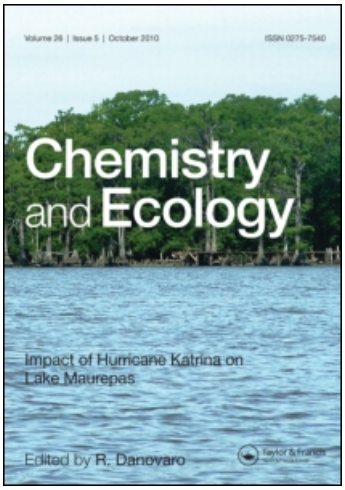

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PROTEIN METABOLIC RESPONSE TO CARBON TETRACHLORIDE IN A FRESHWATER FISH, *SAROTHERODON MOSSAMBICUS*

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Exposure of a freshwater teleost, *Sarotherodon mossambicus*, to a sublethal concentration (26 ppm) of carbon tetrachloride for periods up to 30 days led to significant changes in the soluble protein fractions (albumin and globulins) and free amino acid content. The decrease in total protein content was greater in liver than in muscle. The protein loss was expected due to the impairement of protein synthetic activity during stress conditions.

KEY WORDS: CCl_4, toxicity, fish, muscle and liver, protein metabolism.

INTRODUCTION

Indiscriminate and widespread use of chemicals in various industries has resulted in their discharge into the aquatic ecosystem. Today, chemical pollution is often a major scientific and administrative problem.

Among organic pollutants, halogenated methanes occupy a prime position because they are used extensively as solvents and degreasers in industries and enter into the food chain cycle directly or indirectly, thus affecting ecological homeostasis. Uptake and distribution of halomethane by microorganisms and fish leads to the build up of such compounds in the food chain (Ramanaiah *et al.*, 1991).

Carbon tetrachloride, a hepatotoxin and hepatocarcinogen, causes hepatocellular damage which in turn affects biochemical pathways of animal systems. There is rather little information available on toxicity of CCl_4 to aquatic animals, particularly to edible fish. Hence, in the present study, the protein metabolic response of a freshwater fish to CCl_4 toxicity was studied by quantifying different fractions of tissue proteins.

MATERIALS AND METHODS

Adult samples of *Sarotherodon mossambicus* (13 ± 2 g) were collected from freshwater ponds in and around Tirupati. The LC_{50} of CCl_4 for this species was determined by the probit method of Finney (1964) and found to be 105 ppm for 48 hours exposure. One fourth of LC_{50} was selected as sublethal concentration

(26 ppm) for the present study. A group of six fish were exposed to this sublethal concentration of CCl_4 in tap water for one month. The water was changed once a day and the same concentration of CCl_4 was maintained throughout the experimental period. Control fish were maintained under identical conditions in tap water. Fish were sacrificed on days 7, 14, 21 and 30 after exposure and the tissues isolated for analysis of the following biochemical components. Total protein content was estimated by the method of Lowry *et al.* (1951). Soluble protein fractions (albumins and globulins) were assayed by the method of Cohn *et al.* (1940). Free amino acid content was quantified by the method of Moore and Stein (1954). Statistical significance of the data was assessed using Student's 't' test (Pillai and Sinha, 1968).

RESULTS AND DISCUSSION

Protein profiles of the tissues are indicative of the physiological status of the animal (Harper, 1990). A dynamic equilibrium exists between the synthetic and degradative pathways associated with these protein molecules.

Total protein content decreased in muscle and liver of fish exposed to sublethal concentration of CCl_4 for 30 days (Table I and II). Reduction in protein content was indicative of extensive endogenous utilisation of protein catabolic products for greater energy requirements during CCl_4 stress. The decrease in protein content may be due to enhanced proteolysis or due to diminished protein synthesis. The greater decrease in liver protein content suggests higher protein degradation in liver than in muscle. The halogenated compounds bind covalently to the SH and amino groups of proteins leading to denaturation (Slater, 1972). In support of present findings, Mehendale (1990) reported suppressed hepatocellular regeneration and hepatocellular restoration in CCl_4-administered rats indicating reduced protein synthesis. The studies of Prasada Rao and Mehendale (1990) also showed that the activation of Ca^{2+}-dependent degradative enzymes such as proteases, phospholipases and endonucleases by increased calcium levels may cause cell damage and death during toxic stress, in agreement with our findings.

In view of the changes observed in total protein content, our studies were extended to quantify different fractions of proteins so as to know which fraction was degraded in response to CCl_4 exposure. Since albumins and globulins are sensitive indicators of altered homeostatic and immune response of fish to toxic stress these two fractions were analyzed in the liver and muscle of *Sarotherodon mossambicus.*

Normal levels of plasma albumin are maintained by an equilibrium between hepatic synthesis and degradation or loss. In the present study, the albumin content of muscle + liver tissues rose during initial exposure and later decreased (Table I and II). Since liver is the main centre for the synthesis of albumins, it is presumed that CCl_4 stress might have induced high albumin synthesis initially. Such elevated albumin content has been reported in freshwater mussels under various stress conditions (Sathyavelu Reddy, 1985 and Uma Bhaskaramani, 1989). Hepatic tissue suffered a greater loss in albumin content than muscle after one month exposure to CCl_4. Since hepatic albumin is continuously released into the blood (Harper, 1990)

Table I Changes in total protein content, albumins, α, β-globulins, γ-globulins (mg/g wet weight) and free amino acid content (μ moles of tyrosine equivalents/g wet weight) in the muscle of fish exposed to carbon tetrachloride for a period of one month.

Name of the parameter	*Exposure period*							
	7 days		*14 days*		*21 days*		*30 days*	
	Control	*26 ppm*	*Control*	*26 ppm*	*Control*	*26 ppm*	*Control*	*26 ppm*
Total protein	153.38 ±2.19	136.74 ±2.08 (−10.85)	157.45 ±2.49	120.83 ±2.03 (−23.26)	153.72 ±1.99	107.57 ±2.07 (−30.02)	154.58 ±2.10	100.11 ±2.52 (−35.24)
Albumins	0.781 ±0.04	0.930 ±0.02 (19.08)	0.753 ±0.03	0.573 ±0.04 (−23.90)	0.770 ±0.05	0.468 ±0.01 (−39.22)	0.768 ±0.06	0.395 ±0.01 (−48.57)
α,β-globulins	0.960 ±0.09	0.730 ±0.09 (−23.96)	0.884 ±0.09	1.114* ±0.17 (26.02)	0.923 ±0.14	1.221 ±0.11 (32.29)	0.884 ±0.09	1.301 ±0.12 (47.17)
γ-globulins	10.44 ±0.94	9.15* ±0.42 (−12.36)	11.20 ±0.77	14.01 ±0.57 (25.09)	10.97 ±0.71	7.50 ±0.38 (−31.63)	11.28 ±0.99	17.54 ±0.70 (55.50)
Free amino acid	70.69 ±1.79	79.30 ±1.02 (12.18)	68.12 ±1.72	82.01 ±2.08 (20.39)	73.05 ±2.90	93.22 ±2.28 (27.61)	74.19 ±2.23	97.07 ±1.68 (30.84)

All the values are mean ± S. D. of six individual observations
Values in the parentheses are percent change over control
Values are significant at $P<0.001$ *except significant at $P<0.05$

Table II Changes in total protein content, albumins, α, β-globulins, γ-globulins (mg/g wet weight) and free amino acid content (μ moles of tyrosine equivalents/g wet weight) in the liver of fish exposed to carbon tetrachloride for a period of one month.

Name of the parameter	*Exposure period*							
	7 days		*14 days*		*21 days*		*30 days*	
	Control	*26 ppm*	*Control*	*26 ppm*	*Control*	*26 ppm*	*Control*	*26 ppm*
Total protein	133.72 ±2.58	116.28 ±1.36 (−13.04)	132.36 ±2.23	92.23 ±1.14 (−30.32)	134.01 ±1.60	84.71 ±1.30 (−36.79)	131.07 ±1.22	77.04 ±1.22 (−41.22)
Albumins	0.99 ±0.05	0.781 ±0.03 (−21.11)	0.978 ±0.07	1.062* ±0.08 (8.59)	1.002 ±0.06	0.575 ±0.07 (−42.61)	0.975 ±0.05	0.415 ±0.05 (−57.44)
α,β-globulins	3.65 ±0.09	3.150 ±0.196 (−13.70)	3.69 ±0.14	4.57 ±0.27 (23.85)	3.728 ±0.26	2.50 ±0.17 (−32.94)	3.73 ±0.22	5.77 ±0.20 (54.69)
γ-globulins	21.89 ±0.76	16.93 ±0.38 (−22.66)	20.70 ±0.87	26.05 ±0.66 (25.84)	22.36 ±0.75	30.74 ±0.64 (37.48)	21.93 ±1.08	37.79 ±0.90 (72.32)
Free amino acids	81.42 ±1.69	94.25 ±1.32 (15.75)	83.86 ±1.35	108.29 ±2.25 (29.13)	80.81 ±1.68	110.29 ±1.88 (36.48)	84.28 ±1.55	110.47 ±2.25 (31.07)

All the values are mean ± S. D. of six individual observations
Values in the parentheses are percent change over control
Values are significant at $P<0.001$ *except significant at $P<0.05$

to counter the toxic effects of the applied agent, it was possible that the tissue might be releasing more albumin into the blood to meet the demand thereby depleting tissue albumin levels. The decrease in albumin content in the later phase of exposure might be attributed to histopathological damage caused by CCl_4. When synthesis is reduced in liver disease, the serum albumin concentration and the total body albumin pool decrease until a new equilibrium is established between synthesis and degradation. In cirrhotic patients, a decreased level of serum albumin reflects reduced synthesis or distribution of albumin pool in an expanded extravascular space. A similar possibility may be expected in the present context also since CCl_4 exposure caused accumulation of lipids in liver leading to a cirrhotic condition in fish (Padmavathi, 1993).

α,β and γ-globulins of liver and muscle of freshwater fish showed different responses to CCl_4 exposure. Total globulin content was increased in liver and muscle of CCl_4 exposed fish on day 30 (Table I and II). A similar trend was observed in the freshwater mussel, *Lamellidens marginalis* (Sathyavelu Reddy, 1985) under methylparathion stress. The elevation in γ-globulin level in liver and muscle was seen more in the later phase of exposure than in the early phase. This demonstrates that the animal is orienting its immune system so as to protect the tissues against the toxic effects of CCl_4. The elevation in γ-globulin fraction in response to CCl_4 exposure was higher in liver, which is the main seat of synthesis, than in muscle.

A month-long exposure to CCl_4 resulted in an increased protein catabolism in the tissues of fish with a concomitant increase in the amino acid pool (Table I & II). These catabolic products of protein are very important in the sense that they contribute to the increased energy requirement during stress (Ferguson, 1982). These free amino acids (FAA) may act as the osmotic and ionic agents (Jurss, 1980) bringing ionic balance between loss of ions from the organism and uptake from the medium (Harper, 1990).

Higher levels of FAA in the liver as compared to muscle could also be due to the fact that liver is the major site of nitrogen transfer reactions (Harper, 1990). Elevation in the FAA content in muscle suggests augmented protein degradation by proteolytic enzymes. Thus exposure to the applied toxicant interferes with the structure and function of the muscles of *Sarotherodon mossambicus.*

In general, it can be stated that the enhanced FAA pool attributed to increased proteolysis acts as a possible source of energy to meet increased energy demands of fish under CCl_4 stress.

The hepatotoxicant was found to elevate proteolysis in liver and muscle tissues of *Sarotherodon mossambicus* leading to the accumulation of FAA, which is in turn deaminated, facilitating their metabolic utilization to meet energy demands. The data also suggest that the liver damage is proportional to the length of exposure to CCl_4. Hence it can be stated that halogenated methanes such as CCl_4 affect not only the chemical composition of water (Van Lelyveld and Zoeteman, 1980) but also cause adverse effects on aquatic fauna and also on fish production (Ramanaiah, 1989 and Nageswara Rao, 1990). The findings on CCl_4 toxic studies in animal tissues were found useful to evaluate liver damage caused by the exposure.

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