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Changes in Nitrogen Metabolism in Tissues of Fish (*Sarotherodon mossambicus*) Exposed to Benthiocarb

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The use of organocarbamate pesticides is being increased in the recent years due to the ban or restrictions on chlorinated hydrocarbons (BRETTAL 1979; HAMA 1980). These carbamates rapidly degrade in biological system and their metabolic products persist in irrigation and drainage water canal systems and their effects are certain on aquatic fauna (OSMAN & BELAL 1980). Decreased hatchability, teratogenesis and increased embryonic deformities were reported in hens exposed to carbamates (GHADIRI & GREENWOOD 1966; KHERA 1966; PROCTOR & CASIDA 1975; WEPPFLMAN et al. 1980). Chronic administration of carbaryl resulted in alterations in the reproductive system of rats and increased liver, kidney and adrenal weights (CARPENTER 1961; RYBAKOVA 1966). Acute and chronic carbamate poisoning would also lead to a chain of metabolic changes at cellular level (SESHAGIRI RAO 1982). These carbamates are acutely toxic to many species besides the pests for which they are intended to. The present report deals with the effects of benthiocarb (S-(4-chlorobenzyl)-N, N-diethyl thiolcarbamate) on nitrogen metabolism in tissues of fish. Benthiocarb commonly called as 'Saturn' is popular among rice growers in more than 30 countries including India to control the graminaceous weeds in the rice fields.

MATERIAL AND METHODS

Sarotheradon mossambicus were collected from local fresh water ponds and fed ad libitum with groundnut cake. Prior to use they were acclimatized to laboratory conditions for one week and starved before the day of experimentation (JONES 1972). The LC_{50} (9.5 ppm) was determined by probit analysis (FINNEY 1964). Fishes exposed to a sub-lethal concentration (3 ppm) of benthiocarb (98% W/V) for 24 h, 48 h and 10 days separately were used in the present study. Brain, liver, muscle and gill tissues were isolated

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from the control and experimental fishes and used for the estimation of following parameters.

Determination of ammonia, glutamine and urea levels: 10% Tissue homogenates were prepared in cold distilled water and 15% Perchloric acid (PCA) separately and centrifuged at 750 g for 10 min. The distilled water supernatants were used for the determination of ammonia and glutamine levels by the methods described by BERGEMEYER (1965) and COLOWICK & KAPLAN (1967) respectively. The PCA supernatants were used for the determination of urea levels by the method described by NATELSON (1971).

Estimation of glutamate dehydrogenase (GDH), aspartate (AAT) and alanine (AlAT) transaminases: 5% Tissue homogenates were prepared in cold 0.32 M sucrose solution and centrifuged at 1000 g for 15 min to remove cell debris. A clear cell-free extract was used for the estimation of GDH activity by the method described by NACHLAS et al. (1960) with slight modification. AAT and AlAT activities were estimated by the method of REITMAN & FRANKEL (1957).

Protein content was determined by the method described by LOWRY et al. (1951).

RESULTS

Except in brain, ammonia, urea and glutamine levels were increased significantly with increase in exposure period in all the tissues of fish. Maximum increase in ammonia and urea levels was exhibited by liver followed by muscle, gill and brain tissues (Table 1 Whereas, brain showed maximum increase in glutamine levels followed by liver, muscle and gill tissues.

A decrease in GDH and an increase in AAT and AlAT activities were observed in the tissues of fish exposed to benthiocarb (Table 2). These changes were in parallel with the concentration and duration of pesticide exposure. Maximum changes were observed in the liver as compared to other tissues.

DISCUSSION

The elevated levels of ammonia in tissues of fish during benthiocarb exposure is in support of the earlier report from this laboratory (SREENIVASA MOORTHY et al. 1982). Increased acetyl choline (ACh) levels in the tissues was found to affect the diffusion of ammonia through the gills by increasing the vascular resistance (KEYS & BATEMAN 1932). An increase in ACh levels in the tissues of fish exposed to benthiocarb was observed by the authors (SESHAGIRI RAO 1982). The increased ammonia levels can also be attributed to the increased deamination of the free amino acids during pesticide exposure (RAJENDRA et al. 1980; SESHAGIRI RAO 1982).

Table 1. Changes in levels of ammonia, urea and glutamine in the tissues of fish exposed to sub-lethal (SL) concentration of benthiocarb (Values are expressed in μ moles/gm wet wt. of tissue; each value is mean of ± SD of 6 observations; a = P< 0.001; b = P< 0.01; c = P< 0.05)

Tissue	Component	Control	SL 24h	SL 48h	SL 10days
Brain	Ammonia	2.42	2.50	2.64	2.82
	SD ±	0.52	0.47	0.48	0.50
	% Change		+3.3	+9.1	+16.5
	Urea	0.63	0.66	0.68	0.71
	SD ±	0.17	0.23	0.15	0.30
	% Change		+4.8	+7.9	+12.7
	Glutamine	3.00	3.38	3.90^{a}	4.61^{a}
	SD ±	0.38	0.22	0.24	0.29
	% Change		+12.7	+30.0	+53.7
Liver	Ammonia	12.6	14.0^{c}	15.4^{b}	19.5^{a}
	SD ±	1.00	1.00	1.80	1.70
	% Change		+11.1	+18.2	+54.8
	Urea	3.04	3.60^{b}	3.66^{b}	4.07^{a}
	SD ±	0.26	0.26	0.21	0.28
	% Change		+18.4	+20.4	+33.9
	Glutamine	1.83	2.02	2.42^{a}	2.82^{a}
	SD ±	0.15	0.16	0.12	0.19
	% Change		+10.4	+32.2	+54.1
Muscle	Ammonia	10.0	10.0	11.8^{c}	14.6^{a}
	SD ±	1.00	0.90	1.00	1.50
	% Change		+9.0	+18.0	+46.0
	Urea	0.81	0.91	0.87	1.27^{c}
	SD ±	0.10	0.10	0.06	0.40
	% Change		+12.3	+7.4	+56.8
	Glutamine	7.19	7.90^{c}	8.43^{b}	9.06^{a}
	SD ±	0.43	0.35	0.51	0.56
	% Change		+9.9	+17.2	+20.6
Gill	Ammonia	7.01	7.57	7.98^{b}	7.47
	SD ±	0.50	0.56	0.53	0.42
	% Change		+8.0	+13.8	+6.6
	Urea	1.39	1.53	1.67^{b}	1.98^{a}
	SD ±	0.15	0.14	0.09	0.06
	% Change		+10.1	+20.1	+42.4
	Glutamine	0.37	0.40	0.47^{c}	0.51^{c}
	SD ±	0.06	0.07	0.07	0.11
	% Change		+8.1	+27.0	+37.8

Table 2. Changes in activity levels of GDH (μ moles of formazan/mg protein/hr), AAT and AlAT (μ moles of pyruvate/mg protein/hr) in the tissues of fish exposed to sub-lethal (SL) concentration of benthiocarb (Each value is mean of ± SD of 6 observations; a = P < 0.001; b = P < 0.01; c = P < 0.05)

Tissue	Enzyme	Control	SL 24h	SL 48h	SL 10days
Brain	GDH	0.17	0.16	0.13[a]	0.12[a]
	SD ±	0.005	0.01	0.008	0.004
	% Change		-5.9	-23.5	-29.4
	AAT	0.74	0.79	0.84	1.04[a]
	SD ±	0.03	0.05	0.40	0.07
	% Change		+6.8	+13.0	+40.5
	AlAT	0.84	0.91[b]	0.95[a]	1.11[a]
	SD ±	0.03	0.03	0.05	0.08
	% Change		+8.3	+13.1	+32.1
Liver	GDH	0.28	0.24[a]	0.21[a]	0.16[a]
	SD ±	0.009	0.005	0.008	0.01
	% Change		-14.3	-25.0	-42.9
	AAT	1.16	1.30[a]	1.38[a]	1.62[a]
	SD ±	0.04	0.04	0.06	0.07
	% Change		+12.1	+19.0	+39.7
	AlAT	6.69	7.64[a]	8.33[a]	9.85[a]
	SD ±	0.21	0.21	0.28	0.66
	% Change		+14.2	+24.5	+47.2
Muscle	GDH	0.025	0.024	0.021	0.019
	SD ±	0.004	0.003	0.006	0.007
	% Change		-4.0	-16.0	-24.0
	AAT	0.49	0.53[c]	0.54[c]	0.64[a]
	SD ±	0.02	0.03	0.05	0.06
	% Change		+8.2	+10.2	+30.6
	AlAT	1.13	1.26[c]	1.37[a]	1.62[a]
	SD ±	0.08	0.07	0.06	0.09
	% Change		+11.5	+21.2	+43.4
Gill	GDH	0.016	0.016	0.014	0.013
	SD ±	0.002	0.004	0.005	0.008
	% Change			-12.5	-18.7
	AAT	0.62	0.67	0.72[b]	0.83[a]
	SD ±	0.05	0.03	0.04	0.05
	% Change		+8.1	+16.1	+33.9
	AlAT	0.72	0.81[a]	0.86[a]	1.02[a]
	SD ±	0.03	0.03	0.05	0.05
	% Change		+12.5	+19.4	+41.7

Since ammonia is a toxic metabolite the tissues try to eliminate it by synthesizing urea and glutamine. This is evident from the increased levels of urea and glutamine in the present study. It was also reported that, hyperammonemia increases synthesis of urea in liver and its vascular mobilization, since the operation of urea cycle in extra-hepatic tissues is still doubtfull (SREERAMULU CHETTY 1978). The pronounced increase in brain glutamine suggests its possible enhanced synthesis from glutamate and ammonia, a major detoxification mechanism in extra-hepatic tissues (COHEN & BROWN 1960).

Elevated ammonia levels also accounts for the decreased GDH activity in the present study. It has been reported that increased ammonia and lactate levels inhibit the activity of this mitochondrial enzyme (FLETCHER & HOPKINS 1907; CLELAND 1963; KARLSON et al. 1975). Several environmental and physiological factors influence the aminotransferase activity (KNOX & GREENGARD 1965). The increased AAT and AlAT activities observed in the present study is in consonance with the previous reports (SASINOVICH & VORONINA 1971; POHLAM et al. 1976). KABEER AHMED (1979) has reported an increased affinity of substrate to transaminases in the tissues of fish exposed to malathion. The changes in GDH, AAT and AlAT activities may also be attributed to altered mitochondrial integrity during pesticide exposure (MIVOGLAW 1973).

The present study concludes that during pesticide exposure increased ammonia levels cause a shift in nitrogen metabolism towards synthesis of urea and glutamine. Inhibition of glutamate oxidation to ammonia and α-keto glutarate by GDH suggests the possible adaptive mechanism to reduce the ammonia toxicity by minimising the addition of further ammonia to the existing elevated ammonia pool. Increased transamination facilitates greater feeding of keto acids to impaired oxidative metabolism during pesticide stress (SREENIVASA MOORTHY et al. 1982a).

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