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Effects of Lead on Respiratory Enzyme Activity, Glycogen and Blood Sugar Levels of the Teleost *Oreochromis mossambicus* (Peters) during Accumulation and Depuration

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Abstract

Accumulation and depuration from liver and muscle were measured in *Oreochromis mossambicus* exposed to sublethal levels of lead (Pb) (17.5 and 35 ppm); and activities of two respiratory enzymes, as well as glycogen and blood sugar levels were studied during exposure and recovery. During exposure, a significant decline in succinate dehydrogenase (SDH) activity with a concomitant increase in glyceraldehyde dehydrogenase (GDH) activity was observed in liver and muscle, suggesting a shift in metabolism from aerobiosis to anaerobiosis. Glycogen content of liver and muscle showed a time-and dose-($P<0.01$) dependent decrease, whereas blood sugar level increased. This suggests the breakdown of glycogen to glucose and mobiliza-tion to meet energy requirements. During recovery, activities of respiratory enzymes were re-versed; GDH activity and glycogen content recovered earlier than the activity of the aerobic enzyme SDH. Animals exposed to sublethal levels of Pb produced time-and dose-dependent significant increases in concentrations of Pb in liver ($P<0.05$) and muscle ($P<0.01$). On transfer of the fish to metal-free freshwater after 20 d of exposure, Pb concentrations in tested tissues gradually declined. The rate of recovery or extrapolated time between day 0 and 20 of recovery showed that liver and muscle completely recovered from Pb poisoning on days 54 or 82 and 44 or 62 in animals exposed to 17.5 or 35 ppm, respectively. Animals exposed to 17.5 ppm recov-ered earlier than those exposed to 35 ppm.

Introduction

Lead (Pb) is one of the most toxic of heavy metals and its compounds are included in the grey list of international conventions (Taylor et al. 1985). The annual world input of Pb into the aquatic systems is 33-194 x 10^6 kg·year^{-1} (Nriagu and Pacyna 1988; Nriagu 1989). Environmental contamination is high due to the wide use of Pb in paint, cables, dyes, glazing of ceramics, electrical and electronics and PVC plastics (Hodson et al. 1984). Use of Pb pipes and plastic pipes (stabilized with Pb) contributes to Pb in drinking water. Lead has the tendency to accumulate and undergo foodchain

magnification (Vinikour et al. 1980). Metals interact with ligands in proteins, particularly enzymes, and may inhibit their biochemical and physiological activities (Passow et al. 1961). Succinate dehydrogenase (SDH) and glyceraldehyde dehydrogenase (GDH) are key enzymes in aerobic and anaerobic metabolism, respectively, and their activities greatly change in aquatic animals under toxicant stress. Studies on the key respiratory enzymes, glycogen and blood sugar levels in relation to accumulation and depuration of Pb in fish are few. The present paper reports accumulation and depuration of Pb during exposure to two sublethal levels of Pb and also the activities of two respiratory enzymes and glycogen and blood sugar levels in the tilapia, *Oreochromis mossambicus*, during exposure and recovery periods.

Materials and Methods

O. mossambicus is a cichlid freshwater fish commonly known as tilapia. The fry feeds on phyto-zooplankton but the adult, though chiefly herbivore, accepts all kinds of food. It attains maturity in 2-3 months and breeds throughout the year. It is a sturdy and fast-growing fish. Tilapia responds well to aquarium culture and can tolerate a wide range of environmental conditions.

Experimental animals (400 fish) *O. mossambicus* (Peters) were collected from a local pond (latitude 8° 46; longitude 75°5) near Tuticorin, Tamil Nadu, and transported to the laboratory in plastic containers. They were acclimated for 25 d in 1.2 x 0.9 m tanks under static condition. Water was changed twice daily. During acclimation, animals were regularly fed *ad libitum* with minced fresh beef liver. Unconsumed feed was removed after 2 h of feeding to prevent contamination of the medium.

Acclimated active animals (15 ± 1.5 g) were chosen from the stock and divided into seven groups of six. They were exposed to different toxic concentrations of Pb (0, 30, 55, 80, 105, 130 and 155 ppm) for 96 h to determine LC_{50} value, adopting the static renewable bioassay test (Sprague 1973). Mortality was recorded every 3-h interval up to 24 h, 6-h interval up to 48 h, and 12-h interval up to 96 h. Animals were not fed 1 d prior to the commencement of the experiment and during the bioassay period. Stock solution (ppt) of Pb was prepared by dissolving 1.60 g of lead nitrate ($Pb[NO_3]_2$ - analar grade) in 1 l of distilled water and was diluted with freshwater to obtain the desired concentrations. The 96-h LC_{50} value was determined following the method of Litchfield and Wilcoxon (1949). Based on the LC_{50} value, two sublethal concentrations of Pb, 17.5 and 35 ppm, were selected for the present study.

For the second series of experiments, acclimated *O. mossambicus* (15 ± 1.5 g) were divided into three groups of 10. The first group was exposed to metal-free water and treated as control. Animals belonging to the second and third groups were exposed to 17.5 and 35 ppm of Pb for 20 d. Triplicates were maintained for each concentration in circular plastic troughs containing 40 l test medium. The physico-chemical characteristics of water were: temperature (30 ± 1°C), pH (7.5), dissolved oxygen (5.6 $m10_2 \cdot l^{-1}$) and salinity (0.11‰). Test individuals were fed *ad libitum* with minced beef liver. Test media were

changed daily. After 20 d exposure, the fish were transferred to metal-free water for another 20 d (recovery period). The activities of respiratory enzymes, SDH and GDH, and glycogen content were estimated (Kemp and Kits Van Heijiningen 1945; Kun and Abood 1949) in the liver and in the lateral muscle of the midbody region on days 0, 5, 10 15 and 20 of metal exposure, and on days 5, 10, 15 and 20 of the recovery period. Enzyme activities, glycogen and blood sugar levels on day 20 of exposure were taken as the initial level (day 0) of the recovery period. Enzyme activity is expressed in µg reduced Triphenyl Tetrazolium Chloride (TTC)·100 mg^{-1} wet tissue·h^{-1} and glycogen content in mg·g^{-1} wet tissue.

Blood was collected in a watch glass containing the required amount of 6% Ethylene Diamine Tetra Acetic (EDTA) Acid-disodium salt ([CH2·N(CH_2·COOH) CH_2·COONa]$_2$·$2H_2O$] as an anticoagulant at intervals of 5 d from each of three experimental fishes by severing the caudal peduncle with a sharp knife. Total blood sugar was estimated following the procedure of Roe (1955). Accumulation and depuration of Pb in liver and muscle were analyzed at intervals of 5 d from day 0 to 40. Regression analysis based on the least squares method following Zar (1974) was carried out to predict the pre-exposure levels of enzyme activity, glycogen, blood sugar level and metal concentration in tissues. Analysis of covariance (Snedecor 1961) was applied to test the time- and dose-dependent significance between metal concentrations (17.5 and 35 ppm) and exposure period. The rate of recovery of the chosen parameters was calculated by dividing the difference between day 0 to 20 of the recovery period by the actual recovery period.

Lead Analysis

Total Pb in tissue was estimated on 0.5-1.0 g of wet tissue. Three replicates of samples were digested with a mixture of concentrated nitric and perchloric acids in the ratio of 1:2 until the formation of a white residue at 100°C in a water bath. The cooled residue was dissolved completely by adding 1 N HCl and made up to 25 ml with distilled water (FAO 1972). The solution was filtered through cotton wool and the filtrate subjected to metal analysis by atomic absorption spectrophotometry (Perkin-Elmer 2380). The instrument was calibrated using standards prepared from lead nitrate.

Results

The 96-h LC_{50} value of Pb for *O. mossambicus* was 104.91 ppm (Table 1). No mortality was observed below the concentration of 30 ppm. However, concentrations of 55 ppm and above were observed to be toxic. The 95% confidence limits were 61.99 (lower limit) and 177.54 (upper limit). The slope S was 1.59 (Table 1).

Exposure of *O. mossambicus* to both 17.5 and 35 ppm Pb resulted in a significant ($P<0.05$) decrease in SDH activity and a significant

Table 1. Effect of chosen Pb concentrations on relative percent mortality in *Oreochromis mossambicus* exposed for 96 h. Lethal concentrations, slope function and 95% confidence limits are expressed in ppm.

Concentrations of Pb (ppm)	Dead/ tested	Mortality (%)	Lethal concentration (ppm) at			Slope function (S)	95% confidence limit	
			16%	50%	84%		Lower	Upper
0	0/6	0						
30	0/6	0						
55	1/6	16.7						
80	2/6	33.4						
105	3/6	50	53.98	104.91	130.21	1.59	61.99	177.54
130	5/6	83.4						
155	6/6	100						

($P<0.01$) increase in GDH activity with time in both liver and muscle as compared to control (Fig. 1, Tables 2 and 5). However, the trend was reversed during the recovery period (Fig. 1, Table 2). The SDH and GDH activities in liver and muscle of control fish did not change with time. The extrapolated complete recovery of SDH or GDH activities in liver of *O. mossambicus* exposed to 35 ppm Pb was 60 or 55.5 d, and in muscle it was 53 or 46 d, respectively (Fig. 1). Rate of recovery calculations of enzyme activity to determine the day of return to pre-exposure level of enzymes agrees with results of extrapolations for complete recovery from Pb poisoning. Similar trends were observed in animals exposed to 17.5 ppm Pb. However, activity of both enzymes in tested tissues was recovered earlier than animals exposed to 35 ppm. Fig. 1 illustrates that muscle recovered faster than liver, and that complete recovery from Pb poisoning of the aerobic enzyme SDH was slower than that of the anaerobic enzyme GDH.

Glycogen content in liver and muscle significantly ($P<0.05$) declined, whereas blood sugar level increased in test animals exposed to both Pb concentrations (Fig. 2, Table 3). Glycogen content showed a time- and dose-dependent response against both levels of Pb exposure (see Table 3). When test animals were transferred to metal-free water during recovery, there was a gradual increase in tissue glycogen, and a decrease in blood sugar levels (Table 3). Muscle glycogen and blood sugar recovered earlier than liver glycogen in *O. mossambicus* exposed to both Pb concentrations (Fig. 2).

Animals exposed to both sublethal levels of Pb exhibited a time-and dose-dependent significant increase in Pb accumulation in liver ($P<0.05$) and muscle ($P<0.01$) (see Table 4). *O. mossambicus* exposed to 35 ppm Pb showed 1.62 and 0.98 mg $Pb \cdot g^{-1}$ wet tissue in liver and muscle, respectively, on day 20 of exposure (Fig. 3, Table 4). Animals exposed to 17.5 ppm Pb accumulated lower levels of Pb in the tissue (Fig. 3, Table 4). However, Pb concentrations in liver and muscle declined significantly during recovery. Extrapolated recovery time between day 0 and 20 in liver and muscle from Pb accumulation was 54 or 82 and 44 or 62 d in animals exposed to 17.5 or 35 ppm,

Table 2. Effect of sublethal concentrations of Pb on dehydrogenases of succinate and glyceraldehyde enzymes activities in *Oreochromis mossambicus* as a function of exposure and recovery periods. Each value is the mean of ($\bar{X} \pm SD$) of three observations. Rate of recovery is expressed as mg reduced TTC·100 mg^{-1} wet tissue d^{-1}).

Tissues	Sublethal concentrations (ppm)	Exposure period (d) 0	5	10	15	20	F value	Recovery period (d) 25	30	35	40	F value	Rate of recovery
Succinate dehydrogenase (µg reduced TTC·100 mg^{-1} wet tissue h^{-1})													
	0	54.8 ± 1 1	55.3 ± 0.6	54.6 ± 1.7	55.5 ± 0.9	55.5 ± 0.9		54.8 ± 0.7	55.9 ± 1.2	55.2 ± 1.2	55.4 ± 0.8		
Liver	17.5	54.8 ± 1.1	46.0 ± 1.1	40.0 ± 1.0	34.3 ± 0.9	28.2 ± 1.4	0.406	31.0 ± 0.9	34.5 ± 0.7	36.2 ± 1.5	38.9 ± 0.5	0.475	0.535
	35.0	54.8 ± 1.1	39.6 ± 1.7	35.2 ± 1.3	28.6 ± 1.4	24.1 ± 1.1	NS	26.7 ± 1.0	29.0 ± 0	31.6 ± 1.8	34.4 ± 1.3	NS	0.515
	0	9.13 ± 0.3	9.13 ± 0.3	8.98 ± 0	9.18 ± 0	9.34 ± 0.3		9.25 ± 0.4	9.25 ± 0:4	9.40 ± 6.3	9.34 ± 1.1		
Muscle	17.5	9.13 ± 0.3	8.03 ± 0	6.87 ± 0.19	5.66 ± 0.6	4.88 ± 1.1	2.723	5.35 ± 1.2	5.98 ± 0.9	6.35 ± 0	6.88 ± 0.2	0.002	0.1
	35.0	9.13 ± 0.3	7.19 ± 0.7	6.08 ± 1.2	4.75 ± 1.12	3.94 ± 0.2	NS	4.19 ± 0.7	4.79 ± 1.4	5.25 ± 1.7	5.9 ± 0	NS	0.098
Glyceraldehyde dehydrogenase (µg reduced TTC·100 mg^{-1} wet tissue h^{-1})													
	0	10.3 ± 1.3	10.3 ± 1.3	10.7 ± 0.8	10.6 ± 0.6	10.0 ± 0.6		9.9 ± 1.0	10.8 ± 1.3	10.6 ± 1.3	10.3 ± 1.1		
Liver	17.5	10.3 ± 1.3	16.54 ± 1.6	21.4 ± 1.3	26.0 ± 1.0	31.4 ± 1.1	43.30	28.3 ± 0.4	26.4 ± 0.7	24.2 ± 1.1	21.0 ± 1.0	0.547	0.52
	35.0	10.3 ± 1.3	18.11 ± 1.1	24.3 ± 1.0	30.4 ± 1.2	38.3 ± 1.1	**	36.6 ± 1.4	33.2 ± 0.2	30.5 ± 0.7	28.2 ± 1.0	NS	0.505
	0	3.82 ± 1.3	3.82 ± 0.1	3.87 ± 0.5	3.88 ± 0.5	3.88 ± 0.5		3.71 ± 0.9	3.71 ± 0.9	3.71 ± 0.9	3.80 ± 0.3		
Muscle	17.5	3.82 ± 0.1	4.10 ± 0	4.43 ± 1.1	4.89 ± 1.3	5.70 ± 0.7	4.593	5.48 ± 0.2	5.23 ± 0.4	5.01 ± 0.8	4.60 ± 1.02	0.002	0.055
	35.0	3.82 ± 0.1	4.70 ± 0	5.39 ± 0.2	5.93 ± 0.8	6.34 ± 1.0	NS	6.11 ± 0.7	5.93 ± 0.3	5.72 ± 1.2	5.21 ± 0.3	NS	0.056

Analysis of covariance: NS = Not significant; ** $P < 0.01$.

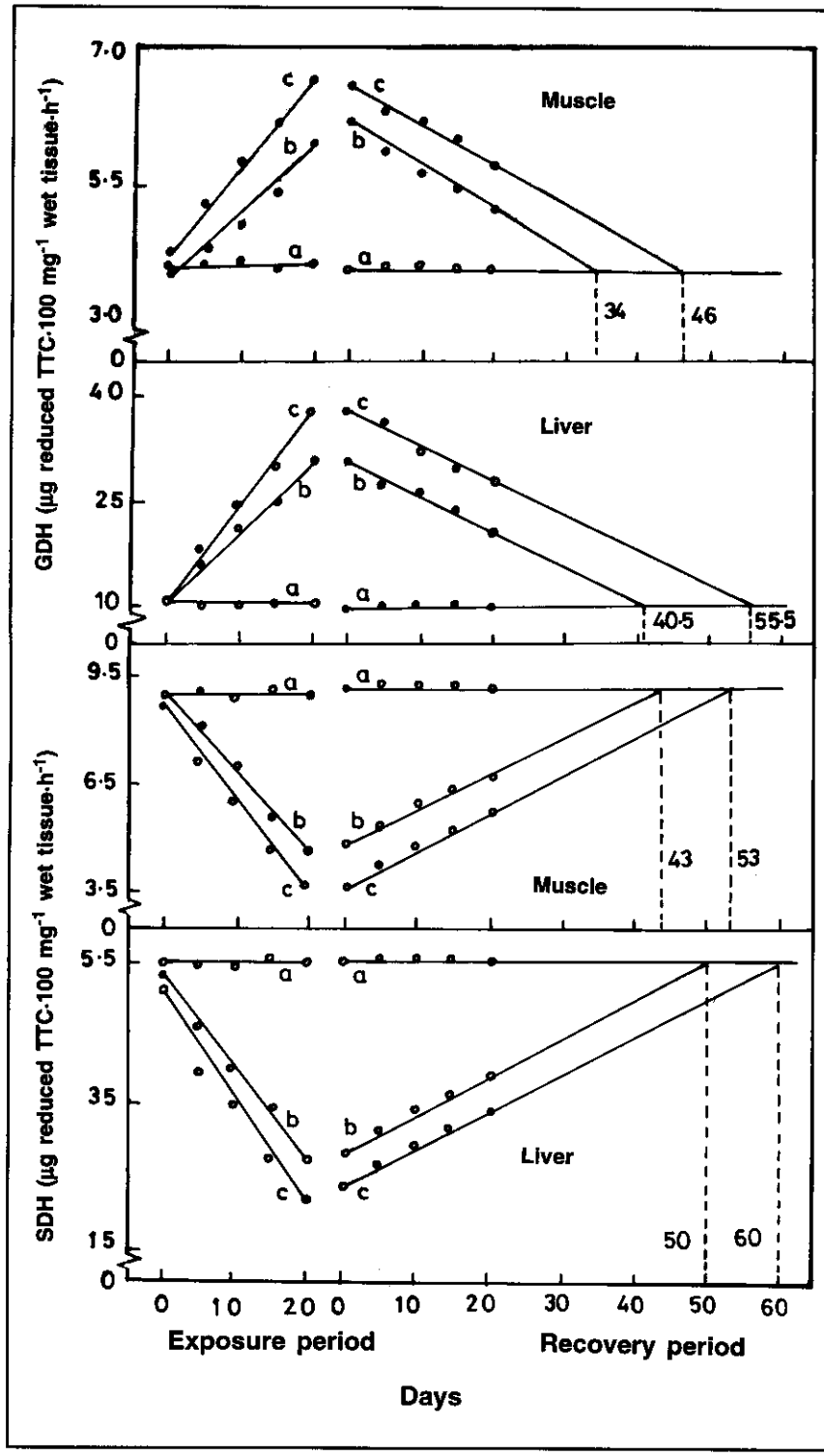

Fig. 1. Activities of succinate dehydrogenase (SDH) and glyceraldehyde dehydrogenase (GDH) in liver and muscle of *Oreochromis mossambicus* exposed to sublethal levels of Pb during exposure and recovery periods. a = metal-free water, b = 17.5 ppm Pb, c = 35 ppm Pb.

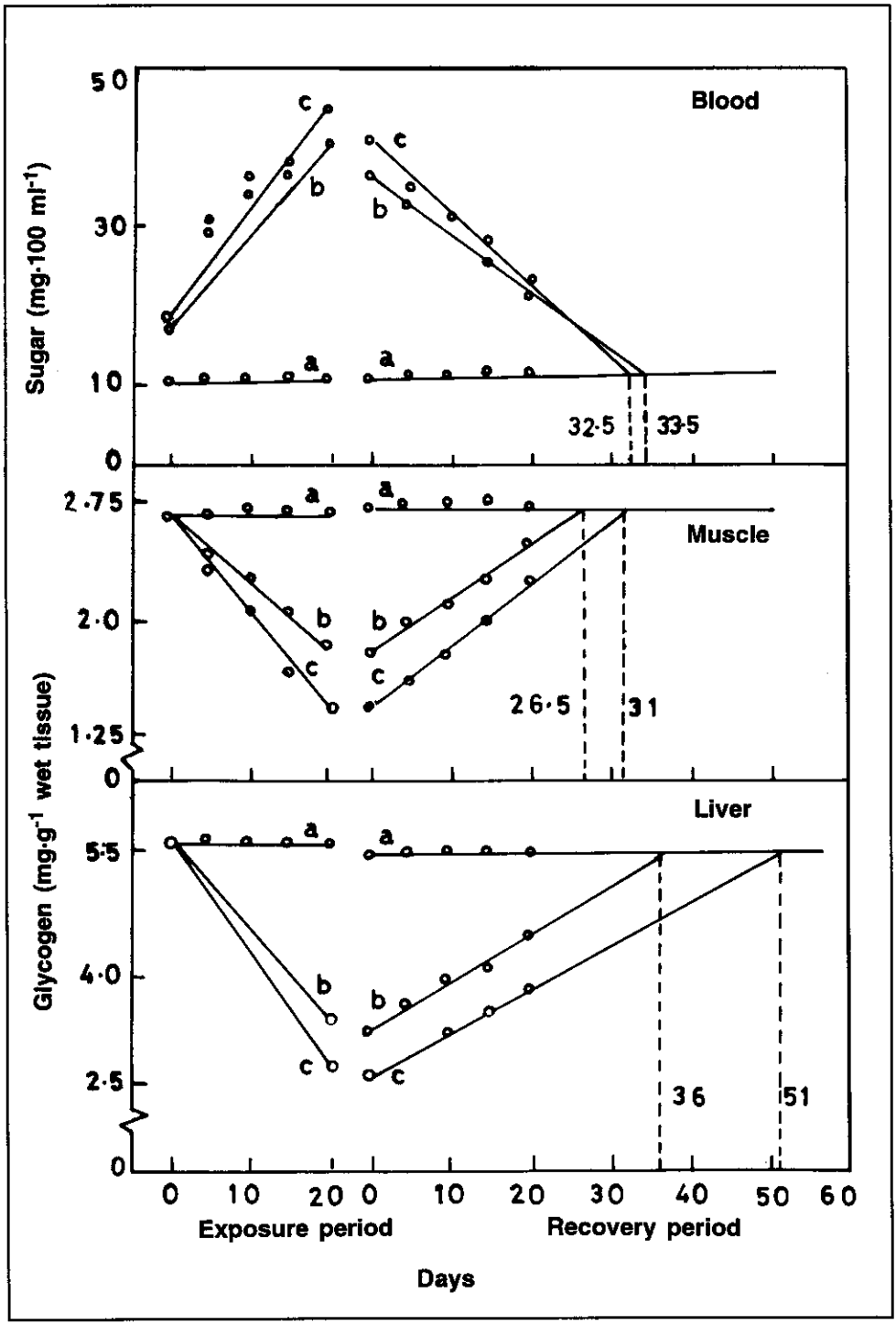

Fig. 2. Blood sugar level and glycogen content in liver and muscle of *Oreochromis mossambicus* exposed to sublethal levels of Pb during exposure and recovery periods. a = metal-free water, b = 17.5 ppm Pb, c = 35 ppm Pb.

Table 3. Effect of sublethal levels of Pb on glycogen and blood sugar as a function of exposure and recovery periods in *Oreochromis mossambicus*. Each value is the mean of ($\bar{X} \pm SD$) of three observations. Rate of recovery is expressed as mg g^{-1} wet tissue d^{-1} for glycogen and mg ·100 ml^{-1} d^{-1} for blood sugar.

Tissues	Sublethal concentrations (ppm)	Exposure period (d) 0	5	10	15	20	F value	Recovery period (d) 25	30	35	40	F value	Rate of recovery
						Glycogen (mg g^{-1} wet tissue)							
Liver	0	5.67 ± 0.3	5.67 ± 0.3	5.70 ± 0.7	5.48 ± 0.1	5.53 ± 0.3		5.53 ± 0.3	5.63 ± 0.7	5.61 ± 0.5	5.61 ± 0.5		
	17.5	5.67 ± 0.3	5.10 ± 0.4	4.48 ± 0.9	4.01 ± 1.1	3.43 ± 0.8	15.12	3.51 ± 0.6	3.89 ± 0.4	4.10 ± 1.07	4.67 ± 0.7	0.252	0.062
	35.0	5.67 ± 0.3	4.95 ± 1.0	4.41 ± 0.8	3.66 ± 0.7	2.71 ± 1.1	**	2.99 ± 0.5	3.29 ± 0.8	3.53 ± 0.7	3.86 ± 0.3	NS	0.058
Muscle	0	2.69 ± 0.2	2.69 ± 0.2	2.76 ± 0.7	2.70 ± 0.3	2.70 ± 0.3		2.75 ± 0.6	2.80 ± 0.3	2.80 ± 0.3	2.79 ± 0.05		
	17.5	2.69 ± 0.2	2.41 ± 0.1	2.26 ± 0.01	2.03 ± 0.01	1.83 ± 0.03	25.70	1.99 ± 0.02	2.12 ± 0.05	2.28 ± 0.08	2.48 ± 0.07	5.30	0.033
	35.0	2.69 ± 0.2	2.34 ± 0.9	2.05 ± 0.7	1.68 ± 0	1.49 ± 0.03	**	1.65 ± 0.01	1.89 ± 0	2.06 ± 0.02	2.26 ± 0	**	0.039
						Blood sugar (mg ·100 ml^{-1})							
	0	10.4 ± 0.3	10.3 ± 0.7	10.7 ± 0.1	10.5 ± 0.9	11.0 ± 0		10.8 ± 0.8	11.3 ± 0.4	11.1 ± 0.2	11.2 ± 0.6		
	17.5	10.4 ± 0.3	29.5 ± 1.6	34.3 ± 2.8	36.4 ± 1.7	36.0 ± 1.0	0.078	32.1 ± 2.0	28.3 ± 0.2	24.8 ± 1.3	20.8 ± 1.7	2.98	0.76
	35.0	10.4 ± 0.3	35.8 ± 1.6	36.1 ± 0.9	37.9 ± 2.1	43.1 ± 1.9	NS	34.5 ± 1.3	31.0 ± 1.6	27.6 ± 0.7	23.0 ± 1.1	NS	1.01

Analysis of covariance: NS = Not significant; ** $P < 0.01$.

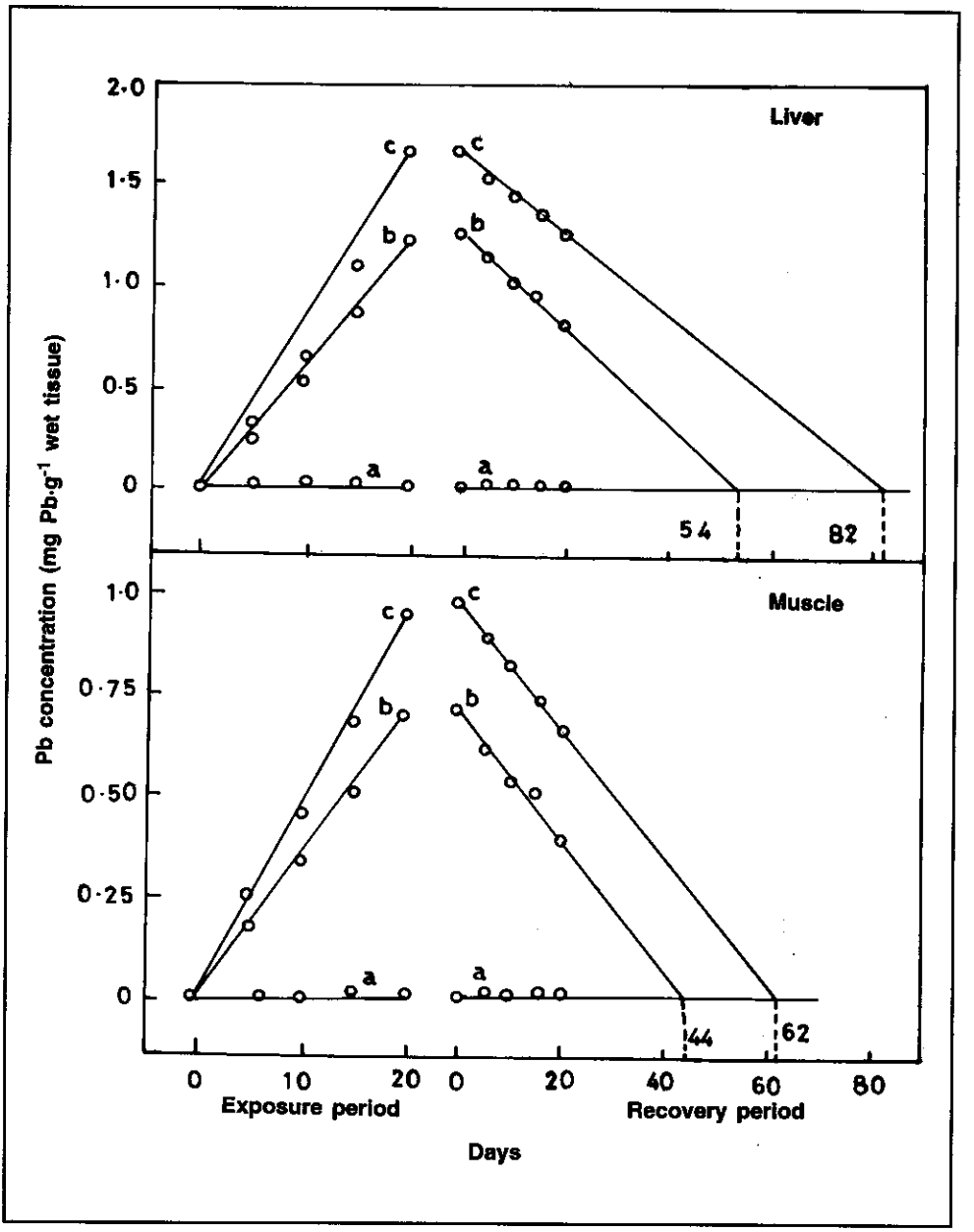

Fig. 3. Lead accumulation and elimination in liver and muscle of *Oreochromis mossambicus* exposed to sublethal levels of Pb during exposure and recovery periods. a = metal-free water, b = 17.5 ppm Pb, c = 35 ppm Pb.

respectively (Fig. 3). Animals exposed to 35 ppm took more time for complete recovery than those exposed to 17.5 ppm.

Discussion

A significant decrease in SDH activity with a concomitant increase in GDH activity was observed in liver and muscle of *O. mossambicus* exposed to sub-

Table 4. Accumulation and elimination of Pb (mg Pb·g^{-1} wet tissue) in *Oreochromis mossambicus* during exposure and recovery periods as a function of sublethal levels of Pb. Each value is the mean ($\bar{x}$ ± SD) of three observations. Rate of recovery is expressed as mg·g^{-1} wet tissue·d^{-1}.

Sublethal concentrations (ppm)	Exposure period (d)						Recovery period (d)					
	0	5	10	15	20	F value	25	30	35	40	F value	Rate of recovery
						Liver						
0	0.019 ±0	0.019 ±0	0.019 ±0	0.019 ±0	0.019 ±0		0.018 ±0	0.018 ±0	0.019 ±0	0.018 ±0		
17.5	0.019 ±0	0.28 ±0.01	0.52 ±0.11	0.86 ±0.19	1.23 ±0.18	11.31*	1.12 ±0.15	0.98 ±0.23	0.87 ±0.03	0.7 ±0.14	12.46*	0.0225
35.0	0.019 ±0	0.31 ±0.09	0.65 ±0.14	1.11 ±0.07	1.62 ±0.04		1.53 ±0.19	1.43 ±0.18	1.34 ±0.13	1.23 ±0.21		0.0195
						Muscle						
0	0.015 ±0	0.015 ±0	0.016 ±0	0.015 ±0	0.015 ±0		0.015 ±0	0.016 ±0	0.015 ±0	0.016 ±0		
17.5	0.015 ±0	0.18 ±0.06	0.34 ±0.04	0.49 ±0.07	0.70 ±0.12	26.11**	0.62 ±0.03	0.55 ±0.09	0.47 ±0.1	0.39 ±0.13	0.097 NS	0.0155
35.0	0.015 ±0	0.23 ±0.08	0.43 ±0.1	0.67 ±0.12	0.98 ±0.05		0.89 ±0.12	0.81 ±0.11	0.73 ±0.13	0.67 ±0.08		0.0155

Analysis of covariance: NS = Not significant; * $P<0.05$; ** $P < 0.01$.

Table 5. Regression equations (Y=a+bx) and correlation coefficient (r) for succinate dehydrogenase and glyceraldehyde dehydrogenase activities, glycogen and blood sugar level in *Oreochromis mossambicus* as a function of Pb exposure and recovery periods in days.

Parameters	Tissues	Pb concentrations (ppm)	Exposure period		Recovery period	
			r	Regression	r	Regression
SDH (µg reduced TTC· 100 mg^{-1} wet wt·h^{-1})	Liver	0	-0.608NS	54.82+0.03	0.078NS	55.32+0.004
		17.5	-0.996	53.66-1.3	0.995	28.44+0.532
		35.0	-0.965	50.94-1.45	0.995	24.08+0.508
	Muscle	0	-0.576NS	9.06-0.009	0.365NS	9.29+0.003
		17.5	-0.998	9.02-0.217	0.998	4.89+0.1
		35.0	-0.989	8.78-0.256	0.991	3.82+0.1
GDH (µg reduced TTC· 100 mg^{-1} wet wt·h^{-1})	Liver	0	-0.169NS	10.44-0.006	0.535NS	10.06+0.026
		17.5	0.998	10.8+1.03	-0.994	31.24-0.498
		35.0	0.998	10.62+1.37	-0.996	32.62-0.526
	Muscle	0	0.910	3.81+0.004	-0.330NS	3.79-0.003
		17.5	0.974	3.67+0.095	-0.991	5.94-0.053
		35.0	0.989	3.98+0.125	-0.974	6.39-0.053
Glycogen ($mg·g^{-1}$ wet wt)	Liver	0	-0.76 NS	5.70-0.009	0.787NS	5.53+0.005
		17.5	-0.990	5.65-0.11	0.968	3.31+0.061
		35.0	-0.995	5.72-0.144	0.999	2.71+0.057
	Muscle	0	0.160NS	2.65-0.006	0.158NS	2.72+0.001
		17.5	-0.996	2.66-0.042	0.997	1.82+0.032
		35.0	-0.995	2.66-0.061	0.999	1.48+0.039
Blood sugar ($mg·100\ ml^{-1}$)		0	0.797NS	10.3+0.028	0.575NS	10.94-0.014
		17.5	0.840	17.7+1.162	-0.999	35.94-0.754
		35.0	0.834	19.16+1.35	-0.984	41.26-0.942
Pb accumulation (mg Pb·g^{-1} wet wt)	Liver	0	0 NS	0.019+0	-0.423NS	$0.019+0.1^{-6}$
		17.5	0.996	-0.019+0.06	-0.997	1.226-0.023
		35.0	0.993	-0.058+0.08	-0.998	1.624-0.019
	Muscle	0	0.354NS	0.015+0.2	0.176 NS	$0.015+0.2^{-6}$
		17.5	0.994	-0.003+0.03	-0.998	0.70-0.015
		35.0	0.996	-0.009+0.047	-0.998	0.972-0.016

NS = Not significant; other values are significant at 5% level.

lethal levels of Pb. This suggests a metabolic shift from aerobiosis to anaerobiosis. The metabolic shift appears as an adapatation for survival under chronic metal stress. Suppression of SDH activity indicates impairment of the oxidative metabolic cycle and reliance on the anaerobic glycolytic pathway to meet energy demands, as an explanation consistent with the elevation of GDH activity. The metabolic shift may be due to mitochondrial disruption (Vasilos et al. 1976), leading to a decrease in activities of oxidative enzymes and an increase in glycolytic enzymes (Deung et al. 1978; James et al. 1992). The significant shift in SDH and GDH activities indicates a higher energy demand in the liver than in muscle of *O. mossambicus* for metabolic coordination of continuous detoxification mechanisms. The liver is a vital organ which demands energy for storage and interconversion of food, and as a center for detoxification. Hodson (1976) reported that activity of red cell δ-amino levulinic acid dehydratase of *Salmo gairdneri* was depressed after exposure to Pb. James et al. (1991, 1992)

found suppression of SDH activity and elevation of GDH activity in *O. mossambicus* exposed to sublethal levels of copper, zinc and cadmium, and in *Heteropneustes fossilis* exposed to sublethal levels of mercury (0.01 and 0.03 ppm). In the present study, the initial level of SDH in liver and muscle was 54.8 and 9.13 μg reduced TTC·100 mg^{-1} wet tissue·h^{-1} compared to 51.9 and 8.05 μg reduced TTC·100 mg^{-1} tissue·h^{-1}, respectively in *Sarotherodon mossambicus* (Ramalingam 1985).

Liver glycogen has a significant role in glucose turnover, and the mobilization of liver glycogen into the blood stream is controlled by glycolytic enzymes. The decrease in glycogen content in the liver and muscle, and increase in blood sugar levels of *O. mossambicus* suggests the elevation of GDH activity in tested tissues for mobilization of glucose from the bound form of tissue glycogen to meet high energy demands due to metal stress. In another study of *O. mossambicus* exposed to sublethal levels of Pb (17.5 and 35 ppm), James et al. (1993a) reported decreased oxygen-carrying capacity of blood due to a reduced RBC count and hemoglobin content, ultimately decreasing overall oxygen consumption. James and Sampath (1995) found that liver showed maximum reduction of tissue glycogen, followed by gill and muscle in *H. fossilis* exposed to mixtures of copper and ammonia. Physiologically, the liver requires more energy than gill and muscle for storage; interconversion and detoxification and, hence demands maximum energy. Lead exposure may stimulate hormones that induce an enhanced breakdown of liver glycogen (Sahib et al. 1983) with increased activity of glycogen-mobilizing enzymes (Simon et al. 1983). James et al. (1991) reported that glycogen reserves significantly declined in liver and muscle of *H. fossilis* exposed to sublethal levels of mercury. Glycogen reserves in liver and muscle of *O. mossambicus* was 5.67 and 2.69 $mg \cdot g^{-1}$ wet tissue, respectively, as compared to 5.83 (Ramalingam 1988) and 2.33 $mg \cdot g^{-1}$ wet tissue (Vasanthi and Ramaswamy 1987) in *Sarotherodon mossambicus*.

Liver accumulates more Pb than muscle, and this may be related to its functions of storage, interconversion and detoxification. The liver is known to synthesize metallothioneins (metal-chelating proteins) for storage and detoxification of metals (Webb 1975). A higher uptake of Pb, Cd and Hg in liver than in muscle of eels *Anguilla anguilla* and roach *Rutilus rutilus* was reported by Barak and Mason (1990). Similar trends have been observed in cadmium-exposed rainbow trout *Salmo gairdneri* (Giles 1988) and in mercury-exposed catfish *H. fossilis* (James et al. 1993b).

The present study showed a significant ($P<0.05$) time- and dose-dependent accumulation of Pb in chosen tissues. Time- and dose-dependent accumulation of mercury was observed in *Tilapia mossambica* (Menezes and Qasim 1984) and in *H. fossilis* (James et al. 1993b). Rodgers and Beamish (1982) reported a linear increase with exposure period in whole body mercury concentrations of *S. gairdneri* fed diets containing methylmercury. Cuvin and Furness (1988) found that uptake of mercury increased in a linear manner in minnows *Phoxinus phoxinus* on exposure to inorganic mercury for 24 d. The tissue burden of cadmium concentration increased linearly with time in liver,

kidney, intestine and stomach of *S. gairdneri* exposed to cadmium for 180 d (Giles 1988).

The ability to eliminate Pb varied between liver and muscle. The rate of depuration of Pb was 0.0225 or 0.0195 and 0.0155 or 0.0155 in liver and muscle of *O. mossambicus* exposed to 17.5 and 35 ppm, respectively. Though Pb depuration rate was faster in liver than muscle, the latter recovered earlier than the former because liver accumulated significantly more Pb ($P<0.05$) and thereby it took more days for complete elimination (see Table 4, Fig. 3). The findings of Holcombe et al. (1976) on *Salvelinus fontinalis* support the present observations. The rate of depuration or extrapolated time between day 0 and 20 of recovery showed that liver and muscle completely recovered from Pb poisoning on days 54 or 82 and 44 or 62 in animals exposed to 17.5 or 35 ppm, respectively (Fig. 3). Animals exposed to 17.5 ppm Pb recovered earlier than those exposed to 35 ppm. Fig. 1 and 3 illustrate that prior to the complete elimination of Pb from both tissues, GDH was recovered earlier than aerobic enzyme SDH. In conclusion, the present study clearly reveals a strong tendency to retain Pb in the liver than in muscle, and it is a strong inhibitor of aerobic enzyme SDH than anaerobic enzyme GDH.

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