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Ethological Responses and Changes in Hemoglobin and Glycogen Content of the Common Carp, *Cyprinus carpio*, Exposed to Cadmium

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Abstract

Specimens of common carp, *Cyprinus carpio*, were exposed to cadmium ranging in concentrations from 5 to 8 mg l^{-1}. The 96 hour LC_{50} was found to be 6.12 mg l^{-1}. Exposed fish showed marked changes in behavioral patterns. Hyperexcitation, fast and jerking movements, convulsion and profuse secretion of mucus were apparent in the fish exposed to cadmium. Severity and frequency of occurrence of these behaviors in-creased in higher concentrations (7-8 mg l^{-1}) in the beginning of treatments. Later, the fish in higher concentrations became less active and settled at the bottom. Behaviors like coughing, yawning, fin flicking and chafe in fish exposed to sublethal concentra-tions (0.25-1.5 mg l^{-1}) of cadmium were found to be concentration dependent also. Depletion of glycogen in the liver and muscle was noted, being greater in muscle (maximum 27%) than the liver (maximum 10%) in the highest sublethal dose (1.5 mg l^{-1}) of cadmium. Hemoglobin concentration of the cadmium-exposed fish was in-creased by 4.4, 6.6, 8.1 and 8.5 per cent in concentrations of 0.25, 0.5, 1.0 and 1.5 mg l^{-1}, respectively.

Introduction

Aquatic organisms are threatened by toxic discharge to the environment from industrial processes, mining, agricultural developments and fossil fuel consumption and which may undergo biological magnification (Beijer and Jornelo 1979; Hamilton and Mehrle 1986). A heavy metal like cadmium, although found in nature, has no known biological function and is extremely toxic to aquatic life (Hiatt and Huff 1975).

Studies indicate that cadmium causes alterations in renal functions of humans (Axelsson and Piscator 1966), horse (Nordberge et al. 1979) and fish (Koyama and Itazawa 1977; Koyama et al. 1979; Yamawaki et al. 1986); testicular structures of mice (Nordberge 1971) and fish (Sangalang and O'Halloron 1972); and carbohydrate metabolism by damaging the liver of fish (Shafi and Qayyum 1978; Roberts et al. 1979; Al-Akel et al. 1988). Watson and Benson (1987) reported that enzymatic activity is suppressed by cadmium. Retarded growth and reproduction were reported by Spehar (1976) after the intoxication of cadmium in fish. Changes in some blood parameters like hemoglobin, RBC, ESR and leucocyte count of fish due to cadmium treatment were reported by Larsson et al. (1976), Majewski and Giles (1981), Houston and Keen (1984), Benson et al. (1987) and Murad and Houston (1988). Larsson and Haux (1982) reported that cadmium inhibits in fish the secretion of insulin which is known to be important for glycogenesis, thus stopping the production of energy reserves. Changes in the behavioral pattern of fish due to cadmium are available in the work of Atchison et al. (1987); Al-Akel et al. (1988) and McNicol and Scherer (1991).

In the present work an attempt was made to study the toxicity of cadmium to common carp, *Cyprinus carpio* L., as measured by its effects on the behavior of fish, on their metabolism of energy reserves, i.e., glycogen, and changes in hemoglobin.

Materials and Methods

Active and healthy specimens of common carp (fork length ranging from 10 to 12 cm and weight from 55 to 60 g), were procured from a fish farm (Deerab) about 80 km south of Riyadh, Saudi Arabia (23° 38'N, 46° 43'E). They were kept in glass aquaria during a two-week period of acclimation to laboratory conditions. During this period the fish were fed a commercial fish food twice daily to satiety. The approximate composition of the food was: 40% protein, 10.9% minerals, 6.5% moisture and 8.7% fat. Laboratory parameters like temperature, pH, dissolved oxygen and hardness measured four times during the experimental period were: temperature 22.0±0.5°C.; pH 7.6±0.4; dissolved oxygen 7.2±0.4 ppm; and hardness 232.58±1.05 ppm as $CaCO_3$.

Acclimation was judged to be complete when normal feeding and activity of the fish resumed. Then ten specimens of *C. carpio*

were transferred into each of six circular jars containing 30 l of water and kept a further 24 hours for acclimation. A solution of $CdCl_2$ was added to bring the water to concentrations of 5.0, 6.0, 6.5, 7.0, 7.5 and 8.0 mg l^{-1}. The water was aerated and feeding was stopped during the period of exposure. A control set was run with the same number of fish and same quantity of water but without cadmium. Dead specimens were removed immediately after death and their numbers registered. The medium was renewed after 24 hours, 48 hours and 72 hours. The LC_{50} for 96 hours was computed from a graph plotted from probit of kill against $\log_{10}$ value of the concentrations as described by Finney (1971).

In another set of experiments, the fish were exposed to sublethal concentrations of 0.25, 0.5, 1.0 and 1.5 mg l^{-1} cadmium for 10 days. A control set was also run simultaneously with the same number of fish and the same amount of water but without cadmium. Each concentration was run in duplicate.

At the end of the experiments five fish from each Cd treatment and from the control were sacrificed and their blood was collected in heparinized vials. Samples of blood which clotted were discarded. Hemoglobin was estimated by the method of Blaxhall and Daisley (1973). A known volume of blood (0.02 ml) was mixed with 4 ml of Drapkin's reagent and left for 30 min for complete conversion of hemoglobin into cyanmethemoglobin. The color intensity was read by spectrophotometer against a blank at 540 μm. The quantity of hemoglobin was expressed as g.100 ml^{-1} blood.

Known quantities of liver (100 mg) and white muscle (500 mg) were taken for the estimation of glycogen. Extraction of glycogen was done by the method of Ashman and Seed (1973) and estimated by the method of Montgomery (1957). Liver and muscle were homogenized in 5 ml of 1.5% KOH and centrifuged. The supernatant was discarded and pellet treated with 1.5% KOH. This process was repeated twice. Lastly, the tissue residue dissolved in 1.5% KOH was heated, cooled and mixed with 5 ml of absolute methanol for the precipitation of glycogen. Glycogen was separated from the solvent by discarding the supernatant after centrifugation. This glycogen was dissolved in a known volume of 1N HCl by heating in a boiling water bath for 30 min and filtering. Glycogen was estimated in the filtrate and expressed as $mg.g^{1}$ wet weight of the tissue.

Yawning, fin flicking, S-jerk, partial jerk, chafe and burst swimming (described in Table 1) were observed directly for 15 min

in each test solution after every 24-hour period. The frequency of each behavior was recorded. The recording time was rotated from morning to evening so that diurnal fluctuations in activity would not be confounded with toxicant effect. One-way analysis of variance was applied to test differences in hemoglobin, liver and muscle glycogen and behavioral data. The least significant difference test was employed to test the significance across the cadmium concentrations.

Table 1. Behavior patterns of *Cyprinus carpio* exposed to various concentrations of cadmium, as described by Henery and Atchison (1986).

Behavior	Description
Cough	Rapid, repeated opening and closing of mouth and opercular covering accompanied by partial extension of fins.
Yawn	Single wide opening of the mouth and opercular coverings accompanied by hyperextension of fins.
S-jerk	Flinching of entire body, moving sequentially from head to tail.
Partial-jerk	Flinching head or tail only.
Fin-flicking	Extension and contraction of fin spines and rays.
Burst swimming	Sudden, rapid spurt of forward movement
Chafe	Rubbing of the body against an inanimate object.

Results and Discussion

Toxicity

Mortality as a function of (Cd) is given in Fig.1. The Lc_{50} value was computed to be 6.12 mg l^{-1}. This value is higher than the value (5.2 mg l^{-1}) reported earlier from this laboratory for another species, *Oreochromis niloticus* (Al-Akel et al. 1988). Pickering and Gast (1972) reported an even higher value (7.2 mg l^{-1}) of cadmium for fathead minnow. The 4-day Lc_{50} was reported as 240 $\mu g \cdot l^{-1}$ for the common carp, *(Cyprinus carpio)* by Rehwoldt et al. (1972), which is less than the value reported in the present investigation. This

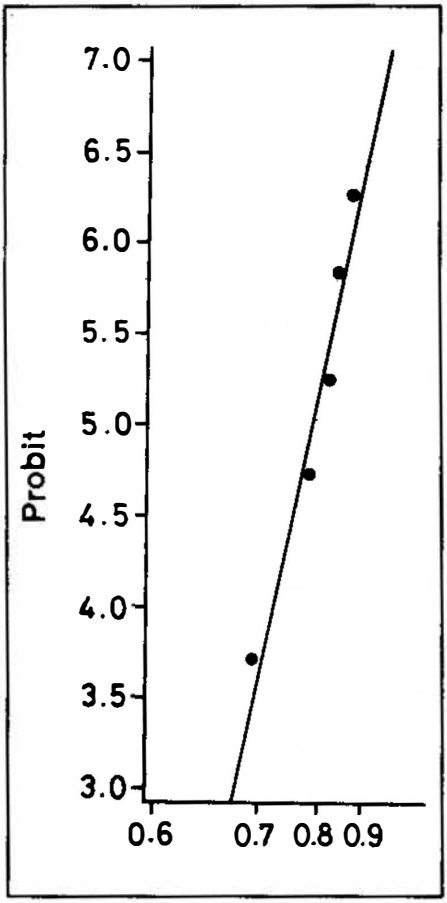

Fig. 1. Relationship between probit kill and $\log_{10}$ concentrations of cadmium.

difference may be attributed to factors like hardness of water, pH and susceptibility of the test specimens. It has been reported that the larval stage (Verma et al. 1981) of fish and sexually mature invertebrates (McCahan and Pascoe 1988) are more susceptible to cadmium. Cadmium toxicity is reduced by increasing the hardness and pH of water (Alabaster and Lloyd 1982). Generally hepatic failure and disturbances in the mechanism of ionoregulation are mainly responsible for mortality following the administration of acutely toxic doses of cadmium (Dudley et al. 1982; Goering and Klaassen 1984).

Behavioral Observations

Remarkable changes in the behavioral patterns of the exposed fish were observed. Fish treated with acute doses exhibited hyperexcitation, fast and jerking movements and occasional convulsion. The effect was more pronounced in the highest dose (mg l^{-1}) and in the beginning of the experiment. Later, (48-96 hours), the frequency of these responses decreased and the specimens secreted large quantities of mucus, showed loss of balance and finally succumbed. It is clear from the data that the frequency of occurrence of such behavior was high in the fish kept in toxic media. The effect of the toxicant was more pronounced in higher concentrations (Table 2). The profuse secretion of mucus by cadmium-exposed fish is probably to coat the body, reduce contact with the toxic environment and get relief from the irritation caused by cadmium. The mucus deposition over the gills undoubtedly interferes

Table 2. Frequency of different behaviors of *Cyprinus carpio* in relation to cadmium concentration. Data are summation of frequencies of five fishes for four-day observations (total 1 hour) averaged for two replicates. Values along a row without a letter in common are significantly different (least-significant-difference test, $P<.05$).

		Concentrations ($mg \cdot l^{-1}$)			
Behaviors	Control	0.25	0.5	1.0	1.5
Cough	5.5^{a}	14.5^{b}	17.5^{c}	22.0^{dc}	25.5^{d}
Yawn	4.5^{a}	10.0^{b}	14.5^{bc}	16.0^{c}	16.0^{c}
S-jerk	5.0^{a}	15.5^{b}	16.0^{b}	16.5^{b}	15.5^{b}
Partial-jerk	3.5^{a}	9.5^{b}	9.5^{b}	10.5^{b}	10.5^{b}
Fin flick	9.5^{a}	22.5^{b}	21.5^{b}	25.5^{bc}	30.0^{c}
Burst swimming	0.5^{a}	1.0^{ab}	2.0^{ab}	2.0^{ab}	2.5^{b}
Chafe	1.0^{a}	1.0^{a}	1.5^{a}	2.0^{a}	3.0^{a}

with gaseous exchange across them. Therefore, the fish cough and yawn to increase gas diffusion across the gills. Both symptoms are regarded as having a clearing effect on gills (Henery and Atchison 1986; Al-Kahem 1989). Loss of balance of the fish may be due to the effect of the metal on the lateral line system.

Carbohydrate Metabolism

The results of the present investigation indicate that glycogenolysis occurs in both liver and muscle tissue of the cadmium-exposed fish (Table 3). The reduction in the glycogen level in the tissues of cadmium-treated fish may be ascribed to increased physical activity, since physical exercise is known to lead to such changes in carbohydrate metabolism in fish (Black et al. 1962; Nakano and Tomlinson 1967; Shamsi and Al-Akel 1986; Al-Akel et al. 1988; Al-Kahem et al. 1990). Nakano and Tomlinson (1967) and Mazeaud et al. (1977) suggested that alterations in carbohydrate metabolism are produced by environmental stress through a primary effect on endocrine glands causing them to release large amounts of hormones such as catecholamines and corticosteroids which are active in carbohydrate metabolism.

Table 3. Hemoglobin and glycogen content in fish, *Cyprinus carpio*, exposed to cadmium. Values are means ± standard deviation. Values along a column without a letter in common are significantly different (least-significant-difference test, $P<.01$).

Concentrations ($mg \cdot l^{-1}$)	Hemoglobin $g \cdot 100\ ml^{-1}$	Glycogen ($\mu g \cdot g^{-1}$)	
		Liver	Muscle
Control	6.64^{a} ±0.15	$8,082.00^{a}$ ±117.64	$2,413.35^{a}$ ±56.75
0.25	6.99^{b} ±0.21	$7,515.37^{b}$ ±205.63	$1,847.73^{b}$ ±41.30
0.5	7.13^{bc} ±0.17	$7,397.86^{c}$ ±103.83	$1,826.60^{cb}$ ±36.48
1.0	7.23^{cd} +0.09	$7,285.20^{cd}$ +75.28	$1,811.89^{d}$ ±32.12
1.5	7.26^{d} ±0.08	$7,298.04^{d}$ ±41.33	$1,754.75^{e}$ ±38.14

Hemoglobin

An increase in the hemoglobin content (Table 3) was registered after exposure of fish to cadmium. As noted, the toxicant-exposed fish experienced greatly increased movements, which would have required increased oxygen supply for the oxidation of fuel molecules to meet their energy requirements. Tissue hypoxia may be expected because of the high requirement or less supply of oxygen due to hindrance in gaseous exchange caused by mucus deposition on the gills. In such hypoxic condition, there is a stress-mediated synthesis of more hemoglobin in older erythrocytes and release of new erythrocytes from the erythropoietic organs to improve the oxygen carrying capacity of the blood (Erslev 1977; Mcleay and Gordon 1977; Yamamoto et al. 1980; Schindler and DeVries 1986; Murad and Mustafa 1989). Majewski and Giles (1981) have attributed the increased hemoglobin in cadmium-exposed fish to the toxicant-induced hyperactivity and impaired gill function. Therefore, the increase of the hemoglobin content of cadmium-exposed fish is hypothesized to be related to an increased level of exercise and/or to a decrease in gaseous exchange across the gills due to excess mucus secretion.

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