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Effect of Lethal and Sublethal Concentrations of Cadmium on Energetics in the Gills of Fry and Fingerlings of *Cyprinus carpio*

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There has been rapid and continuous increase in the worldwide production and use of cadmium since 1925 (Moore and Ramamoorthy 1984). Contamination of the freshwater environment by cadmium has increased recently due to effluents from various sources such as oil refineries, combustion of fuels, pesticides, degradation of tires, phosphate fertilizers, mine leachate etc. (Shaikh and Smith 1984). Cadmium, even in minute concentrations of ppb; is lethal to freshwater organisms, particularly fishes, and may affect growth and reproduction (Moore and Ramamoorthy 1984). Cadmium binds irreversibly with sulfhydryl groups of catalytic proteins and inhibits normal biochemical functions (Cherian and Goyer 1978). The capacity of any freshwater organism to resist this metal stress, however, depends on its energetic efficiency (Dhavale et al 1988). So, a measure of energetics is considered to be a sensitive indicator of metal toxicity. No published information is available on the effects of cadmium on freshwater fishes comparing their energetics at lethal and sublethal concentrations. The present study was undertaken with the commercially important freshwater fish *Cyprinus carpio* to determine rate of oxygen consumption, succinate and lactate dehydrogenase activity and pyruvate and lactate levels. The study is made in gills, the respiratory organ and the primary entry points of metal from the water column. As the period of exposure and size of the fish are important factors influencing toxic effects (Suresh 1992), our study was carried out on fry and fingerlings at days 1, 2, 3 and 4 in lethal and days 1, 7, 15 and 30 in sublethal concentrations of cadmium.

MATERIALS AND METHODS

Fry and fingerlings of *Cyprinus carpio* weighing 150 $\pm$ 10 mg (S.E.) and 20 $\pm$ 1 g (S.E.), respectively, procured from the State Fisheries Department, were maintained in the laboratory in 1.5 x 0.9 x 0.9 M cement tanks, fifty in each. Water with pH 7.6 $\pm$ 0.2, total hardness 100 $\pm$ 5 mg/L Ca CO_3, temperature 28 $\pm$ 1°C and oxygen content 5.79 $\pm$ 0.4 mg/L was obtained from local wells. The fish were fed daily with commercial food pellets having about 40% protein. Water was changed daily to replenish the oxygen. The animals were held in the laboratory for ten days prior to experimentation. Later, groups of thirty fish of the two stages were exposed separately to eight different concentrations, ranging from 2.0 to 9.0 mg/L and 10 to 24 mg/L of cadmium for

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fry and fingerlings, respectively. The LC_{50}s for 96-hr tests were derived from the percent and probit mortality versus log concentration curves (Finney 1971), and were subsequently verified by Dragstedt and Behren's method (Carpenter 1975). The 96-hr LC_{50}s for fry and fingerlings were 4.26 and 17.05 mg cadmium/L, respectively, which were used as lethal concentrations. One-fifth of these LC_{50}s, i.e., 0.86 and 3.45 mg/L, respectively, were used as sublethal concentrations in further experimentation. Groups of ten fish were exposed to the respective lethal and sublethal concentrations of cadmium, and at the end of days 1, 2, 3 and 4 in lethal and days 1, 7, 15 and 30 in sublethal concentrations, the gills were removed from surviving fish and transferred into cold fish ringer solution (Ekberg 1958). The rate of oxygen uptake was measured in a Gilson 5/6 oxygraph, and succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) activity and pyruvate and lactate levels were estimated using the standard procedures described by Nachlas et al. (1960), Srikanthan and Krishnamoorthi (1955) modified by Govindappa and Swami (1965), Friedman and Hangen (1941), and Barker and Summerson (1941) modified by Huckabee (1961), respectively. Similarly, control fish were measured in the same manner. The data were statistically computed with mean, standard deviation, t-test and F values.

RESULTS AND DISCUSSION

Since heavy metals gain entry mainly through the gills of fish, disturbance in the normal respiratory epithelium may disrupt the rate of oxygen consumption (Jones 1947). SDH being an important link between the electron transport system and oxidative phosphorylation, can define the rate of operation of TCA cycle in the gill of fish exposed to toxic stress (Radhakrishnaiah 1986).

The mean rate of oxygen consumption and mean SDH activity decreased significantly ($P < 0.05$), relative to controls, in gills of both fry and fingerlings over time when subjected to lethal concentrations of cadmium. This decrease either in oxygen consumption or SDH activity was more pronounced in gills of fry than fingerlings. Interestingly, LDH activity increased significantly ($P < 0.05$) at days 1 and 2 in the gills of fry, but declined at days 3-4. SDH steadily increased with exposure time in the gills of fingerlings (Table 1). Pyruvate and lactate levels revealed two different trends in the gills of fry and fingerlings exposed to lethal concentrations Cd. Although a significant ($P < 0.05$) increase was observed in both these parameters, the increase in pyruvate in fry was on the order day $2 < 1 < 3 < 4$ and in lactate day $1 > 4 > 2 > 3$, whereas it was the reverse in fingerlings, i.e., pyruvate day $1 > 2 > 3 > 4$ and lactate day $1 < 2 < 3 < 4$ (Table 2).

The progressive decrease in the rate of oxygen consumption and SDH activity in gills of fry and fingerlings with the increase in exposure time in lethal concentrations of cadmium indicates a suppression of oxidative metabolism due to acute toxic stress. Damage may have been caused to the gill structure (Suresh 1992) on continuous exposure and a greater precipitation of mucus on the gill filaments leading to clogging of the gills (Usha Rani and Ramamurthi 1987). This could lead to suffocation (Jones 1947) and necrosis of the epithelial and inter-epithelial cells of the gill (Suresh 1992). This may have been the reason for the marked decrease in oxidative metabolism. The fish may have switched over from oxidative metabolism to anaerobic glycolysis to derive energy, hence an increase was observed in LDH activity and lactate

Table 1. Rate of oxygen consumption (μM C_2consumed/g wet wt/min) and activities of SDH (succinate dehydrogenase) and LDH (lactate dehydrogenase) (μM/mg protein/hr) in the gills of fry (F) and fingerlings (FL) of C. carpio at different periods of exposure to lethal and sublethal concentrations of cadmium. Percentage change relative to controls is given in parentheses. Each value is based on six estimations.

S.D.± : Standard Deviation F : Variance ratio LOS = 0.05

Parameter	Stage	Control	Exposure Period in Days — Lethal					Exposure Period in Days — Sublethal				
			1	2	3	4	F	1	7	15	30	F
O_2 consumption	F	3.480	2.276	1.806	1.312	0.874	6.40	2.913	3.873*	4.336	5.029	6.27
	S.D.±	0.154	0.164	0.128	0.008	0.063		0.185	0.396	0.348	0.184	
	%		(-34.6)	(-48.1)	(-62.3)	(-74.9)		(-16.3)	(+11.3)	(+24.5)	(+44.5)	
	FL	1.480	1.274	1.146	0.735	0.559	7.39	1.254	1.223	1.523*	1.795	6.53
	S.D.±	0.120	0.087	0.102	0.062	0.051		0.075	0.123	0.168	0.094	
	%		(-13.9)	(-22.6)	(-50.3)	(-62.2)		(-15.3)	(-17.4)	(+2.9)	(+21.3)	
SDH	F	0.220	0.161	0.116	0.089	0.031	4.30	0.175	0.243*	0.264	0.355	5.48
	S.D.±	0.018	0.007	0.013	0.008	0.003		0.012	0.019	0.023	0.027	
	%		(-26.8)	(-47.3)	(-59.6)	(-85.9)		(-20.5)	(+10.5)	(+20.0)	(+61.4)	
	FL	0.189	0.151	0.139	0.113	0.071	5.41	0.162	0.133	0.209*	0.257	5.72
	S.D.±	0.017	0.010	0.014	0.007	0.005		0.004	0.014	0.018	0.026	
	%		(-20.1)	(-26.5)	(-40.2)	(-62.4)		(-14.3)	(-29.6)	(+10.6)	(+36.0)	
LDH	F	0.041	0.055	0.060	0.049	0.034	5.04	0.054	0.052	0.050	0.047	5.23
	S.D.±	0.004	0.003	0.007	0.004	0.002		0.005	0.002	0.002	0.003	
	%		(+34.2)	(+46.3)	(+19.5)	(-17.1)		(+31.7)	(+26.8)	(+22.0)	(+14.6)	
	FL	0.054	0.060*	0.067	0.072	0.076	5.70	0.065	0.064	0.064	0.062	5.71
	S.D.±	0.004	0.007	0.004	0.006	0.008		0.005	0.006	0.005	0.004	
	%		(+11.1)	(+24.1)	(+33.3)	(+40.7)		(+20.4)	(+18.5)	(+18.5)	(+14.8)	

* Denotes not significantly different from controls ($P > 0.05$)

Table 2. Levels of pyruvate and lactate (mg/g wet wt) in gills of fry (F) and fingerlings (FL) of C. carpio at different exposure periods in lethal and sublethal concentrations of cadmium. Percentage change relative to controls is given in parentheses. Each value is based on six estimations.

S.D.± : Standard Deviation F : Variance ratio LOS = 0.05

Parameter	Stage	Control	Exposure Period in Days: Lethal 1	Lethal 2	Lethal 3	Lethal 4	F	Sublethal 1	Sublethal 7	Sublethal 15	Sublethal 30	F
Pyruvate	F	0.143	0.206	0.181	0.234	0.260	5.67	0.180	0.172	0.131*	0.113	5.75
	S.D.±	0.012	0.017	0.009	0.021	0.025		0.011	0.015	0.014	0.010	
	%		(+44.1)	(+26.6)	(+63.6)	(+81.8)		(+25.9)	(+20.3)	(-8.4)	(-21.0)	
	FL	0.126	0.196	0.180	0.167	0.158	5.93	0.170	0.116	0.162	0.118*	5.92
	S.D.±	0.007	0.020	0.012	0.008	0.015		0.013	0.011	0.010	0.013	
	%		(+55.6)	(+42.9)	(+32.5)	(+25.4)		(+34.9)	(+31.8)	(+20.6)	(-6.4)	
Lactate	F	3.68	5.62	5.30	5.17	5.54	6.26	4.99	4.70	4.32	3.34*	6.32
	S.D.±	0.27	0.44	0.38	0.53	0.49		0.26	0.17	0.35	0.36	
	%		(+52.7)	(+44.0)	(+40.5)	(+50.5)		(+35.6)	(+27.7)	(+17.4)	(-9.2)	
	FL	7.93	9.26	9.71	10.79	11.46	6.24	10.85	10.36	9.93	9.47	6.25
	S.D.±	0.82	0.60	0.35	1.16	0.72		1.09	0.67	1.01	0.83	
	%		(+16.8)	(+22.5)	(+36.1)	(+44.5)		(+36.8)	(+30.6)	(+25.2)	(+19.4)	

* Denotes not significantly different from controls ($P > 0.05$)

levels. However, the increase in anaerobic glycolysis decreased at day 3 and was suppressed at day 4 in the fry, with a rapid increase in the accumulation of pyruvate and maintenance of a steady lactate level (Table 2). Hence, the energy contribution even from anaerobic glycolysis was also inhibited in the gills of fry on prolonged exposure. With the activation of anaerobic glycolysis from days 1 to 4 in fingerlings, there was a significant decrease in the accumulation of pyruvate and increased retention of lactate. This suggests that the fingerlings may have tried to counter the acute toxic stress at least by deriving some amount of energy from anaerobic glycolysis. However, the marked increase in the accumulation of lactate could result in hyperlacticemia (Radhakrishnaiah et al. 1992). It is clear from the data that during lethal stress there is a steep suppression in the energetically more efficient oxidative metabolism in both fry and fingerlings. The efforts to activate anaerobic glycolysis to meet energy needs appeared greater in fingerlings and lower in fry.

In contrast to the changes observed at lethal concentrations, there was an initial decrease in oxygen consumption and SDH at day 1 in fry and at day 1 and 7 ($1 < 7$) in fingerlings exposed to Cd at sublethal concentrations. This was followed by an increase at the remaining exposure periods in the order day $7 < 15 < 30$ in fry and day $15 < 30$ in fingerlings. Concurrently, a notable increase occurred in LDH activity at day 1, which gradually decreased in the order day $1 > 7 > 15 > 30$ in both size groups (Table 1). Pyruvate in fry increased at day 1 and 7 ($1 > 7$) and declined at days 15 and 30 ($15 < 30$). Lactate increase reached its highest level at day 1 and then subsequently declined. Pyruvate levels in fingerlings increased up to day 15 in the order $1 > 7 > 15$ with an insignificant ($P > 0.05$) decrease at day 30. Lactate levels were elevated at all four exposure periods in the order day $1 > 7 > 15 > 30$ (Table 2).

The initial decrease followed by elevation in the rate of oxygen consumption and SDH activity in fry and fingerlings exposed to sublethal concentrations of cadmium reflect their ability to derive energy to overcome chronic toxic stress during prolonged exposure. As fry possess a high protein synthetic potential, it is possible that the synthesis of metal-binding 'metallothioneins' (Kagi and Nordberg 1979) prevented cadmium from interacting with the oxidative enzymes. The active elimination of metal ions from a gill site (Suresh et al. 1993) might also have facilitated the animal to absorb more oxygen without any hindrance. Since greater amounts of energy are needed for the effective elimination of metal ions and/or for enhanced protein synthesis, operation of the oxidative metabolic cycle were enhanced in the gills of fry on prolonged exposure to sublethal levels of Cd. Counteracting the rise in oxidative metabolism, the enhancement in anaerobic glycolysis observed at day 1 was slowly suppressed as evidenced by the decrease in pyruvate and lactate levels and insignificant increase of LDH at day 30. Although the oxidative metabolism in fingerlings was suppressed at days 1 and 7, the fish could overcome the sublethal stress on further exposure. This could probably be done by activating detoxification mechanisms and metal- elimination processes. As the rise in oxidative metabolism is slow, in order to derive required energy for compensatory processes, greater elevation is observed in anaerobic glycolysis in the gills of fingerlings than in the fry. This elevation, however, slowly decreased with the rise in oxidative metabolism. These findings revealed that the fry of *C. carpio* are more sensitive than fingerlings to acute cadmium stress. Greater suppression of oxidative metabolism and failure in activating

anaerobic glycolysis on prolonged exposure are evidenced from the significant changes in fry. Even though oxidative metabolism is inhibited in the gills of both fry and fingerlings during the initial days of sublethal exposure to cadmium, a rapid metabolic reorganization is observed in the gills of fry than in the fingerlings. This disparity in the metabolic reorganization between fry and fingerlings could be due to their differential resistance capacity. Fry, being the smaller animals with higher weight-specific metabolic rates (Ringwood 1990), can quickly become sensitive to acute metal stress, but can rapidly activate compensatory mechanisms under tolerable concentrations.

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