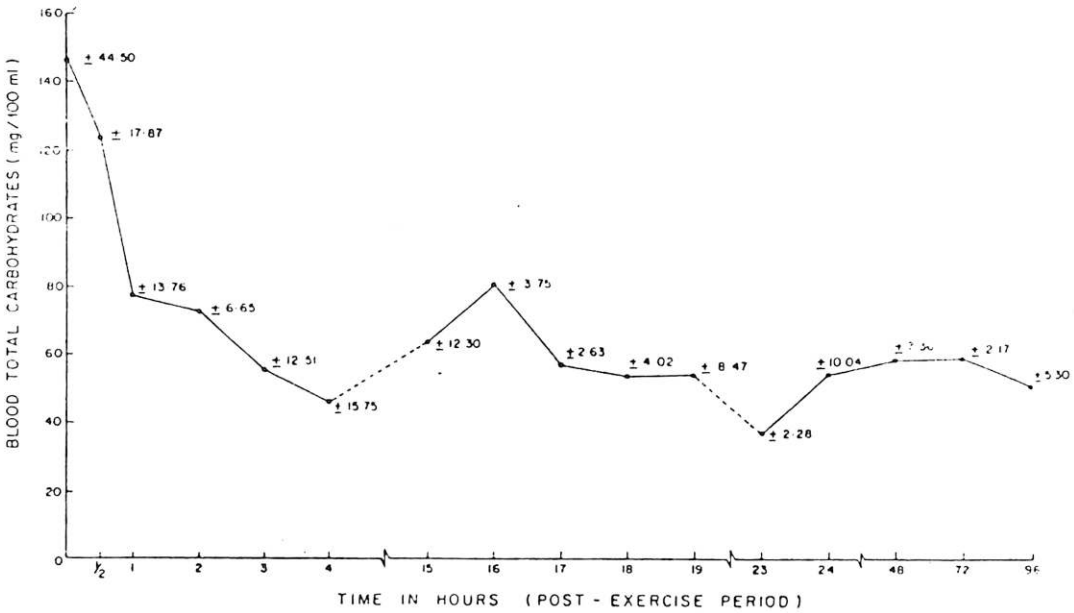

Fig. 2. Blood total Carbohydrate level in *S. mossambicus* after exercise.

For all experiments, only male specimens were used. The time of blood sampling was restricted between 09 and 10 hours. Blood was taken by severing the tail. Potassium oxalate (0.8%) mixed with Ammonium oxalate (1.2%) was used as the anticoagulant. The blood total carbohydrate was estimated by the anthrone method of Roe (1955). Carroll *et al.* (1956) was followed for tissue glycogen and free sugars estimation. Blood and tissue lactic acid were determined by the method of Barker & Summerson (1941) using para-hydroxydiphenyl reagent. The tissue total protein was determined by Lowry *et al.* (1951) method. Liver and muscle tissues were taken from fishes after blood sampling. The values of control and experimental groups were compared by Student 't' test of significance (at 0.05 level).

RESULTS

The blood total carbohydrate levels after transportation and exercise stress are presented in Figs. 1 & 2. Blood total carbohydrate showed higher levels upto 4th day after transport. The levels showed uniformity from 5th day upto 28th day. In exercise stressed fishes, higher level was noticed after 0, 1/2, 1, 2, 15 and 16 hours. In other intervals the values remained uniformly. Table I shows the concentrations of liver and muscle metabolites in endosulfan treated fishes. In this, the blood total carbohydrate increased significantly after 7 and 15 days. It increased by five fold after 7 days and two fold after 15 days. In the same intervals, the blood lactic acid increased by eleven fold and seven fold respectively. The liver lactate content increased significantly by six fold only after 7 days. On the contrary, the muscle lactate increase significantly after both 7 and 15 days intervals. The glycogen content in both liver and muscle decreased significantly during the experimental periods, as compared to control. The total protein content in liver decreased significantly after 15 days while in muscle it declined significantly at both intervals.

DISCUSSION

The results on transportation stress (Fig. 1) indicate that *S. mossambicus* acclimated to laboratory conditions within short intervals after they were brought from the field. Such physiological feature of quick acclimation can be taken as an important

Table I. Blood and tissue metabolites levels in endosulfan (Thiodan) exposed *S. mossambicus.* (0.01 ppm of 4% Wettable Powder).

Metabolites	7 days		15 days	
	control	expt.	control	expt.
Blood Sugar (mg%)	35.85 ± 2.63	170.43± 13.65	38.76 ± 2.44	65.87± 1.16*
Blood Lactate (μg/ml)	14.75 4.12	163.33 34.96*	22.41 10.37	158.00±46.21*
Liver Lactate (μg/100 mg)	17.52 2.61	101.07 15.03*	16.14 8.85	19.69 ± 6.01*
Muscle Lactate (μg/100 mg)	32.32 ± 8.38	100.50 15.95*	21.99 2.69	105.59 ± 9.68*
Liver Free Sugars (mg%)	3.85 0.58	27.83 2.33*	3.12 0.34	17.84 ± 0.52*
Muscle Free Sugars (mg%)	0.27 0.02	4.48 0.42*	0.267 0.02	8.64 ± 1.53*
Liver Glycogen (mg%)	5.32 0.59	1.72 0.50*	5.88 1.07	1.07 ± 0.33*
Muscle Glycogen (mg%)	0.16 0.02	0.12 0.05*	0.18 0.03	0.09 ± 0.00*
Liver Protein (mg%)	17.50 ± 2.73	15.03 ± 0.42*	17.85 0.55	8.15 ± 0.54*
Muscle Protein (mg%)	12.07 1.62	5.03 0.12	11.42 1.35	4.92 ± 0.48*

(Tissue - Wet wt.)

*Statistically significant (Student t-test) (P = 0.05)

prerequisite for experimental studies. The results on exercise stress (Fig. 2) also reveal that the animals recovered from hyperglycemia within short postexercise period. As glycogen stores in tissues are mobilised for the elevation of blood carbohydrates, the short period recovery is an advantageous physiological correlate in this species. The above capacity of recovery from stress may have a genetic basis, and this could be considered as an important criterion in aquaculture. A comparision of the above results with those of temperate fishes revealed that in salmons, *Salvelinus namaycush* and *Salmo gairdneri* higher levels of blood glucose persisted after muscular exercise upto 24 hours (Black, 1957; Black *et al.*, 1960). The above difference between tropical and temperate fishes could be attributed to temperature and salinity of water and their consequent influence on metabolism also, besides species specific tolerance capacity.

The effects of endosulfan revealed (Table I) both hyperglycemia and hyperlactemia in blood. The liver and muscle tissue also showed higher lactate content. The formation and accumulation of lactic acid in tissues are due to insufficient supply of oxygen (hypoxia) and due to the functioning of LDH (lactic dehydrogenase) enzyme in reverse pathway to convert more pyruvate into lactate. Ramalingam (1985) has shown that the LDH isoenzymes pattern changes characteristic of hypoxic condition and also noticed a decrease in the SDH (succinic dehydrogenase) activity of these tissues in *S. mossambicus* exposed to DDT. Similar results have been demonstrated in fishes as well rodents (Zimmerman *et al.*, 1971; Bostrom & Johansson, 1972; Truhaut *et. al.*, 1973). The results thus suggest that endosulfan affects the fish metabolism similar to DDT. The depletion of glycogen and elevation of free sugars in tissue indicate glycogenolysis and the consequent feeding of them for pyruvate to lactate conversion process. The significant decline of protein after 15 days in the liver and after both intervals in muscle suggest that protein may also contribute to lactate formation through gluconeogenesis. Such a contributory role of protein in addition to carbohydrates during stress has also been recorded previously (Umminger, 1970; Ramalingam & Ramalingam, 1982). The low level of glycogen (in normal condition) in muscle as compared to liver may be attributed for the significant decline of protein in it after 7 days. Coleman (1968) has noticed a significant decline in the bound hexosamines of serum of rats treated with endrin. Ramalingam & Ramalingam (1982) also revealed the solubilisation of tissue proteins by the increase in the serum protein fractions electrophoretically. Intensive tissue proteolysis resulting in free amino acid pool increase and conversion of them into keto acids during toxic stress have been reported by Kabeer Ahamad *et al.* (1978). The enhancement of transaminase enzymes for the above conversion has also been reported by several workers (Mckim *et. al.*, 1970; Lane & Scura, 1970; Sakaguchi & Hamaguchi, 1975). The over all results thus reveal that both carbohydrates and proteins contribute to energy release during toxic stress. The contribution of protein in different tissues may be dependent upon the total reserve glycogen available and the period of exposure of the animals to toxic stress. The importance of carbohydrates in the productivity of fish in aquaculture has recently been elucidated by Shimeno (1974). Hence draining of this energy reserve by toxic stress in fishes appears to be a cause for concern.

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REFERENCES

BARKER, S.B. & SUMMERSON, W.H. 1941. The colorimetric determination of lactic acid in biological matter. *J. Biol. Chem. 138* : 535 - 554.

BLACK, E.C. 1957. Alterations in the blood level of lactic acid in certain satmonid fishes following muscular activity. II. Lake trout. *Salvelinus namaycush. J. Fish Res. Bd. Can. 14* : 645 - 649.

BLACK, E.C., ROBERTSON, A.C., HANSLIP, A.R. & CHIU, W.G. 1960. Alterations in glycogen, glucose and lactate in rainbow trout and kamloops trout *Salmo gairdneri* following muscular activity. *Ibid. 17 :* 487-500.

BLACK, E.C., MANNING, G.T. & HAYASHI, K. 1966. Changes in levels of haemoglobin, oxygen, carbon dioxide pyruvate and lactate in venous blood of rainbow trout *Salmo gairdneri* during and following severe muscular activity. *Ibid. 23* : 783-795.

BOSTROM, S.L. & JOHANSSON, R.G. 1972. Effects of PCP on enzymes involved in energy metabolism in the liver of the eel. *Comp. Biochem. Physiol. 41* (B) : 359-369.

CARROLL, N., LONGLEY, R.W. & ROE, J.H. 1956. The determination of glycogen in liver and muscle by use of anthrone reagent. *J. Biol. Chem.*

CHAVIN, W. & YOUNG, J.E. 1970. Factors in the determination of normal scrurn glucose levels of gold fish *Crassius auratus. Comp. Biochem. Physiol. 33* : 629-633.

COLEMAN, R.L. 1968. Endrin induced alteration in bound carbohydrates in rat serum. *Bull. Jpn. Soc. Sci. Fish. 41* : 238 - 290.

KABEER AHAMED SAHIB, I., BEGUM. Md., SIVAIAH. S. & RAMANA RAO, K.V. 1978. Effects of malathion on free amino acids, total proteins, glycogen and some enzymes of pelecypod *Lamellidens marginalis* (Lamack). *Proc. Indian. Acad. Sci. 87* : 377-381.

LANE, G.E. & SCURA, E.D. 1970. Effects of dieldrin on glutamic oxaloacetic transaminase in *Poecilia latipinna. J. Fish. Res. Bd. Can. 27* : 1869-1871.

LE RESCHE, R.E., SEAL, U.S., KERN, P.D. & FRANZMANN, A.W. 1974. A review of blood chemistry of moose and other Cervidae with emphasize on nutritional assessment. *Natl. Can. Ottawa. 101* : 263-290.

LOWRY, D.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem. 193.* 265-275.

MOUNT, D.I. & STEPHAN, C.E. 1967. A method for establishing acceptable toxicant limits for fish-malathion and the butoxethanol ester of 2, 4-D. *Trans. Am. Fish. Soc. 96* : 195-199.

RAMALINGAM, K. & RAMALINGAM, K. 1982. Effects of sublethal levels of DDT, malathion and mercury on tissue proteins of *Sarotherodon mossambicus* (Peters). *Proc. Indian Acad. Sci. 91* : 501-505.

RAMALINGAM, K. 1985.Effects of DDT and malathion on tissue succinic dehydrogerase activity (SDH) and lactic dehydrogerase iso-enzymes (LDH) of *Sarotherodon mossambicus* (Peters). *Ibid. 94* : 527 : 531.

ROE, J.H. 1955. The determination of sugar in blood and spinal fluid with anthrone reagent. *J. Biol. Chem. 212* : 335-343.

SAKAGUCHI, H. & HAMAGUCHI. A. 1975. Physiological changes in the serum and hepatopancreas of yellow tail injected with carbon tetrachloride. *Bull. Jpn. Soc. Scient. Fish. 41* : 283-290.

SHIMENO, S. 1974. *Studies on carbohydrate metabolism in fish.* Amerind Publishing Co., New Delhi.

SOIVION, A. & OIKARI, A. 1976. Haematological effects of stress on a teleost *Esox lucius.* L. *J. Fish. Biol. 8* : 397-411.

TRUHAUT, R., NGUYEN. PHULICH., NGOC TRAM., LE QUANG THUAN *et* HELINE DUTERTE CATELLA, 1973. Etude de quelques activities enzymatiques serique et de quelques constituents bio-chemiques sanguins an cours des intoxications subaigues avec le tetra chloro 1, 1, 1, 2 ethane chez le lapin. *J. Env. Toxicol. 2* : 81-84.

WIEME, R.J. & VAN MAERCKE, Y. 1961. The fifth electrophoretically slowest lactic dehydrogerase as an index of liver injury. *Ann. N.Y. Acad. Sci. 94* : 898-911.

ZIMMERMAN, H.J., KENDLER, J. & KOFF. R.S. 1971. Intraperitoneal halothane administration: Evidence of hepatic and muscle injury. *Proc. Soc. Exp. Biol. Med. 138* : 678-682.