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Hematology

ERYTHROCYTE SYSTEM OF RAINBOW TROUT, *SALMO GAIRDNERI* RICH. AFFECTED BY PROLONGED SUBACUTE PHENOL INTOXICATION

UKŁAD CZERWONOKRWINKOWY PSTRĄGA TĘCZOWEGO *SALMO GAIRDNERI* RICH. W PRZEDŁUŻONEJ PODOSTREJ INTOKSYKACJI FENOLOWEJ

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Basic erythrocyte indices, erythrocyte osmotic resistance as well as quantitative and qualitative analysis of the erythrocyte pattern in the peripheral blood, spleen and kidney of rainbow trout affected by phenol intoxication (2 mg dm^{-3} doses applied for 2 months in winter) are presented.

INTRODUCTION

Phenol although present in water bodies in concentrations lower than lethal, is rather common. Dougharty (in Metelev et al., 1971) has regarded the compound as a strong toxin. It brings about a general intoxication with no specific symptoms; its action is both local – corrosive or irritating, depending on concentration – and systemic (Reichenbach-Klinke, 1965; Waluga, 1975). The coumpound causes the fish nerval system disfunction (Flerow, 1970; Gdovskij et al., 1974; Lukjanenko, 1974). By damaging blood cells it

affects the abundance of various types of blood cells (Halsband and Halsband, 1963; Waluga, 1975; Własow, 1980).

In the course of chronic phenol intoxication in fish, juvenile erythrocytes in the peripheral blood were found to occur in increased abundance (Waluga, 1975), which points out to intensified erythropoiesis in phenol-intoxicated fish. In the present work, the rainbow trout erythrocyte pattern was studied in order to find out if prolonged subacute intoxication results in impaired or intensified erythropoiesis.

MATERIALS AND METHODS

The materials studied consisted of 196 rainbow trout: (*Salmo gairdneri* Rich.) individuals aged 1+. The fish originated from the Szwaderki Fish Farm and were in good condition. The mean length and weight were 22.6 cm (s_x = 1.9 cm) and 123.4 g (s_x = 30.4 g), respectively.

Pure grade phenol produced by the Oświęcim Chemical Plants was used in the experiments.

The experiments were carried out in 250 dm^3 aquaria fed with aerated lake water (Table 1). Water aeration, temperature, and pH were checked daily; the results of measurements are presented in Table 2.

Based on preliminary tests, the phenol concentration and exposition were set at 2 mg dm^{-3} and 2 months, respectively. The experimental solution was obtained by diluting the fresh stock solution; it was changed daily. The experiment was carried out in winter (December 1978 – February 1979) as the rainbow trout is then more resistant to phenol (Własow, 1980) and a more pronounced response from the hemopoietic system can be expected.

The rainbow trout were adapted to conditions in the aquaria for 2 weeks. The experimental and control groups consisted of 112 and 84 individuals, respectively, 14 individuals per aquarium. The fish were fed with pelleted food.

On the outset of the experiment, blood for hematologic assays was collected from the heart. Also the hemopoietic organs: spleen and kidney, were examined. The following assays were performed: erythrocyte count and hemoglobin content, by means of procedures generally applied in fish studies (Klontz and Smith, 1968; Blaxhall and Daisley, 1973); hematocrit (relative erythrocyte volume), by micromethod in heparin capillaries (Krawczyński and Osiński, 1967); mean hemoglobin content (MCH), mean corpuscles hemoglobin content (MCHC), and mean cell volume (MCV), calculated with Wintrobe's formulae (Krawczyński and Osiński, 1967).

Erythrocyte osmotic resistance was determined by acidic erythrograms modified for fish (Telitchenko and Govorova, 1962) in a Specol (λ = 580 nm). The osmotic resistance index was calculated according to Terskov and Gitelzon (1957).

The erythrocytes were examined in panchromatically stained (Pappenheim's MGG technique) smears. Percentages of various blood cells in the spleen and kidney were

Table 1

Chemical characteristics of the water used in experiment

N_{NH_4}	N_{NO_2}	N_{NO_3}	$Fe_{tot.}$	SiO_2	P_{PO_4}	Cl	SO_4	CO_3	HCO_3	Ca	K	Na	Oxidability
mg/dm^3													mg O_2/dm^3
0.12	0.016	0.54	0.29	24.5	0.061	12.2	9.7	0.0	219	49	3.1	9.3	10.6

Table 2

Oxygen content, temperature and pH of the water

Groups	Oxygen content mgO_2/dm^3		Water temperature °C		Water pH	
	RANGE	MEAN	RANGE	MEAN	RANGE	MEAN
Exp.	5.4 – 10.8	8.3	6.0 – 14.5	9.3	7.2 – 7.6	7.4
Control	6.8 – 9.6	7.8	6.0 – 14.5	9.5	7.3 – 7.6	7.5

determined from prints stained as the smears. When calculating the per cent composition of blood cells in the spleen and kidney, 500–800 cells were examined, as in mammalians myelo- and splenograms (Barański et al., 1962).

The results were treated statistically by means of analysis of variance with Fisher's F test.

RESULTS

Peripheral blood

The erythrocyte count in the experimental fish averaged 1.05×10^6 mm^{-3}, which is by 5.4% lower than the average in the control (Table 3), the difference being statistically non-significant. On the other hand, the erythroblast proportion in the experimental fish peripheral blood was decidedly higher (11.5%) than that in the control (3.3%).

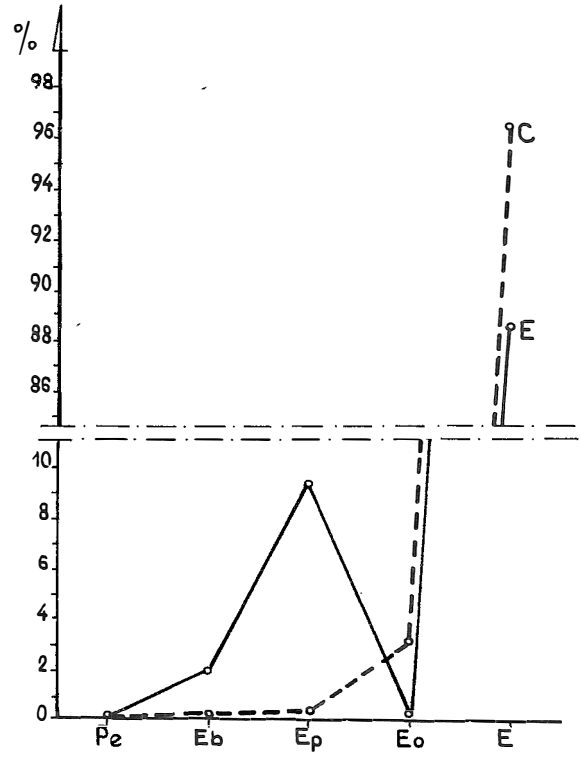

Fig. 1. The blood. Red blood cells maturity of rainbow trout (*S. gairdneri Rich.*) after 2 – month exposure to 2 mg/dm^3 of phenol. E – experimental, C – control. Pe – proerythroblasts, Eb – basoph. erythroblasts, Ep – polichromatophilic erythroblasts, Eo – orthochromerythroblasts, E – erythrocytes.

The predomination of juvenile erythrocytes evidences an intensified erythropoiesis. In the phenol-exposed rainbow trout the erythrocyte renewal was disturbed both quantitatively and qualitatively. The peripheral blood showed an out-of-proportion increase in the polychromatic erythroblasts and a decrease in ortochromatic erythroblasts (Fig. 1).

The erythrocytes occurring in the experimental fish blood were of different size (anisocytosis) and varied in shape (poikilocytosis) (Fig. 2). The cells underwent structural alterations resulting from degenerative changes. Cytoplasm was unevenly thickened (Fig. 3), with basophil granulations (Fig. 4), the nucleus frequently transferred to the cell

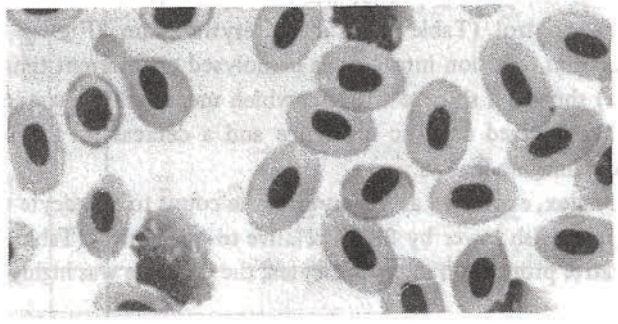

Fig. 2. The blood. Anisocytosis, poikilocytosis, erythrocytic shadows. MGG. x 1250

Phot. C. Nagięć

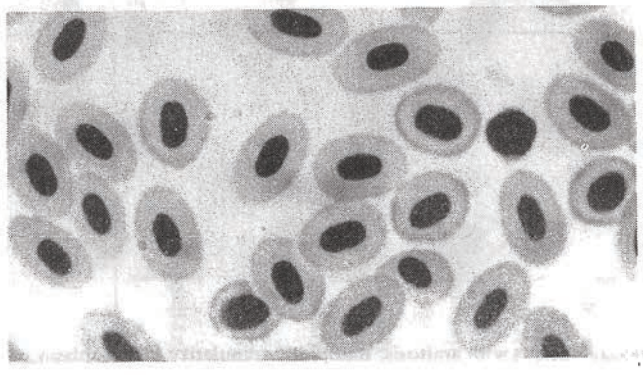

Fig. 3. The blood. Structural changes in cytoplasm of red blood cells. MGG.x1250

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margin (Fig. 2). Nuclei of altered erythroblasts and erythrocytes had thinned chromatin with a clear separation of basochromatin strands. That was a rather common process, although not a very intensive one. More rare were nuclei in the amitotic division, narrowed or segmented (Fig. 4). The phenol-exposed fish blood more frequently contained pre-hemolytic spherocytes and cellular shadows (Fig. 2).

The relative erythrocyte volume (hematocrit) of the experimental fish was 35.9%, i.e., by 11.6% lower than that of the control fish.

The hemoglobin concentration in the experimental fish was also significantly lower (by 13%) relative to the control (Table 3). Similarly, the mean cell hemoglobin content (MCH) decreased by 8.9% relative to the control. On the other hand, the mean corpuscle hemoglobin content (MCHC) and the mean erythrocyte volume (MCV) of the experimental fish showed no significant differences with the control values (Table 3).

The osmotic resistance of the phenol-intoxicated fish erythrocytes was by 7% lower than that in the control (Table 3). A mean erythrogram (Fig. 5) illustrating the erythrocyte population partition into groups hemolysed at different time (thus differing in their resistance) showed a shift to the left, which means an increase in the amount of erythrocytes with a lowered osmotic resistance and a decrease in the amount of those highly resistant to damaging factors.

The blood cell index, expressed as the erythrocyte count to leukocyte count ratio, was in the phenol-exposed fish lower by 28.3% relative to the control (Table 3), pointing out to a decreased relative proportion of erythrocytes; the decrease was highly significant.

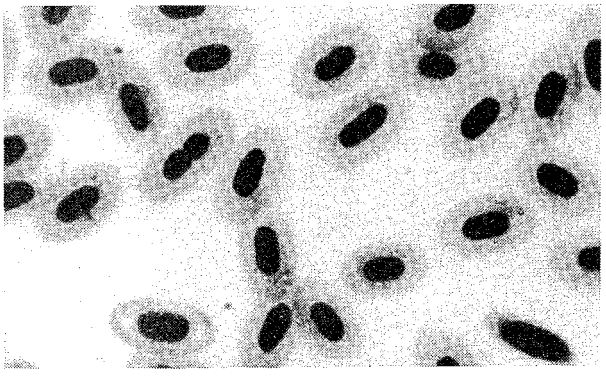

Fig. 4. The blood. Nucleus with amitosis. Basophilic granularity in cytoplasm of erythrocytes MGG. x 1250

Phot. C. Nagięć

Table 3

Haematological indices of rainbow trout (S. gairdneri Rich.) after 2-month exposure to 2 mg/dm^3 of phenol

Parameters	Experimental group		Control group		Significant + non-significant – differences	
	$\bar{x}$	$S_{\bar{x}}$	$\bar{x}$	$S_{\bar{x}}$		
Erythrocytes (x10^6/mm^3)	1.05	0.20	1.11	0.20	–	P = 0.05
Erythroblasts (%)	11.5	3.4	3.3	1.1	+	P < 0.01
Red: white cell ratio	231	193	322	183	+	0.01 < P < 0.05
Haematocrit – PCV (%)	35.9	4.8	40.6	4.4	+	P < 0.01
Haemoglobin (g %)	7.5	1.4	8.7	1.0	+	P < 0.01
MCH (pg)	72	12	79	14	+	P < 0.01
MCHC (%)	20.7	2.4	21.7	2.2	–	P = 0.05
MCV (μM^3)	351	63	369	63	–	P = 0.05
Index of osmotic resist. of erythrocytes	0.93	0.11	1.00	0.07	+	0.01 < P < 0.05

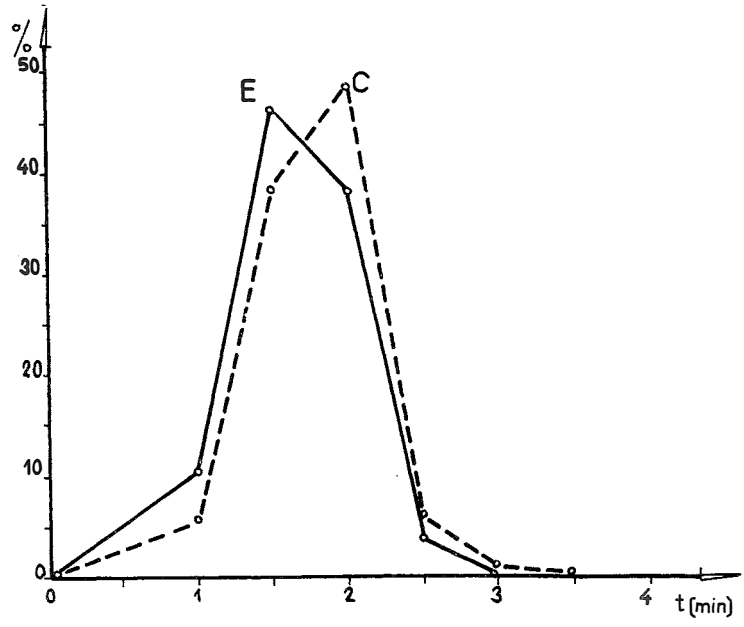

Fig. 5. Acidic erythrogramme of erythrocyte resistance of rainbow trout (*S. gairdneri* Rich.) after 2 – month exposure to 2 mg/dm^3 of phenol. E – experimental, C – control, t – time, % – of haemolysed erythrocytes

HEMOPOIETIC ORGANS

The spleen of the experimental fish showed the same proportion of proerythroblasts as that in the control (Table 4), the proportion of basophil erythroblasts being markedly higher (by 17%) than that in the control. The differences between the experimental and control fish in poly- and ortochromatic erythroblasts were non-significant.

The erythrocyte system maturity curve (Fig. 6) in the spleen of the intoxicated fish confirms the presence of intensified erythropoiesis with respect to basophil erythroblasts. This phenomenon can be accounted for by an intensified erythroblast amitosis accompanying mitotic divisions. However, apart from intensified erythropoiesis, the organ showed degenerative changes which resulted in the emergence of paraerythroblasts, atypical cells with narrowed parts and projections. Some of them were unusually large ellipsoid in shape, with obliterated margins. Paraerythroblast nuclei were atypical, often pear-shaped or excessively elongated (Fig. 7). Numerous erythroblasts showed a differential maturation of nuclei and cytoplasm, the nuclei being more mature than the cytoplasm.

In the kidney of the phenol-exposed fish, the proportion of paraerythroblasts decreased by 38.5% as compared with the control, the decrease being statistically significant (Table 4). No difference in basophil erythroblasts between the experimental and control fish was recorded. On the other hand, the proportion of polychromatic

Table 4

Red blood cells in the spleen and kidney of rainbow trout (S. gairdneri Rich.) after 2-month exposure to 2 mg/dm^3 of phenol. (In percentage of all cells)

Cells in the spleen:	Experimental group $\bar{x}$	Experimental group $S\bar{x}$	Control group $\bar{x}$	Control group $S\bar{x}$	Significant + non-significant – differences	
proerythroblasts	0.1	0.2	0.1	0.2	–	P = 0.05
alkal. erythrobl.	4.4	2.2	1.6	1.7	+	P < 0.01
polychr. erythrobl.	4.3	2.3	5.0	3.5	–	P = 0.05
orthochr. erythrobl.	1.1	0.8	1.5	1.7	–	P = 0.05
erythrocytes	21.7	8.3	24.9	8.2	–	P = 0.05
in the kidney:						
proerythroblasts	1.6	1.5	2.6	1.5	+	0.01 < P < 0.05
alkal. erythrobl.	4.4	3.1	4.0	2.5	–	P = 0.05
polychr. erythrobl.	4.6	3.0	6.8	4.7	+	0.01 < P < 0.05
orthochr. erythrobl.	2.7	1.8	1.8	0.7	+	0.01 < P < 0.05
erythrocytes	7.0	6.4	8.5	5.1	–	P = 0.05

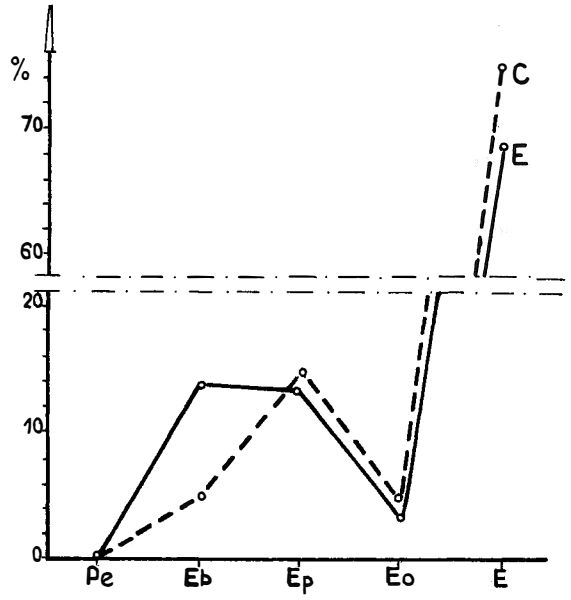

Fig. 6. The spleen. Red blood cells maturity of rainbow trout (*S. gairdneri* Rich.) after 2 – month exposure to 2 mg/dm^3 of phenol. E – experimental, C – control.
Symbols used as in fig. 1.

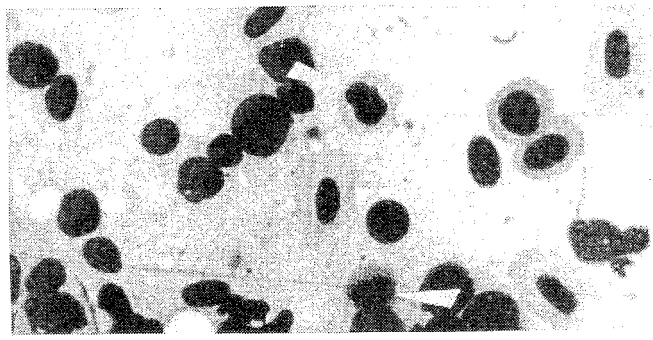

Fig. 7. The spleen. Paraerythroblastes with irregular nucleus. MGG. x 1250.

Phot. C. Nagięć

erythroblasts decreased by 32.4% in the intoxicated fish. Apart from a retarded erythropoiesis in the kidney with respect to proerythroblasts and polychromatic erythroblasts, the experimental fish showed a 50% increase in ortochromatic erythroblasts as compared to the control. The erythrocyte system maturity curve was irregular (Fig. 8), evidencing a deficit of maternal cells and polychromatic erythroblasts.

The erythrocyte pattern showed the presence of paraerythroblasts, as was the case in the experimental individuals spleen. Both these cells and erythroblasts showed unevenly thickened cytoplasm and displaced nucleus (Fig. 9). Moreover, vacuolar degeneration of the cytoplasm and nucleus was much more pronounced than in the spleen (Figs. 9, 10). A considerable amount of cells in mitosis showed lytic changes (Fig. 10).

DISCUSSION

The erythrocyte system of the rainbow trout subject to prolonged subacute intoxication showed a number of alterations of varying directions and intensity. Some of those alterations, such as the peripheral blood erythroblastosis or a strong increase in basophil erythroblast proportion in the spleen indicate an intensified erythropoiesis in organisms exposed to phenol. Such a non-specific reaction as the blood erythroblastosis was observed in phenol intoxications of bream (Waluga, 1966, 1975) and other species (Metelev et al., 1971). However, the kidney which is the main blood-producing organ in the rainbow trout (Fijan, 1961; Klontz, 1972; Ostroumova, 1957), showed a retarded erythropoiesis, particularly with respect to proerythroblasts, a stage very important for the process.

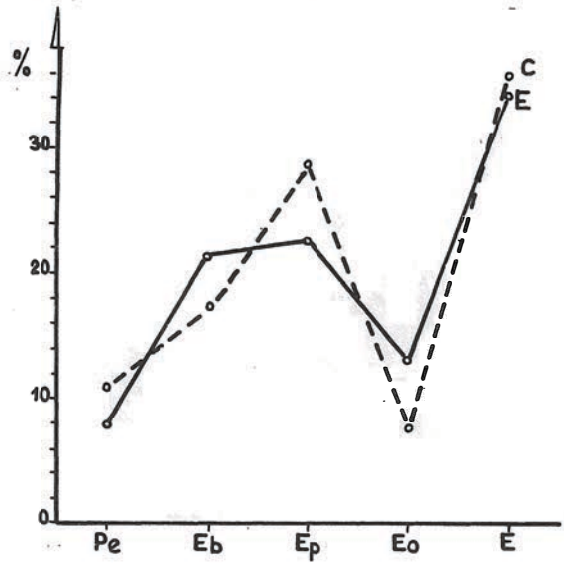

Fig. 8. The kidney. Red blood cells maturity of rainbow trout (*S. gairdneri* Rich.) after 2 – month exposure to 2 mg/dm^3 of phenol. E – experimental, C – control.

Symbols used as in fig. 1.

Apart from a deficit in maternal red cells in that organ, a significant reduction in polychromatic erythroblasts was observed, which generally gives a negative prognosis with respect to erythropoiesis in the phenol-exposed rainbow trout.

The kidney erythropoietic function, presumably impaired by phenol, was intercepted by the spleen which showed intensified erythropoiesis. This phenomenon confirms the hemopoietic function replacement between these two organs (Fijan, 1971). However, one should consider the spleen erythropoietic capacity in view of the blood-destroying processes operative in the blood itself and in the blood-producing organs. The processes were evidenced by more numerous spherocytes and cellular shadows in the experimental fish as well as by the decreased erythrocyte osmotic resistance typical of spherocytosis (Ławkowicz and Krzemińska-Ławkowiczowa, 1973).

A reduced erythrocyte osmotic resistance was observed in the rainbow trout exposed to sublethal and lethal phenol concentrations (Własow, 1980) and also in the fish exposed to radioactivity (Telitchenko and Govorova, 1962). The reduction of this parameter means an elevated susceptibility of the cells to hemolytic agents as well as changed physical and chemical structures of cell membranes.

Additionally morphotic (aniso-, poikilo-, and schizocytosis) and structural (degeneration and necrobiosis) changes were observed in the blood and in the hemopoietic organs. Those changes were more intense in the kidney, which would explain the retarded erythropoiesis in the organ.

The blood-destroying effects of phenol are further evidenced, apart from morphotic

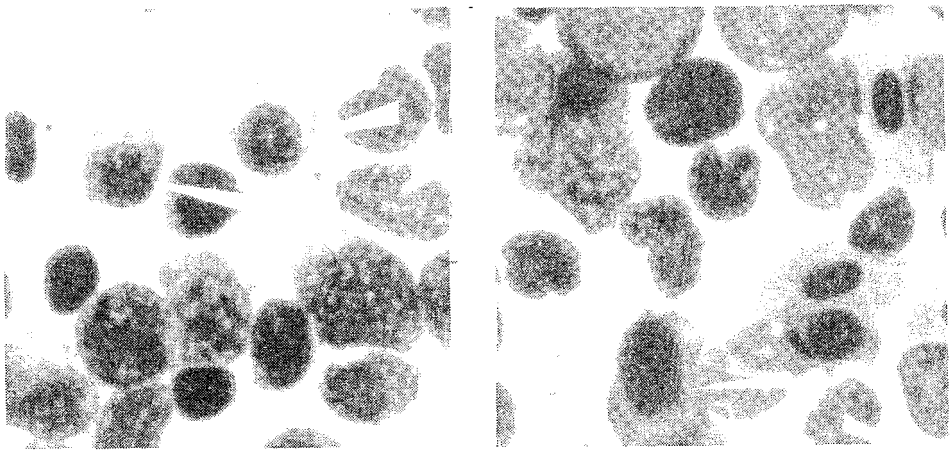

Fig. 9. The kidney. Paraerythroblastes with untypical intracellular structure. Note dyslocated nuclei. MGG. x 1250.

Phot. C. Nagięć

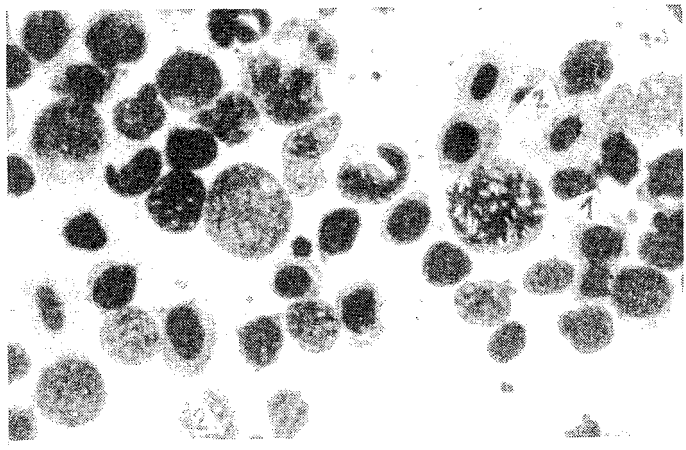

Fig. 10. The kidney. Lytic changes in mitotic stage of erythroblast. Vacuole degeneration in cytoplasm (1). Paraerythroblastes (2). MGG. x 1250

Phot. C. Nagięć

changes and reduced erythrocyte osmotic resistance, by the decreased hematocrit, hemoglobin content, and mean cell hemoglobin content. The decreased hematocrit in the experimental fish blood was a symptom of a decreased erythrocyte volume. Other workers not always were able to give unambiguous data with respect to that parameter. Swift (1978) reported no change in hematocrit of phenolexposed rainbow trout; on the other hand, Halsband and Halsband (1963) when workin with similar phenol concentrations, recorded a 12% increase. Similarly, an unchanged hematocrit was observed in

pike subject to subacute concentrations (McKim et al., 1976). Thus the parameter discussed should be treated with caution, the more so that, as stated by some authors (Barański et al., 1962), its diagnostic value refers to a non-differentiated erythrocyte population only.

Among various hematologic parameters studied, the erythrocyte index was decreased in the experimental fish, which points out to a reduced proportion of erythrocytes relative to leukocytes. The role of leukocytes in the prolonged subacute phenol intoxication of the rainbow trout is discussed elsewhere (Własow, in press).

CONCLUSIONS

1. Erythroblastosis occuring in the blood of the rainbow trout affected by prolonged phenol intoxication does not evidence intensified erythropoiesis. Similarly, the increase in basophil erythroblasts in the spleen is no proof of intensified erythropoiesis in view of:
   - strongly retarded erythropoiesis at the basic stage in the kidney, the principal hemopoietic organ of the rainbow trout,
   - The presence of hypobiotic changes in erythrocytes of the peripheral blood and hemopietic organs, the kidney in particular.
2. In spite of a compensatory reaction, the erythrocyte system can be regarded as impaired and giving no favourable prognosis.

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Własow, T., Układ białokrwinkowy pstrąga tęczowego Salmo gairdneri Rich. w przedłużonej podostrej intoksykacji fenolowej [praca przygotowywana do druku w Acta ichthyol. et piscat.] (Leukocyte system of rainbow trout Salmo gairdneri Rich. in prolonged sub-acute phenol intoxication (paper submitted for Publication in AIP).

Translated: Dr. Teresa Radziejewska

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UKŁAD CZERWONOKRWINKOWY PSTRĄGA TĘCZOWEGO *SALMO GAIRDNERI* RICH. W PRZEDŁUŻONEJ PODOSTREJ INTOKSYKACJI FENOLOWEJ

STRESZCZENIE

Badano układ czerwonokrwinkowy pstrąga tęczowego (196 szt.) poddanego działaniu fenolu (2 mg/dm^3 2 miesiące).

Stwierdzono obniżenie Ht, stężenia hemoglobiny (Hb) w krwi i MCH oraz spadek oporności osmotycznej erytrocytów przy zachowaniu liczby erytrocytów, ich średniej objętości (MCV) i średniego stężenia Hb w krwince (MCHC).

Analiza ilościowa komórek badanego układu wykazała statystycznie istotną erytroblastozę w krwi obwodowej, zahamowabie erytropoezy na szczeblu proerytroblastów w nerce i nieproporcjonalny wzrost udziału erytroblastów zasadochłonnych w śledzionie.

W komórkach układu czerwonokrwinkowego stwierdzono szereg zmian morfotycznych i strukturalnych wynikających z zaburzeń wstecznych. Zmiany były nasilone zwłaszcza w nerce, co wyjaśnia zahamowanie erytropoezy w tym narządzie.

Mimo istnienia pewnej reakcji wyrównawczej działalność układu czerwonokrwinkowego była upośledzona i rokująca niepomyślnie o dalszym przebiegu erytropoezy pstrąga.

Власов Т.

СИСТЕМА КРАСНЫХ КРОВЯНЫХ ТЕЛЕЦ У РАДУЖНОЙ ФОРЕЛИ SALMO GAIRDNERI RICH. ПРИ ПРОДОЛЖЕННОЙ ПОДОСТРОЙ ФЕНОЛЬНОЙ ИНТОКСИКАЦИИ

Р е з ю м е

Исследовали систему красных кровяных телец у радужной форели (196 особей), подвергавшейся действию фенола (2 мг/дм^3, 2 месяца).

Установили снижение Ht, концентрации гемоглобина (Hb) в крови и СГЭ (MCH), а также снижение осмотического сопротивления эритроцитов при одновременном постоянном уровне: числа эритроцитов, их среднего объёма (MCV) и средней концентрации Hb в тельце (MCHC).

Количественный анализ клеток исследованной системы показал статистически существенный эритробластоз периферической крови, торможение эритропоэза на уровне проэритробластов в почке и непропорциональное увеличение доли базофильных эритробластов в селезёнке.

По клеткам системы красных кровяных телец установлено ряд морфологических и структурных изменений как результат патологических вырождений. Резкие изменения наблюдались особенно в почке, что подтверждает торможение эритропоэза в этом органе.

Не смотря на некоторую компенсационную реакцию, действие системы красных кровяных телец отличалось дефектностью что прогнозировало неблагоприятное дальнейшее поведение эритропоэза у форели.

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