Aquat. Living Resour., 1989, **2**, 169-174

Cellular changes in the blood and haemopoietic tissues of common carp exposed to sublethal concentration of ammonia

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Received September 9, 1988; accepted April 11, 1989.

Wlasow T., H. Dabrowska. *Aquat. Living Resour.*, 1989, **2**, 169-174.

Abstract

Carps, *Cyprinus carpio* L., were exposed to a sublethal concentration of ammonia NH_3 (0.102 ± 0.059 mg.dm^{-3}) during 3 weeks. The sensitivity of the blood picture and haemopoietic organs were evaluated. Ammonia intoxication resulted in erythroblastosis of circulating blood. Concurrently, a significant decrease in polychromatophilic erythroblasts in kidney and orthochromatic erythroblasts in spleen tissues were observed in exposed fish compared with controls. The relative proportion of erythrocytes in peripheral blood decreased and coincided with a highly significant ($p<0.01$) increase of erythrocytes in spleen imprints of intoxicated carps compared with controls. Peripheral leucopenia, mainly lymphopenia as an intoxication response, was observed. In the pronephric cells significant differences in the proportion of young and adult eosinophils and the proportion of plasmocytes with vacuolized cytoplasm were found between those animals exposed to the toxic stress and the controls. Thus, the examination of haemopoietic organs has a great potential in carp health assessment.

Keywords : Blood cells, haemopoietic tissues, sublethal ammonia concentration, carp.

Modifications cellulaires du sang et des tissus hématopoiétiques chez la carpe commune exposée à une concentration subléthale d'ammoniac.

Résumé

Des carpes, *Cyprinus carpio* L., ont été soumises à une concentration subléthale d'ammoniac NH_3 ($0,102 \pm 0,059$ mg.dm^{-3}) durant 3 semaines. La sensibilité des cellules du sang et des organes hématopoiétiques a été évaluée. L'intoxication par l'ammoniac se caractérise par une érythroblastose des cellules en circulation. Parallèlement, une diminution significative des érythroblastes polychromatophiles dans le rein et une baisse des érythroblastes orthochromatiques de la rate ont été observées chez les poissons exposés, comparées aux poissons non soumis à ces concentrations d'ammoniac. La proportion d'érythrocytes du sang périphérique diminue et coïncide avec une augmentation significative ($p<0,01$) des cellules de la rate des poissons intoxiqués. L'intoxication se traduit par une leucocytose et principalement par une lymphocytose. Dans le pronéphron, les différences de proportion de leucocytes éosinophiles jeunes et adultes diffèrent de façon importante, ainsi que le pourcentage de plasmocytes dont le cytoplasme comporte des vacuoles. L'examen des organes hématopoiétiques présente donc un intérêt potentiel pour déterminer l'état de santé des carpes.

Mots-clés : Cellules sanguines, tissus hématopoiétiques, concentration subléthales d'ammoniac, carpe.

INTRODUCTION

The response of fish to many external factors normally includes changes in haematological parameters (Golovina *et al.*, 1977; Hickey, 1976; Hoffmann and Lommel, 1984). However, more specific responses are required which will challenge classical haematology techniques and allow the development of alternative methods to assess responses to environmental or internal factors. It has been observed that the peripheral blood picture has low specificity as an indicator of ammonia intoxication in fish (Dabrowska and Wlasow, 1986; Metelev *et al.*, 1971; Waluga and Flis, 1971). The haemopoietic role of the kidney and spleen has been recognized in fish for a long time (Fijan, 1964), but since the counting techniques are laborious, they are only occasionally used in fish pathology and toxicology (John and Mahajan, 1979; Peters and Schwarzer, 1985; Wlasow, 1984, 1985). Information is particularly needed concerning the effect of sublethal exposure to toxicants which may influence indirectly fish health, immunological response, production capacity, etc. The present study is aimed at identification of quantitative and qualitative changes in peripheral blood and in basic haemopoietic carp organe when fish were exposed to a common toxicant of fish culture systems, ammonia, at sublethal level.

MATERIAL AND METHODS

In addition to the results presented here, this experiment also provided data on certain biochemical and haematological indicators in carp after sublethal intoxication (Dabrowska and Wlasow, 1986). The experimental carp, *Cyprinus carpio* L.,· were healthy and in good condition, with an average weight 116 ± 19 g. The fish were acclimated to the experimental aquarium for 2 weeks in 250 dm^3 volume of water at 19°C. Before the trial and during the experiment, fish were fed with pellets containing 38% protein.

An open circulation of water with constant aeration and thermoregulation was employed. During the experiment, oxygen content, pH and temperature of the water were measured daily. Ammonia concentration was determined every second day. The level of un-ionized ammonia was calculated according to Emmerson *et al.* (1975). The results are presented in *table* 1.

Table 1. — Average values of water characteristics monitored during the experiment.

Parameters	Control	Experimental
Temperature (°C)	19.20 ±0.6	19.00 ±0.8
Oxygen content ($mg.dm^{-3}$)	6.80 ±0.4	7.08 ±0.2
pH	7.59 ±0.1	7.50 ±0.08
N/NH_4 ($mg.dm^{-3}$)	0.35 ±0.08	7.91 ±4.58
NH_3 calculated ($mg.dm^{-3}$)	0.004±0.002	0.102±0.059

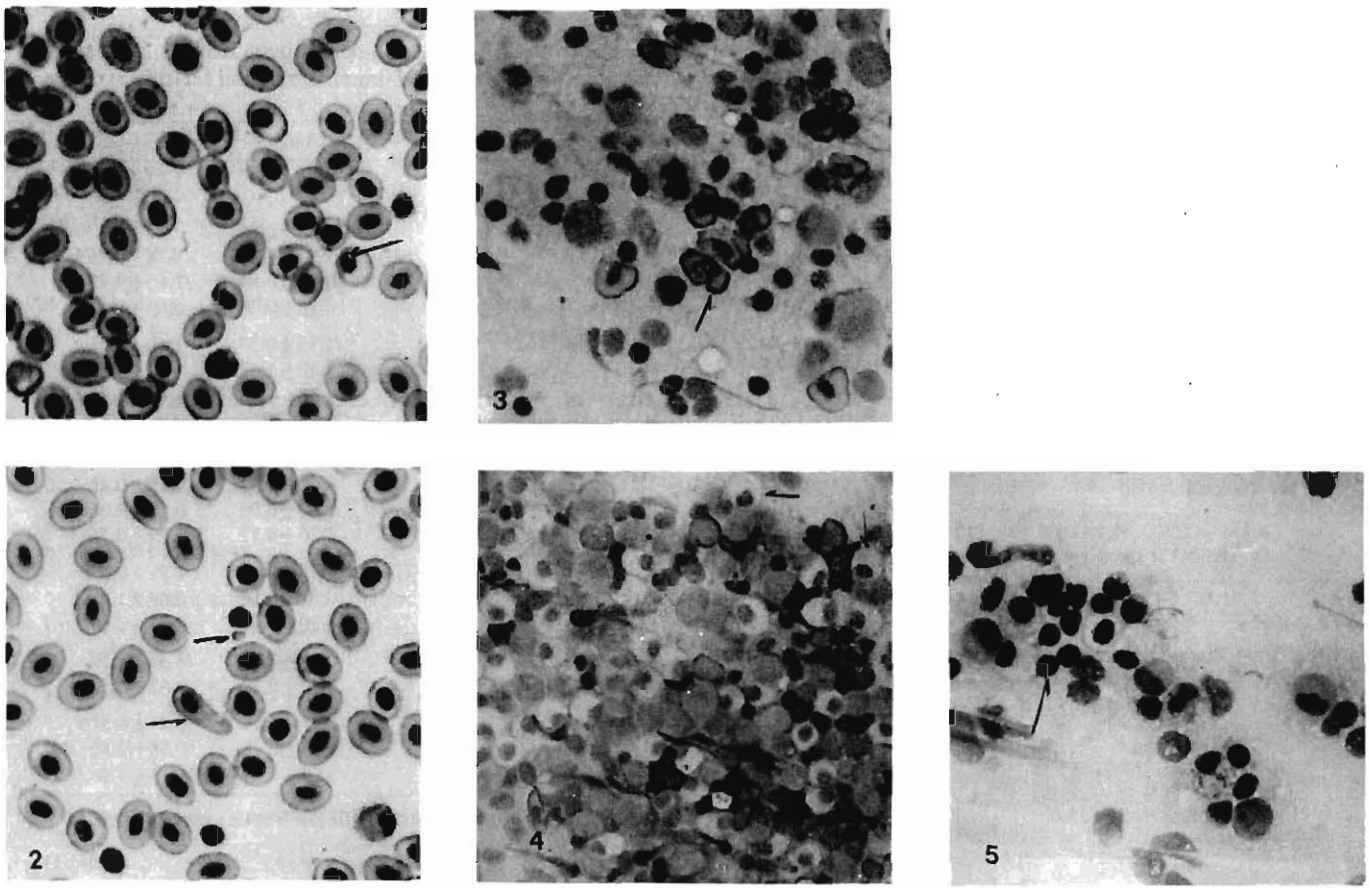
Two groups of fish (12 and 12) were exposed for a period of 3 weeks to a sublethal concentration of ammonia NH_3 (0.102 ± 0.059 $mg.dm^{-3}$). The control fish also consisted of two groups. A solution of ammonium chloride (reagent grade) was added to the water using fixed-speed pumps as the water entered the test aquaria. After completion of a 3 weeks long trial, blood was collected from the caudal vessels and haematological parameters were determined using standard methods (Blaxhall and Daisley, 1973; Klontz and Smith, 1968). The number of trombocytes was not included in the leucocyte counts. The haemopoietic organs, kidney and spleen were collected from 12 exposed and 12 control fish. Imprints from individual fish tissues were made on alcohol cleaned slides and stained with May-Grunwald-Giemsa (MGG). Differential cell counts of the imprints were made under a 900× magnification. About 500-600 cells from two slides for every fish were counted and the results were expressed as percentages of the total number of both red and white blood cells present. Cells were identified according to Fijan's terminology (1964). The term "Mott's cells" is used for plasma cells with multiple vacuoles (Baranski *et al.*, 1962; Bessis, 1972). The results were subjected to statistical analysis with Student's *t*-test.

RESULTS

A noticeable increase in the proportion of erythroblasts was observed in the peripheral blood of exposed fish compared with the control group (*table* 2), whereas the total red blood cell count was slightly lower in the exposed group. This erythroblastosis was due to polychromatophilic forms. In several erythroblasts, a disturbance of the cytoplasmatic structure and a shift in the position of the nucleus were noted (*fig.* 1 and 2). The number of leucocytes was significantly lower in the exposed group compared with the controls (*table* 2), and leucopenia was associated with relative eosinophilia and lymphocytopenia. The mean numbers of eosinophils in the blood of exposed and control carp were 843.5 and 895.3 mm^{-3} respectively. Differences in the proportion of neutrophils were insignificant.

Sublethal exposure to ammonia had no significant effect ($p < 0.05$) on white blood cells in the spleen of common carp (*table* 3). However, exposure resulted in pronounced changes in the red blood cell system, particularly a decrease in the proportion of orthochromatic erythroblasts, with a simultaneous increase in the erythrocytes (*table* 3).

In the pronephros of carp exposed to ammonia, the quantitative changes were more extensive (*table* 4) than in the spleen. Among the erythroblastic cells the proportion of polychromatophilic cells decreased significantly, whereas an increase of proerythroblasts was insignificant.



(*Photos J. Waluga*)

Figure 1. – The blood of common carp after sublethal exposure to ammonia; erythroblastosis, structural changes in cytoplasm of red blood cells, (→) dyslocated nuclei. MGG × 1 008.

Figure 2. – The blood of common carp after sublethal exposure to ammonia: erythroblastosis, schizocytes (→), erythrocyte with pycnotic and dyslocated nucleus (→) source for schizocytes and microcytes. MGG × 1 109.

Figure 3. – The head kidney of common carp after sublethal exposition to ammonia; irregular forms of red blood cells (→), some nuclei with lytic changes. MGG × 1 109.

Figure 4. – The head kidney of common carp after sublethal exposure to ammonia: Mott's cells-plasmocytes with vacuolized cytoplasm (→). MGG × 806.

Figure 5. – The head kidney of common carp after sublethal exposure to ammonia; group of plasma cells, note vacuolization and multiple naked nuclei (→). MGG × 1 008.

Irregular forms of red blood cells (*fig.* 3), with nuclei showing lytic changes or dislocation, were observed in exposed fish kidney imprints. White blood cells in this tissue exhibited a decreasing proportion of eosinophils from 39.3% in control to 34.8% in exposed fish. This change was mostly due to a significant decrease in juvenile eosinophils despite an increase of adult eosinophils.

An increase in lymphoid and reticular cells was not significant in exposed fish (*table* 4). On the other hand, the proportion of plasmocytes with vacuolized cytoplasm, so called Mott's cells (*table* 4, *fig.* 4 and 5) was significantly higher. In addition to the vacuolisation of cytoplasm, multiple naked nuclei were observed (*fig.* 5).

DISCUSSION

The sublethal ammonia concentration resulted in disorder in erythropoiesis of the common carp. In peripheral blood, it was manifested by erythroblastosis, with the same polychromatophilic character as in two-year carp under the influence of ammonia water (Waluga and Flis, 1971). This phenomenon may be an indication of erythrocyte renewal (Waluga and Flis, 1971; Dabrowska and Wlasow, 1986). However, such a conclusion would not be fully justified without information from the erythropoietic tissue. In the present study, there was a significant decrease in the number of immature red blood cells: orthochromatic erythroblasts in spleen and polychromatophilic erythroblasts in the head kidney were

Table 2. — Some haematological parameters of common carp after sublethal exposure to ammonia.

Parameters	Control	Exposed
Erythrocyte count (10^6.mm^{-3})	1.23±0.07	1.12±0.06
Erythroblast total (%)	11.52±0.91	24.70±3.50 (**)
Paraerythroblast (%)	0.00±0.00	0.12±0.07
Erythroblast		
Polychr. (%)	7.13±0.88	20.36±3.56 (**)
Orthochr.	4.48±1.17	4.25±0.61
Erythrocyte (%)	88.39±1.14	75.27±3.49 (**)
Leucocyte count (10^3.mm^{-3})	34.70±3.41	18.10±1.48 (**)
Red: white cell ratio	39.00±4.22	64.00±4.53 (**)
White blood cells (%):		
Lymphocyte	92.58±1.00	89.00±1.13 (*)
Eosinophil	2.58±0.66	4.66±0.70 (*)
Neutrophil-band form	0.83±0.38	0.58±0.23
Neutrophil-segmented form	1.04±0.20	0.75±0.25

Mean+S.D.; (*) significantly different from control ($0.01<p<0.05$); (**) significantly different from control ($p<0.01$).

Table 3. — Percentage composition of both red and white blood cells from the spleen of common carp after sublethal exposure to ammonia.

Cells	Control	Exposed
Procrythroblast	0.90±0.41	0.46±0.76
Erythroblast		
Basophil	1.43±0.39	1.05±0.79
Polychr.	9.75±5.97	10.95±7.47
Orthochr.	56.35±3.65	6.25±10.00 (**)
Erythrocyte	3.43±2.24	57.08±10.61 (**)
Myeloblast	0.48±0.39	0.52±0.34
Myelocyte	0.38±0.29	0.25±0.15
Neutrophil-band form	0.00±0.00	0.05±0.12
Neutrophil-segmented form	0.02±0.06	0.05±0.12
Eosinophil	1.08±0.39	1.71±0.97
Monoblast	0.00±0.00	0.02±0.05
Promonocyte	0.02±0.06	0.12±0.14
Monocyte	0.33±0.16	0.28±0.41
Lymphoblast	0.90±0.44	0.67±0.61
Prolymphocyte	7.35±3.51	4.19±2.58
Lymphocyte	13.78±5.02	10.27±2.98
Reticular cell	3.20±1.18	2.86±3.13
Macrophage	0.38±0.21	2.02±1.56

Mean±S.D.; (**) significantly different from control ($p<0.01$).

observed in exposed carp. It reflects attenuation of regeneration in the red cell system rather than restoration under the influence of ammonia. A similar decrease of polychromatophilic erythroblasts in the pronephros along with some disorders in the spleen took place in phenol exposed trout (Wjasow, 1984). But the depletion of procrythroblasts in the head kidney of these trout was the most important change. It led to the supposition that kidney disorders were more fundamental than those in the spleen. A comparison of the changes in both haemopoietic organs in exposed carp suggests a similar conclusion. However, erythroblast decrease in the spleen of trout exposed

Table 4. — Percentage composition of both red and white blood cells from the head kidney of common carp after sublethal exposure to ammonia.

Cells	Control	Exposed
Procrythroblast	0.65±0.32	1.27±0.74
Erythroblast		
Basophil	9.33±1.83	7.07±4.12
Polychr.	15.90±6.46	4.34±3.75 (**)
Orthochr.	10.55±2.89	11.67±3.29
Erythrocyte	0.30±0.20	0.69±0.49
Myeloblast	1.35±0.33	1.60±1.01
Promyelocyte	3.40±1.62	1.47±0.83
Myelocyte	1.38±1.03	1.45±1.14
Eosinophil-young	35.05±5.86	20.45±6.47 (**)
Eosinophil-adult	4.25±1.82	14.38±7.99 (**)
Monoblast	0.08±0.09	0.18±0.25
Promonocyte	0.00±0.00	0.18±0.25
Monocyte	0.20±0.17	0.29±0.26
Lymphoblast	1.28±0.76	1.71±0.62
Prolymphocyte	0.98±0.56	2.09±1.13
Lymphocyte	6.20±2.76	9.67±2.69
Reticular cells	2.30±0.85	4.53±1.29
Macrophages	0.33±0.33	0.82±0.51
Plasmoblast	0.53±0.19	0.44±0.37
Plasmocyte	0.93±0.48	0.58±0.42
Mott's cell	5.05±1.71	15.11±4.06 (**)

Mean±S.D.; (**) significantly different from control ($p<0.01$).

to stress was more pronounced in comparison with kidney (Peters and Schwarzer, 1985). On the other hand, an increase in erythrocyte number and a decrease in orthochromatic erythroblasts in the spleen of exposed carp (*table* 3) could be explained as functional disorders in this organ. Fish spleen with such disorders or under stress may be overloaded with old erythrocytes displacing immature cells-erythroblasts (Benfey and Sutterlin, 1984). Thus, the carp spleen worked as a resorvoir of adult erythrocytes under sublethal ammonia exposure.

The effects of ammonia were manifested also in changes of the quantitative composition of white blood cells in the peripheral blood and haemopoietic organs of carp. Similar lymphocytopenia and leucopenia have been observed in fish from other toxicological experiments (Waluga and Flis, 1971; Piotrowska 1980). Such changes in the blood picture are recognised as effects of stress on fish immune systems (Peters and Schwarzer, 1985; Angelidis *et al.*, 1987). On the other hand ammonia is recognised as an agent, causing stress in fish by changing their metabolic processes (Nemcsok *et al.*, 1984).

The increased proportion of lymphocytic series cells in the head kidney of exposed carps was not significant (*table* 4), but a large increase in the number of vacuolized forms of plasmocytes—Mott's cells—was observed. Similar pathological forms of plasmocytes were noticed during the course of certain infectious diseases with toxication in humans (Aleksandrowicz

and Lisiewicz, 1975). Strong vacuolization in cytoplasm of the macrophage-like cells within haemopoietic organs were detected at trout under influence of stress (Peters and Schwaizer, 1985).

In our experiment, we observed an increase in the proportion of eosinophils in the peripheral blood of carp. But this increase cannot be regarded as a reactive eosinophilis because the number of these cells per mm^3 of blood was reduced. Additionally, an assessment of granulopoietic processes in fish, can be observed in haemopoietic organs. A decrease in the proportion of young eosinophils in the head kidney indicates reduced regeneration of these cells (*table* 4). A high proportion of adult eosinophils in this organ in exposed carp suggests granulopoiesis disorder because, in contrast to mammals, haemopoietic tissue of healthy carp does not enclose large masses of adult granulocytes. These cells are gradually and slowly supplied to the periphery (Yasuda *et al.*, 1984). Perhaps this supply is changed in conditions of ammonia exposure or in pathological situations in which eosinophils can invade certain tissues (Ellis, 1982).

No changes in the proportion of neutrophils within the blood of exposed carp were observed. In contrast, Waluga and Flis (1971) reported a rise of neutrophil number in the blood of carp exposed to ammonia water. Similar neutrophilia took place in carp blood in acute toxicity of ammonia when ammonium nitrate solution was used (Wlasow and Dabrowska, manuscript in preparation). Neutrophils are less frequent in the blood of healthy carp in comparison with eosinophils, but their number can rise in the peripheral blood of inflamed carp (Yasuda *et al.*, 1984).

Increase of neutrophil number in the spleen of exposed carp was adventicious. We were unable to detect neutrophils in the pronephric cells of carp from both groups, control and exposed, despite the fact that large numbers of granulocytes in this organ in carp are characterized as neutrophils (Bayne, 1986; Temmink and Bayne, 1987). However, all granulocytes observed in the head kidney of trout controls and those exposed to phenol had a neutrophilic character and constitued 17-18% of total cells (Wlasow, 1985). The type and proportion of leucocytes in the blood and haemopoietic tissues of fish can change in response to environmental fluctuations. Different results may be obtained depending upon the methods used and the species examined.

Acknowledgements

We would like to thank Prof. K. Dabrowski, School of Natural Resources, The Ohio State University, 2021 Coffey Rd. 210, Columbus, Ohio 43210-1085 (USA), for critical reading of the first draff. We thank Mrs. I. Morawska and eng. E. Ziomek for technical help. This research was supported by the Inland Fisheries Institute (State Programme PR-4), Ministry of Agricultuure, Poland.

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