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Comparison of the Sublethal Effect of Mercury and Lead on Visceral Dehydrogenase System in Three Inland Teleosts

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Summary

The sublethal effect of mercury and lead was investigated on visceral (liver, muscle, gill, kidney and brain) succinic, malic and lactic dehydrogenases in *Labeo rohita, Clarias batrachus* and *Chana punctatus* in acute experiments. The highest decrease of succinic, malic and lactic dehydrogenases was recorded in the hepatic tissue in comparison to muscle, brain, kidney and gill. This decrease was greater in *L. rohita* than in *C. batrachus* or in *C. punctatus*. Mercury was more effective than lead. Marked variations in the activities of the three dehydrogenases in dark tissues (liver, kidney) were noted after exposure to mercury than lead in the above mentioned species. The observed dehydrogenase variations are discussed in relation to the breakdown of gas exchange at the lamellar level, to visceral hypoxia, hypoglycaemia, impaired aerobic and anaerobic pathways, formation of a metalloenzyme complex and alterations in mitochondrial electron transport.

Key words

Succinic dehydrogenase – Lactic dehydrogenase – Malic dehydrogenase

Introduction

Metabolic disturbances, inhibition of mitochondrial respiration, mitochondrial electron transport, membrane physiology, failure in the synthesis of enzymes, decrease in energy reserves, alterations in ketoacid induction in the tricarboxylic acid cycle, lactic acid accumulation, hypoxia in the viscera and impairment of oxidative metabolism are changes recorded in a variety fish species when exposed to the lethal and sublethal concentrations of heavy metals (Gagne *et al.* 1990, Jackson 1991, Jagadeesh and Shaffi 1990, James *et al.* 1992, Jeelani and Shaffi 1989).

In the present investigation the sublethal effect of mercury and lead on visceral (liver, muscle, brain, kidney and gill) succinic dehydrogenase, malic dehydrogenase and lactic dehydrogenase were studied in three fresh water teleosts living in tropical environment, namely *Labeo rohita* (Ham), *Clarias batrachus* (L.) and *Channa punctatus* (Bloch).

Material and Methods

Mature, live and healthy *L. rohita, C. batrachus* and *C. punctatus* (standard length 18-20 cm) were obtained locally and adapted in the laboratory for 10 days. Seven fish of each species were dissected to obtain organs for estimations of succinic dehydrogenase, lactic dehydrogenase and malic dehydrogenase activities.

Thirty-five fishes of each species were exposed to sublethal levels of mercury nitrate or lead nitrate for a period of 50 h. An equal number of fishes was kept subsequently in tap water as controls for the same period. After 24 h or 48 h control and exposed fish were killed and the above mentioned viscera were excised in the three species studied.

The liver, muscle, brain, kidneys and gills were homogenized in a chilled 0.01 M phosphate buffer (pH 7.4) to obtain a 10 % homogenate. The succinic dehydrogenase activity in these tissue homogenates was assayed by the colorimetric method of Kun and Abood (1949) which is based on the principle that tissue homogenates in the presence of succinate in a buffered (pH 7.4) medium reduce the colourless tetrazolium salt to formazon, which is red and insoluble in water.

The tissues for estimation of lactic and malic dehydrogenase activity were homogenized in a cold 0.25 M sucrose solution. The homogenates were centrifuged at 150xg for 10 min. The clear supernatant fluid which was adjusted with a sucrose solution and was used as the source of enzymes according to Srikanthan and Krishna Murthy (1955).

The experiment was carried out in seven separate samples pf each fish species. The data were evaluated by Student's t-test.

Results

The differential responses of succinic dehydrogenase, lactic dehydrogenase and malic dehydrogenase in the liver, muscle, brain, kidneys and gills in *L. rohita, C. batrachus* and *C. punctatus* exposed to sublethal concentration of mercury and lead under the acute conditions are shown in Tables 1-6. Mercury reduced succinic dehydrogenase activity most in the liver and less in the muscle, brain, kidneys and gills of *L. rohita* (Tab. 1). The differences in succinic dehydrogenase in the viscera of *C. batrachus* were similar as those observed in *L. rohita*. A considerable fall in succinic dehydrogenase activity was recorded in the renal tissue of *C. punctatus* (Tab. 1).

The mercury-induced decline in malic dehydrogenase activity was greatest in liver of *C. batrachus* (Tab. 2), followed by muscle, gill, kidney and brain. In *L. rohita* the maximum fall of malic dehydrogenase was also induced by mercury in the liver and less in the remaining organs. The pattern in *C. punctatus* was similar as in *L. rohita* (Tab. 2).

The fall in lactic dehydrogenase induced by mercury was maximal in liver of *L. rohita*. The changes seen in organs of *C. batrachus* and *C. punctatus* were somewhat smaller (Tab. 3).

Lead also inhibited the succinic dehydrogenase activity to a greater extent in the liver than in the muscle, brain, kidney and gill of *L. rohita* (Tab. 4). The changes in *C. batrachus* or *C. punctatus* were more or less the same as in *L. rohita* (Tab. 4). Malic and lactic dehydrogenases were lowered in the hepatic tissue by sublethal concentrations of lead more in *L. rohita* than in *C. batrachus* or *C. punctatus*. Other changes were similar to those of succinic dehydrogenase (Tables 5-6).

Out of the two metals investigated, mercury was more effective than lead. Among the three enzymes, succinic dehydrogenase was affected more in the viscera of all three fish species studied than lactic dehydrogenase and malic dehydrogenase.

Discussion

Slower enzyme synthesis, enhanced accumulation of metabolites and the binding of toxicants on the active site of enzymes resulted in a distorted functional state of the organism (Diamond *et al.* 1991, Shaffi and Dubey 1989, Shaffi 1992 a,b)

In the present investigation, the fall of succinic dehydrogenase, malic dehydrogenase and lactic dehydrogenase due to the exposure to sublethal levels of mercury and lead in the visceral organs of *L. rohita*, *C. batrachus* and *C. punctatus* might be due to a reduction in oxidative phosphorylation because required amount of oxygen is not available to the viscera due to the breakdown at the site of gas exchange at the lamellar level.

It has been established that sublethal heavy metal intoxication causes visceral glycogenolysis, hypoglycaemia and a rise in blood lactate and pyruvate concentration which indicate that the exposed organisms experienced hypoxic conditions. This causes the inactive state of fish during pollution due to stress (Pascoe *et al.* 1983, Shaffi 1978, 1981, 1992). Such changes might prevail in the present experiments so that variations in the activity of the studied dehydrogenases in the viscera of *L. rohita*, *C. batrachus* and *C. punctatus* may be explained by the above interpretation.

Decreased activity of succinic, malic and lactic dehydrogenases indicates that both aerobic and anaerobic metabolic pathways, such as succinic dehydrogenase, are impaired. Heavy metals exert a direct inhibitory effect on the activity of this mitochondrial enzyme. Owing to this, succinic dehydrogenase was inhibited more by mercury than by lead (Katz 1979, Zaba and Harris 1978). Heavy metallic ions interact with proteins through their sulphydryl groups and cause the precipitation of metalloenzyme complexes. In the present investigation, the decrease in visceral dehydrogenase activity exposed to mercury and lead may be due to metalloenzyme complex formation. This was highest in the viscera of *L. rohita* as compared to *C. batrachus* or *C. punctatus* exposed to mercury. The efficacy of mercury upon the dehydrogenases was more than lead what might indicate a greater affinity of mercury to dehydrogenases (Jagadeesh and Shaffi 1990, James *et al.* 1992, Jeelani and Shaffi 1982, 1988, Pascoe 1983, Shaffi and Jeelani 1985).

The reduction in mitochondrial respiration, electron transport, oxidative phosphorylation and a number of hitherto unknown mechanisms certainly influenced the dehydrogenase activity in the present investigation. The observed fall ight have been due to the interference of heavy metals with the basic function of mitochondria which act as a "power house" for the cell. Among the organs, dark tissues such as the liver and kidney exhibited higher variations in the activities of the three dehydrogenases than white tissues. These variations may be due to a larger number of red blood cell mitochondria and blood in dark than in white tissues. Mercury was more effective than lead and this might be due to the different affinity between metal and enzyme proteins. Out of these three species, *L. rohita* was more suscceptible to both metals than *C. batrachus* or *C. punctatus*. At present it seems that this is probably due to the biochemical heterogeneity of the visceral organs that differ in three species studied (Shaffi 1979, Jeelani and Shaffi 1988, Jeelani and Shaffi 1989, Shaffi and Jeelani 1985).

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Reprint Requests

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Table 1
Effect of sublethal mercury concentration on tissue succinic dehydrogenase in three fresh water teleosts.

	Duration of exposure			
Organ	Control	24 h	48 h	Decrease (%)
L. rohita				
Liver	0.648 ±0.088	0.366 ±0.016	0.115 ±0.013	82.25
Muscle	0.290 ±0.013	0.224 ±0.019	0.153 ±0.018	46.55
Brain	0.213 ±0.017	0.180 ±0.021	0.136 ±0.012	36.15
Kidney	0.140 ±0.013	0.124 ±0.016	0.100 ±0.010	28.57
Gill	0.080 ±0.010	0.068 ±0.014	0.058 ±0.011	27.50
C. batrachus				
Liver	0.703 ±0.035	0.445 ±0.026	0.225 ±0.022	67.99
Muscle	0.395 ±0.021	0.305 ±0.032	0.280 ±0.018	29.11
Brain	0.261 ±0.019	0.213 ±0.014	0.181 ±0.027	30.65
Kidney	0.163 ±0.015	0.137 ±0.020	0.122 ±0.016	25.15
Gill	0.093 ±0.020	0.078 ±0.017	0.064 ±0.012	31.18
C. punctatus				
Liver	0.881 ±0.050	0.644 ±0.042	0.485 ±0.035	44.94
Muscle	0.480 ±0.041	0.405 ±0.025	0.277 ±0.020	42.29
Brain	0.295 ±0.036	0.205 ±0.017	0.179 ±0.015	39.32
Kidney	0.136 ±0.012	0.083 ±0.013	0.069 ±0.009	49.26
Gill	0.104 ±0.019	0.092 ±0.016	0.084 ±0.011	19.23

Mean values ±S.D. (micrograms of formazan/mg protein/h) of 7 samples.

Table 2
Effect of sublethal mercury concentration on tissue malic dehydrogenase in three fresh water teleosts.

Organ	Duration of exposure			
	Control	24 h	48 h	Decrease (%)
		L. rohita		
Liver	0.368 ±0.022	0.295 ±0.019	0.150 ±0.013	59.23
Muscle	0.222 ±0.030	0.168 ±0.022	0.108 ±0.010	51.35
Brain	0.115 ±0.012	0.098 ±0.111	0.074 ±0.016	35.65
Kidney	0.105 ±0.016	0.106 ±0.014	0.085 ±0.011	19.04
Gill	0.055 ±0.010	0.050 ±0.011	0.045 ±0.012	18.18
		C. batrachus		
Liver	0.408 ±0.041	0.387 ±0.025	0.151 ±0.014	62.99
Muscle	0.410 ±0.030	0.364 ±0.015	0.191 ±0.021	53.41
Brain	0.115 ±0.012	0.098 ±0.017	0.089 ±0.013	22.60
Kidney	0.113 ±0.010	0.098 ±0.015	0.085 ±0.020	24.77
Gill	0.046 ±0.011	0.038 ±0.009	0.032 ±0.008	30.43
		C. punctatus		
Liver	0.537 ±0.070	0.376 ±0.030	0.276 ±0.021	48.60
Muscle	0.280 ±0.024	0.239 ±0.052	0.180 ±0.019	35.71
Brain	0.183 ±0.019	0.168 ±0.014	0.151 ±0.016	17.48
Kidney	0.155 ±0.020	0.149 ±0.012	0.133 ±0.018	14.48
Gill	0.090 ±0.010	0.020 ±0.020	0.075 ±0.012	16.66

Mean values ±S.D. (micrograms of formazan/mg protein/h) of 7 samples.

Table 3
Effect of sublethal mercury concentration on tissue lactic dehydrogenase in three fresh water teleosts.

Duration of exposure				
Organ	Control	24 h	48 h	Decrease (%)
L. rohita				
Liver	0.333 ±0.042	0.263 ±0.032	0.078 ±0.028	76.75
uMscle	0.213 ±0.036	0.122 ±0.020	0.093 ±0.024	56.33
Brain	0.100 ±0.028	0.077 ±0.015	0.061 ±0.017	39.00
Kidney	0.103 ±0.014	0.091 ±0.028	0.074 ±0.013	28.15
Gill	0.053 ±0.015	0.044 ±0.010	0.037 ±0.009	30.18
C. batrachus				
Liver	0.397 ±0.058	0.210 ±0.050	0.183 ±0.042	53.90
Muscle	0.220 ±0.040	0.195 ±0.020	0.127 ±0.035	44.29
Brain	0.129 ±0.030	0.100 ±0.028	0.084 ±0.021	34.88
Kidney	0.120 ±0.032	0.109 ±0.026	0.098 ±0.018	18.33
Gill	0.063 ±0.021	0.057 ±0.025	0.050 ±0.009	20.63
C. punctatus				
Liver	0.430 ±0.047	0.313 ±0.049	0.225 ±0.036	46.67
Muscle	0.251 ±0.039	0.193 ±0.036	0.155 ±0.024	39.04
Brain	0.160 ±0.020	0.145 ±0.010	0.108 ±0.018	32.50
Kidney	0.101 ±0.014	0.093 ±0.021	0.077 ±0.021	23.76
Gill	0.082 ±0.030	0.077 ±0.016	0.069 ±0.017	15.85

Mean values ±S.D. (micrograms of formazan/mg protein/h) of 7 samples.

Table 4
Effect of sublethal lead concentration on tissue succinic dehydrogenase in three fresh water teleosts

Duration of exposure				
Organ	Control	24 h	48 h	Decrease (%)
L. rohita				
Liver	0.645 ±0.079	0.533 ±0.038	0.191 ±0.023	70.38
Muscle	0.290 ±0.041	0.232 ±0.024	0.179 ±0.019	38.27
Brain	0.212 ±0.022	0.185 ±0.030	0.141 ±0.014	33.49
Kidney	0.135 ±0.014	0.116 ±0.019	0.106 ±0.013	21.48
Gill	0.071 ±0.013	0.064 ±0.010	0.059 ±0.015	16.90
C. batrachus				
Liver	0.707 ±0.088	0.522 ±0.047	0.322 ±0.023	54.45
Muscle	0.396 ±0.029	0.282 ±0.032	0.201 ±0.019	49.24
Brain	0.268 ±0.030	0.236 ±0.020	0.193 ±0.016	27.98
Kidney	0.171 ±0.021	0.150 ±0.014	0.142 ±0.017	16.95
Gill	0.090 ±0.016	0.087 ±0.012	0.073 ±0.010	16.90
C. punctatus				
Liver	0.856 ±0.060	0.645 ±0.043	0.492 ±0.036	42.53
Muscle	0.488 ±0.042	0.414 ±0.032	0.363 ±0.029	25.61
Brain	0.264 ±0.032	0.250 ±0.015	0.234 ±0.021	21.47
Kidney	0.181 ±0.022	0.162 ±0.018	0.156 ±0.021	13.81
Gill	0.105 ±0.014	0.097 ±0.013	0.089 ±0.016	15.23

Mean values ±S.D. (micrograms of formazan/mg protein/h) of 7 samples.

Table 5
Effect of sublethal lead concentration on tissue malic dehydrogenase in three fresh water teleosts.

Organ	Control	24 h	48 h	Decrease (%)
		Duration of exposure		
		L. rohita		
Liver	0.361 ±0.048	0.237 ±0.052	0.188 ±0.015	47.92
Muscle	0.222 ±0.031	0.187 ±0.020	0.131 ±0.019	40.99
Brain	0.116 ±0.018	0.098 ±0.016	0.085 ±0.012	26.72
Kidney	0.111 ±0.012	0.115 ±0.018	0.090 ±0.014	18.91
Gill	0.058 ±0.010	0.057 ±0.011	0.050 ±0.014	13.79
		C. batrachus		
Liver	0.407 ±0.021	0.335 ±0.036	0.233 ±0.015	42.75
Muscle	0.239 ±0.016	0.209 ±0.030	0.162 ±0.012	32.21
Brain	0.142 ±0.015	0.138 ±0.014	0.112 ±0.018	21.11
Kidney	0.112 ±0.010	0.099 ±0.011	0.094 ±0.014	16.07
Gill	0.075 ±0.016	0.064 ±0.010	0.055 ±0.019	26.66
		C. punctatus		
Liver	0.531 ±0.040	0.425 ±0.050	0.318 ±0.056	40.11
Muscle	0.280 ±0.024	0.237 ±0.021	0.204 ±0.037	27.14
Brain	0.180 ±0.017	0.166 ±0.028	0.151 ±0.035	16.11
Kidney	0.154 ±0.020	0.148 ±0.035	0.134 ±0.025	12.98
Gill	0.096 ±0.014	0.086 ±0.021	0.079 ±0.032	17.70

Mean values ±S.D. (micrograms of formazan/mg protein/h) of 7 samples.

Table 6

Effect of sublethal concentration of lead on tissue lactic dehydrogenase in three fresh water teleosts

	Duration of exposure			
Organ	Control	24 h	48 h	Decrease (%)
		L. rohita		
Liver	0.332 ±0.042	0.241 ±0.035	0.114 ±0.023	65.66
Muscle	0.213 ±0.039	0.187 ±0.032	0.116 ±0.028	45.53
Brain	0.100 ±0.023	0.090 ±0.034	0.071 ±0.020	29.00
Kidney	0.111 ±0.032	0.094 ±0.022	0.084 ±0.018	24.32
Gill	0.046 ±0.009	0.045 ±0.011	0.040 ±0.008	13.04
		C. batrachus		
Liver	0.402 ±0.046	0.311 ±0.041	0.148 ±0.029	63.18
Muscle	0.225 ±0.038	0.174 ±0.026	0.138 ±0.032	38.66
Brain	0.134 ±0.035	0.118 ±0.041	0.090 ±0.022	33.11
Kidney	0.120 ±0.026	0.121 ±0.022	0.108 ±0.025	10.00
Gill	0.061 ±0.010	0.059 ±0.012	0.056 ±0.008	08.19
		C. punctatus		
Liver	0.439 ±0.049	0.308 ±0.030	0.188 ±0.026	57.17
Muscle	0.254 ±0.035	0.203 ±0.018	0.163 ±0.020	35.82
Brain	0.163 ±0.021	0.146 ±0.028	0.129 ±0.031	20.85
Kidney	0.100 ±0.035	0.094 ±0.019	0.088 ±0.022	12.00
Gill	0.086 ±0.018	0.076 ±0.011	0.073 ±0.015	15.11

Mean values ±S.D. (micrograms of formazan/mg protein/h) of 7 samples.