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A shift in metabolic pathway of *Sarotherodon mossambicus* (Peters) exposed to thiodon (endosulfan)

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Abstract. Total oxygen consumption, glycogen content and succinic dehydrogenase levels in liver, muscle and heart in normal and thiodon-exposed (to sub-lethal concentration for 48 h and to lethal concentration for 6 h) *Sarotherodon mossambicus* (Peters) were studied. While oxygen consumption of the fish dropped to 43 and 35% respectively under sub-lethal and lethal exposures, the magnitude of decrease in tissue glycogen content as well as tissue succinic dehydrogenase level followed a uniform order of liver > muscle > heart in both the exposures. Reduction in oxygen uptake and tissue glycogen levels were indicative of the onset of hypoxia involving utilization of stored glycogen by the fish under thiodon exposure. The inhibition of succinic dehydrogenase levels in the 3 tissues of thiodon-exposed *Sarotherodon mossambicus*, while indicating the impairment of aerobic metabolism, is also suggestive of a shift from aerobic to anaerobic metabolism in the fish under pollution stress.

Keywords. *Sarotherodon mossambicus*; thiodon; succinic dehydrogenase; aerobic metabolism; anaerobic metabolism.

1. Introduction

Pesticides are known to affect the oxygen consumption and metabolic pathways of non-target species like fish in freshwater ecosystems. Effect of pesticides on the oxygen consumption of fish has been studied in *Mystus vittatus* (Reddy and Gomathy 1977) and *Colisa lalia* (Reddy *et al* 1977; Uthaman 1977). Utilization of stored glycogen by the fish under pesticide stress was reported in *Heteropneustes fossilis* (Qayyam and Shaffi 1977), *Labeo rohita*, *Ophiocephalus punctatus* and *Clarias batrachus* (Shaffi 1979), *Channa punctatus* (Shah and Dubale 1983) and *Tilapia mossambica* (Vasanthi 1983) exposed to different pesticides. Inhibition of succinic dehydrogenase (SDH) (EC 1·3·99·1) enzyme activity has also been uniformly observed in *Tilapia mossambica* (Koundinya and Ramamurthi 1978), *Channa striatus* (Natarajan 1981), *H. fossilis* (Dubale and Mohini Awasthi 1982) and *T. mossambica* (Kabeer *et al* 1983) under pesticide-exposed conditions. While the effect of pesticide on oxygen consumption, stored glycogen content and SDH activity levels of tissues were studied in different fishes exposed to different pesticides (providing only fragmentory informations), the present paper provides a comprehensive information on the effects of a particular pesticide on the metabolic pathway of a particular fish by studying total oxygen uptake, stored glycogen levels and SDH activity levels of liver, muscle and heart of normal *S. mossambicus* (Peters) and of those exposed to an organochlorine pesticide, thiodon (endosulfan).

2. Materials and methods

Thiodon, an organochlorine as well as organic sulphite pesticide, is in wide usage in

the eradication of cotton pest in Coimbatore District. The pesticide washes from cotton fields were observed to pollute 4 major lakes in and around Coimbatore City which are the chief sources of inland fisheries. *S. mossambicus*, locally called as Jilebi Kendai, is the major fish variety available from these lakes throughout the year.

Samples of *S. mossambicus* (5–9 g), obtained from 4 major lakes in and around Coimbatore City, were maintained in cement tanks at 28 ± 1°C and fed with cooked rice regularly. Feeding was discontinued one day before the experiment.

A stock solution of technical grade thiodon (supplied as 'Hochest' product by Parry Limited, Bombay) in acetone was used to prepare different concentrations used in the static bioassay study. Sub-lethal (0·001 ppm) and lethal (0·005 ppm) concentrations were determined employing repeated exposure experiments and Probit analysis method (Finney 1964).

Fishes were exposed (in glass jars of 3 l capacity) to 0·001 ppm thiodon for 48 h and to 0·005 ppm thiodon for 6 h. The pesticide water containing 0·001 ppm thiodon was renewed every 12 h to maintain the dissolved oxygen content and pesticide concentration constant throughout the period of exposure. Throughout this investigation, control fishes were also treated with 0·25 ml acetone/l which formed the largest aliquot of stock used in the pesticide water.

2.1 *Measurement of oxygen consumption*

Oxygen consumption of control and thiodon-exposed (TE) fish was measured using a simple respiratory chamber by measuring the loss of oxygen content (due to the respiration of the fish) of water in the respiratory chamber. Oxygen content of water sample was estimated using Winkler's method (Welsh and Smith 1960). The oxygen consumption of the fish was expressed in ml/kg/h.

Weighed samples of liver, muscle and heart tissues were dissected out from the control and TE fish by keeping the stunned (by a blow on the head) fish in an iced trough and used for estimation of glycogen content and SDH activity levels.

2.2 *Estimation of glycogen content*

The glycogen content of liver, muscle and heart was estimated by employing the method of Kemp and Kits (1954). The weighed tissue sample was homogenized in 5 ml of 80% methanol and centrifuged. The residue was collected and treated with 5 ml of 5% tricarboxylic acid (TCA) (w/v). The mixture was boiled for 5 min in a water bath, again made upto 5 ml by adding 5% TCA and centrifuged. To 2 ml of the supernatant, 6 ml of concentrated sulphuric acid (Analar grade) was added and heated in a boiling water bath for exactly 6·5 min. The final mixture was cooled and the colorimetric reading was obtained at 530 μ using a photoelectric colorimeter (Erma, AE–11 model, Japan). Using the colorimetric readings, glycogen values were obtained from a standard graph already prepared using known glucose standard solutions. The glycogen contents of tissues were expressed in mg/g of tissue.

2.3 *Estimation of SDH activity*

SDH enzyme activity level of the 3 tissues were estimated using the method of

Nachlas *et al* (1960). For the assay of SDH activity, a 5% homogenate of the tissue was prepared with 0·25 M cold sucrose solution. The homogenate was centrifuged at 3000 rpm for 15 min to remove the cell debris. Enzyme reaction mixture was prepared with 0·1 ml of 0·5 M sodium succinate; 1·0 ml of phosphate buffer (pH 7·4); 1·0 ml of INT solution [(2-*p*-iodophenyl)-(3-*p*-nitrophenyl-5-phenyl tetrasolin chloride)]; 0·5 ml of 5% homogenate supernatant and 0·4 ml of distilled water. The reaction was stopped after 30 min of incubation at 37°C by adding 6·0 ml of acetic acid. The formazon formed was extracted into 6·0 ml of toluene after the mixture was kept in freeze for 12 h. The optical density (OD) of pink colour formed was read at 495 μ using a photoelectric colorimeter (Erma, AE-11 model, Japan). A reagent blank (to set the colorimeter) was prepared as above except 0·5 ml of supernatant. Instead, 0·5 ml of distilled water was added. From a standard graph of formazon formed, μmol of formazon formed in the enzyme reaction mixture was obtained. SDH activity levels of the tissues were given as μmol formazon/mg tissue/h.

Changes in oxygen consumption, tissue glycogen content and SDH activity levels of TE fish from that of control fish were expressed in percentages and tested for statistical significance using Student's '*t*' test.

3. Results

Oxygen consumption of control and TE *S. mossambicus* are presented in table 1. Table 2 provides the glycogen content in liver, muscle and heart of control and TE fish. Levels of SDH activity in liver, muscle and heart of control and TE fish are given in table 3.

S. mossambicus showed a 43% reduction in oxygen consumption when exposed to sub-lethal concentration (0·001 ppm) of thiodon for 48 h. When exposed to lethal concentration (0·005 ppm) of thiodon for 6 h, the fish also showed a significant drop in oxygen uptake to the tune of 35% (table 1).

Of the 3 tissues examined, liver appears to be the major site of stored glycogen content. Exposure of *S. mossambicus* to thiodon concentrations caused significant drop in glycogen content of all the 3 tissues studied. Under sub-lethal exposure, the drop in glycogen content of liver, muscle and heart were 78, 73 and 67% respectively. When exposed to lethal concentration of thiodon, the liver showed a minimum 9% drop in its glycogen content which was statistically not significant. This indicates

Table 1. Oxygen uptake of control and thiodon-exposed *S. mossambicus* expressed in ml/kg/h ± S.E.

	Thiodon-exposed (ppm)	
Control	0·001	0·005
210·00[a] ± 20·12	118·90[b] ± 16·38 (−43) $P < 0·01$	137·20[c] ± 17·91 (−35) $P < 0·05$

[a]Mean value of 6 observations; [b]Mean value of 5 observations; [c]Mean value of 7 observations.

'−' denotes % decrease from control level. (% Changes from control levels are given in parentheses).

Table 2. Glycogen levels in liver, muscle and heart of control and thiodon-exposed *S. mossambicus* expressed in mg/g of tissue ± S.E.

Tissues	Control	Thiodon-exposed (ppm)	
		0·001	0·005
Liver	4·69 ± 0·38	1·05 ± 0·10 (−78) $P < 0·01$	4·28 ± 0·66 (−9) NS
Muscle	2·33 ± 0·04	0·63 ± 0·10 (−73) $P < 0·05$	0·48 ± 0·05 (−79) $P < 0·01$
Heart	2·40 ± 0·10	0·79 ± 0·12 (−67) $P < 0·01$	0·55 ± 0·05 (−77) $P < 0·01$

NS, Not significant statistically ($P > 0·05$).
'−' denotes % decrease from control level.
Values are means of 7 observations (% changes from control levels are given in parentheses).

Table 3. SDH activity in liver, muscle and heart of control and thiodon-exposed *S. mossambicus*, expressed in μmol formazon/mg tissue/h.

Tissues	Control	Thiodon-exposed (ppm)	
		0·001	0·005
Liver	0·57 ± 0·17	0·18 ± 0·02 (−68) $P < 0·01$	0·37 ± 0·14 (−35) $P < 0·01$
Muscle	0·19 ± 0·03	0·17 ± 0·01 (−11) $P < 0·01$	0·17 ± 0·03 (−11) $P < 0·01$
Heart	0·29 ± 0·02	0·26 ± 0·06 (−10) $P < 0·01$	0·28 ± 0·08 (−4) NS

NS, Not significant statistically ($P > 0·05$).
'−' denotes % decrease from control level.
Values are means of 6 observations ± S.E. (Changes from control levels are given in parentheses).

that utilization of stored liver glycogen occurs only upon prolonged exposure. On the other hand, muscle and heart tissues showed increased magnitudes of drop in glycogen contents to about 79 and 77% respectively (table 2) which implies that utilization of stored glycogen content increased with increase in concentration of pesticide irrespective of the period of exposure. This variation in the drop in glycogen content of the 3 tissues indicates that different tissues behave differently under pesticide exposure.

The data on tissue SDH activity levels clearly indicate an inhibition of SDH activity to about 68, 11 and 10% respectively in liver, muscle and heart tissues of

S. mossambicus exposed to sub-lethal concentration of thiodon (table 3). However, exposure of fishes to lethal concentration of thiodon elicited only a lesser magnitude of drop in SDH activity level in the liver tissue (35%). Muscle tissue showed a similar drop in SDH level as that of 0·001 ppm TE fishes under lethal exposure (11%). The non-significant minimum drop (4%) in SDH activity level of heart tissue under lethal exposure of the fish is noteworthy for discussion (table 3).

4. Discussion

A perusal of table 1 clearly indicates that exposure of fish to sub-lethal and lethal concentrations of thiodon caused a definite reduction in oxygen uptake of the fish. A similar depression in oxygen uptake has been reported in *C. lalia* (Reddy *et al* 1977; Uthaman 1977) and *Mystus vittatus* (Reddy and Gomathy 1977) exposed to different pesticides. The decreased oxygen uptake in TE *S. mossambicus* (in the present study) could be suggested as a sequel to gill damage upon pesticide exposure as reported in *C. striatus* (Natarajan 1981) or due to hypochromic microcytic anaemia caused due to pesticide toxicity as reported in *S. mossambicus* (Koundinya and Ramamurthi 1979), *C. striatus* (Natarajan 1981) and *C. batrachus* (Goel *et al* 1984). The drop in oxygen uptake of TE *S. mossambicus*, observed in the present investigation, indicates the onset of a severe hypoxia in the fish following pesticide exposure which will trigger on some biochemical changes in different tissues of the fish body.

Anoxia or hypoxia is known to increase carbohydrate consumption (Dezwaan and Zandee 1972). The unequivocal reductions in stored glycogen content in liver, muscle and heart of 0·001 ppm TE fish and in muscle and heart of 0·005 ppm TE fish indicate the utilization of stored glycogen possibly through anaerobic glycolysis to meet the energy requirement under hypoxia caused due to pesticide stress. A similar reduction in stored tissue glycogen content has been reported in *H. fossilis* exposed to mercuric nitrate (Qayyam and Shaffi 1977), *L. rohita*, *O. punctatus* and *C. batrachus* following copper sulphate intoxication (Shaffi 1978), *A. scandens* exposed to zinc sulphate (Natarajan 1981), *T. mossambica* exposed to lindane (Vasanthi 1983) and methyl parathion (Siva Prasada Rao and Ramana Rao 1979) and *C. punctatus* exposed to malathion (Shah and Dubale 1983).

The data on tissue SDH activity levels, obtained in the present study (table 3), clearly indicate an inhibition of SDH activity in liver, muscle and heart of 0·001 ppm TE fish and in liver and muscle of 0·005 ppm TE fish. A similar drop in SDH activity levels of different tissues has also been reported in *S. mossambicus* exposed to lindane (Lakshmi 1984), *C. striatus* exposed to metasystox (Natarajan 1981), *H. fossilis* (Dubale and Mohini Awasthi 1982) and *T. mossambica* under malathion stress (Kabeer *et al* 1983). SDH being a key enzyme in TCA cycle, its inhibition under toxic condition, as observed in liver, muscle and heart of *S. mossambicus* exposed to sub-lethal concentration of thiodon (in the present study) and also reported in other fishes exposed to different pesticides could be considered as indication of the fish showing a shift towards anaerobic metabolism (utilizing stored metabolites like glycogen) under toxic conditions. Based on SDH and LDH studies, Koundinya and Ramamurthi (1978) suggested the operation of anaerobic glycolysis in different tissues of *T. mossambica* exposed to sumithion. Exposure of fish to pesticide, thus, causes impairment of an important enzyme system (SDH activity) besides other toxic effects.

The observed insignificant 4% drop in SDH activity level of heart tissue under lethal exposure of the fish, inspite of a significant 77% reduction in stored heart glycogen content under similar exposure, indicates a continued operation of aerobic breakdown of glycogen in the heart tissue to meet energy demand during early periods of exposure. This further indicates that the heart is aerobic possibly due to increased oxygen supply to the heart in order to prevent the onset of lactic acidosis in the heart tissue which will occur normally during hypoxic conditions. Thus the heart tissue is found adaptive in maintaining itself physiologically fit for effective cardiac function during early periods of pesticide exposure.

In conclusion, it could be stated that exposure of *S. mossambicus* to thiodon elicited a severe hypoxia resulting in the utilization of stored glycogen by way of anaerobic glycolysis to meet the energy demand during pesticide stress. The inhibition of SDH activity levels in different tissues of TE *S. mossambicus*, while suggesting the failure of aerobic metabolic pathway, also hints upon the possibility of a shift from aerobic to anaerobic mode of energy metabolism in tissues.

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