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Hematological Characteristics of Rainbow Trout, *Salmo gairdneri* (Richardson), in Response to Cadmium Exposure

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In recent years there has been a growing interest in examining the various blood cell stages and their possible role in the immune and physiological stress response in fish (Ellis 1976; Wedemeyer and McLeay 1981; Donaldson 1981). The study of Lehmann and Sturenburg (1975) have described the differential blood cell types in rainbow trout, but there have been only limited reports on the baseline observations of red and white blood cell ratios in trout (Wedemeyer and Yasutake 1977). Furthermore, relatively little work has studied the influence of chemicals or contaminants exposure on the differential blood cell ratios in this species (Spannhof et al 1979; Niimi and Lowe-Jinde 1984). Cadmium is a well known industrial and environmental contaminant and in our recent report (Lowe-Jinde and Niimi 1984) of cadmium (4-36 ug Cd/L) effects on blood and other tissues in rainbow trout (Salmo gairdneri), it was found that short-term exposure caused significant reduction in tissue glycogen levels while long-term exposure increased the size of the hemopoietic tissue, the liver. Therefore, it seems feasible to extend these investigations to further examine the peripheral blood. Thus, in this study the spleen somatic index, total and differential erythrocyte and leucocyte counts in cadmium-exposed rainbow trout were measured. The lower working concentrations include levels (1.5-6.3 ug Cd/L) recommended by the USEPA (1980) for protection of freshwater aquatic life.

MATERIALS AND METHODS

Hatchery-reared rainbow trout were maintained in the laboratory according to the conditions previously described (Lowe-Jinde and Niimi 1984). Briefly, groups of 40 fish were held in 300-L holding tanks supplied with aerated water at 10±1°C and fed ad libitum on alternate days.

There were three sets of experiments and the experimental procedures have been described in detail previously (Lowe-Jinde and Niimi 1984). Fish were exposed to 36 ug Cd/L for 1, 2, 3 and 42 days, to 12 ug Cd/L for 7, 14 and 21 days, to 4 ug Cd/L for 14, 28, 56 and 84 days. Corresponding groups of control fish were also maintained under the same conditions. Ten exposed and five

control fish were sampled at each interval in each of the experiments except fish at 36 ug Cd/L where five fish were sampled after 42 days. The feeding regime was identical for both exposed and control fish. Food was withheld at least 48 h. prior to sampling. Also, fish in 36 ug Cd/L were not fed during the first three days of cadmium exposure.

After the specific cadmium exposure, individual fish was anesthesized with 200 mg/L tricaine methane sulphonate (MS-222) and a blood sample taken from the caudal vein within 1 min. After blood was removed, the fish was killed and spleen removed, blotted dry and weighed. Counts of total erythrocytes and leucocytes were made using a Neubauer haemocytometer. Blood was diluted 1:100 using a modified Rees-Ecker solution (Wedemeyer and Yasutake 1977). Both erythrocyte and leucocyte counts were made from the same slide preparation. Blood smears were fixed in methanol and later stained with Leishman Giemsa stain for differential cell ratio counts. A minimum of 200 erythrocyte and leucocyte cells were counted under oil immersion according to the description of Lehmann and Sturenburg (1975). Erythrocytes were identified as mature erythrocytes, erythroblasts including proerythrocytes, or degenerating erythrocytes (stages I-IV), and leucocytes according to types characterized as lymphocytes, thrombocytes, or granulocytes including granuloblasts. The number of each erythrocyte cell stage and leucocyte cell type was estimated for each fish by multiplying the total cell count by the differential cell ratio. Analysis of variance (ANOVA) was used to test for statistically significant differences of the measured parameters.

RESULTS and DISCUSSION

Cadmium concentrations in the low, medium and high exposure tanks were 4.4 ± 1.1 (N=8), 12.6 ± 1.5 (N=4), 36.5 ± 3.5 (N=9) ug Cd/L respectively which showed good agreement with intended concentrations at a dilution of 1:4000 as previously reported (Lowe-Jinde and Niimi 1984). Mortality occurred only among the 40 fish exposed to the 36 ug Cd/L cadmium concentration. Two fish died after 5 and 22 days exposure. Fish weight averaged 135 ± 28 g and 110 ± 24 g for control and exposed fish at 4 ug Cd/L, 89 ± 22 g for control and 111 ± 24 g for exposed fish at 12 ug Cd/L and 158 ± 30 g for control and 159 ± 27 g for exposed fish at 36 ug Cd/L respectively. There were no significant differences in weight between exposed and control fish sampled on the same day at all exposure levels.

The spleen somatic index (SSI = spleen weight x 100/body weight) was not significantly changed in the control or exposed fish at each sample interval. The SSI averaged 0.13 ± 0.05% for control fish and 0.14 ± 0.05% for exposed fish of 4 ug Cd/L, 0.19 ± 0.14% for control and 0.2 ± 0.12% for exposed fish at 12 ug Cd/L, and 0.11 ± 0.03% for control and 0.11 ± 0.03% for exposed fish at 36 ug Cd/L. However, a decline in erythrocyte counts was suggested with prolonged cadmium exposure and was significantly reduced ($P<0.05$)

after 21 days at 12 ug Cd/L (Table 1). The total erythroblast-proerythrocyte abundance was not decreased on days 1 and 3 but their abundance was significantly reduced on days 2 ($P<0.05$) and 42 ($P<0.01$) at 36 ug Cd/L (Table 1). The observed decrease in erythrocytes is consistent with the previous report of anemia in cadmium exposed fish (Sjobeck et al 1984). The mild anemia was probably not due to increased destruction of erythrocytes since the spleen size was unchanged but might be due to a decrease in synthesis or release of erythrocytes into the circulation. The observed reduction in the number of erythroblasts-proerythrocytes would suggest a reduction in erythropoiesis. It was also suggested in the recent report by Houston and Keen (1984) that cadmium impeded the formation of red cell in goldfish, Carassius auratus. Also the accumulation of cadmium in various organs particularly the kidney and liver has been observed (Kumada et al 1980), and it is conceivable that the activity of these haemopoietic tissues may be suppressed. Furthermore, cadmium-induced anemia appears to be associated with a defect in iron metabolism caused by a deficiency in intestinal absorption (Richardson et al 1974).

In Table 2 at 4 ug Cd/L on day 14, total leucocytes were significantly increased ($P<0.05$) compared to control values while on day 56 the leucocytes were significantly decreased ($P<0.01$) compared to control values. At 36 ug Cd/L leucocytes decreased compared to control values on day 3 ($P<0.01$). Subsequently, values returned to control levels after 42 days of exposure. Also, comparisons of differential leucocyte abundance between controls and exposed fish on a given day indicated that lymphocytes significantly increased ($P<0.05$) on day 14 and then decreased ($P<0.01$) on day 56 at 4 ug Cd/L. Lymphocytes were also significantly reduced ($P<0.05$) on day 3 at 36 ug Cd/L. In addition, granulocyte abundance was increased in fish exposed to 36 ug Cd/L and was significantly increased ($P<0.01$) on day 2 compared to control values. After 42 days at 36 ug Cd/L, the granulocytes returned to control values. There were some changes in the total and differential cell counts among the sample days in the controls and so the effects of individual fish variability and/or undetermined environmental changes cannot be discounted in this study.

A variety of stressors including cold shock, social stress and pollutants result in moderate to severe leucopenia. In this study the leucopenia seemed to be accompanied by lymphopenia and granulocytosis. These secondary stress responses are probably partly mediated by increased pituitary-interrenal activity (Donaldson 1981; Wedemeyer and McLeay 1981). It has also been reported that the effects of stress and corticosteroid may vary depending on the physiological state of the fish such that cortisol may cause either lymphocytosis or lymphopenia in Fundulus heteroclitis (Pickford et al 1971). Thus, the presently observed temporary leucopenia, lymphopenia and granulocytosis may represent secondary response to stress.

Table 1. Cadmium effects on total and differential erythrocyte counts in trout. Results are expressed as mean values ± SD. Days of exposure within parentheses. Significant differences at $P < 0.05$ (*) or $P < 0.01$ (**).

	Erythro. x $10^6/mm^3$	Mature Erythro. x $10^6/mm^3$	Degen. Erythro. x $10^4/mm^3$	Erythrobl. Proerythro. x $10^4/mm^3$
		Exposure to 4 ug Cd/L		
Control (14)	1.26±0.12	1.16±0.12	6.15±3.73	4.34±2.61
Exposed (14)	1.38±0.16	1.30±0.18	4.65±5.22	3.54±2.66
Control (28)	1.18±0.13	1.08±0.11	4.28±2.24	5.12±6.17
Exposed (28)	1.07±0.21	1.01±0.20	3.11±2.06	3.02±3.38
Control (56)	1.01±0.13	0.88±0.12	7.20±1.73	5.62±2.31
Exposed (56)	1.02±0.21	0.84±0.17	6.98±3.36	10.36±5.32
Control (84)	1.15±0.18	0.97±0.12	8.50±5.74	9.31±1.00
Exposed (84)	1.03±0.11	0.88±0.12	9.23±4.26	6.70±3.20
		Exposure to 12 ug Cd/1		
Control (7)	1.22±0.30	1.12±0.32	3.26±2.52	6.90±2.99
Exposed (7)	1.09±0.21	1.01±0.21	3.60±2.12	4.66±4.20
Control (14)	1.19±0.20	1.11±0.15	2.12±2.10	6.02±6.98
Exposed (14)	1.03±0.17	0.92±0.17	2.57±2.21	7.80±4.48
Control (21)	1.25±0.22	1.18±0.27	2.25±2.60	3.16±4.24
Exposed (21)	1.02±0.14*	0.93±0.14	2.78±3.05	6.98±4.59
		Exposure to 36 ug Cd/L		
Control (1)	1.36±0.19	1.19±0.18	8.86±2.77	8.44±4.85
Exposed (1)	1.22±0.20	1.09±0.19	5.16±2.72*	7.73±4.91
Control (2)	1.19±0.15	1.06±0.13	5.66±2.97	7.32±0.62
Exposed (2)	1.30±0.21	1.20±0.21	4.71±1.69	5.05±2.62*
Control (3)	1.30±0.08	1.13±0.11	8.20±3.24	8.25±5.95
Exposed (3)	1.23±0.17	1.08±0.16	6.08±2.31	8.67±3.97
Control (42)	1.15±0.18	0.97±0.12	8.50±5.73	9.42±0.82
Exposed (42)	0.94±0.19	0.84±0.17	5.97±3.22	4.10±2.08**

Table 2. Cadmium effects on total and differential leucocyte counts in trout. Results are expressed as mean values ± SD. Days of exposure within parentheses. Significant differences at $P < 0.05$ (*) or $P < 0.01$ (**) level.

	Leuco. $\times 10^4/mm^3$	Lympho. $\times 10^4/mm^3$	Thrombo. $\times 10^3/mm^3$	Granulo. $\times 10^3/mm^3$
		Exposure to 4 ug Cd/L		
Control (14)	3.93±0.72	3.53±0.98	2.94±2.13	1.06±1.10
Exposed (14)	6.27±1.48*	5.98±1.62*	2.32±3.43	0.81±0.78
Control (28)	4.16±1.10	3.89±1.06	1.95±1.00	0.71±0.57
Exposed (28)	5.08±1.41	4.93±1.38	1.03±1.73	0.43±0.28
Control (56)	5.83±1.42	5.43±1.43	2.86±2.25	1.16±1.04
Exposed (56)	3.68±0.92**	3.47±0.81**	1.55±1.23	0.64±0.58
Control (84)	3.81±1.13	3.14±0.88	6.26±4.09	0.48±0.24
Exposed (84)	4.96±1.69	4.38±1.62	4.89±3.96	0.91±0.67
		Exposure to 12 ug Cd/L		
Control (7)	3.49±0.36	3.03±0.46	3.54±1.74	1.06±0.27
Exposed (7)	4.29±0.88	3.79±0.94	3.62±2.80	1.39±1.09
Control (14)	3.78±0.56	2.98±0.77	4.57±2.89	3.42±4.16
Exposed (14)	4.06±1.01	3.46±0.85	4.14±2.55	1.77±1.29
Control (21)	4.95±1.73	4.25±1.78	5.11±1.80	1.86±0.65
Exposed (21)	3.95±0.60	3.57±0.58	2.07±1.44**	1.71±0.84
		Exposure to 36 ug Cd/L		
Control (1)	4.53±1.35	4.14±1.46	3.04±2.17	8.30±5.30
Exposed (1)	4.54±1.44	3.93±1.34	5.10±4.49	10.59±12.54
Control (2)	3.78±0.66	3.26±0.65	5.46±1.17	1.90±0.84
Exposed (2)	3.90±0.49	3.07±0.77	6.83±4.43	12.28±7.43**
Control (3)	4.33±0.55	3.31±0.41	9.48±4.22	11.98±13.29
Exposed (3)	2.83±0.90**	2.14±1.14*	5.65±3.64	20.57±26.24
Control (42)	3.84±1.11	3.05±0.94	6.28±4.07	4.89±2.42
Exposed (42)	4.23±1.44	3.49±1.30	6.69±3.18	6.78±7.00

The lymphocytes of fish have been regarded as immunocompetent cells (Ellis 1976), and in higher vertebrates, immunological responses have been shown with cadmium. In rats, cadmium suppressed antibody production. This could be partly due to the decrease in number of the B type lymphocytes which differentiate into antibody-producing cells (Koller and Brauner 1977). Also, in brown trout (Salmo trutta), the antibody titer was decreased after cadmium treatment (O'Neill 1981). It is, therefore, suggested that the presently observed leucocytic and lymphocytic changes may be associated with an immune response induced by cadmium.

Previous efforts to enumerate erythrocytes and leucocytes in fish to assess the sublethal effects of contaminants such as mercury and chlorobenzenes at concentrations approaching environmental levels have shown no significant changes in cell ratios (Niimi and Lowe-Jinde 1984). However, present work observed some changes in erythrocytes and leucocytes in cadmium exposed trout.

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