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Hematology

**THE LEUKOCYTE SYSTEM IN RAINBOW TROUT,
SALMO GAIARDNERI RICH. AFFECTED BY
PROLONGED SUBACUTE PHENOL INTOXICATION**

**UKŁAD BIAŁOKRWINKOWY PSTRĄGA TĘCZOWEGO
SALMO GAIARDNERI RICH. W PRZEDŁUŻONEJ
PODOSTREJ INTOKSYKACJI FENOŁOWEJ**

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The paper gives a quantitative and qualitative analysis of leukocytes in the peripheral blood, spleen, and pronephros of rainbow trout affected by phenol intoxication (2 mg phenol dm^{-3} applied for 2 months in winter).

INTRODUCTION

The present paper continues to present the results of a prolonged subacute test on phenol effects on rainbow trout blood. Having discussed the changes and responses of the erythrocyte system in the phenol-exposed trout in the previous paper (Własow, in press a), the author proceeds to describe the leukocyte system, a rather labile one in fish, in the phenol-intoxicated rainbow trout.

MATERIALS AND METHODS

For details on the materials and experimental procedures in aquaria, see the previous paper (Własow, in press a).

To determine the leukocyte count as well as proportions of various leukocyte types in the peripheral blood, spleen and kidney, the techniques used also for the erythrocyte system (Własow, in press a) were applied.

The analysis of variance with Fisher's F test was used as a statistical treatment of the results.

RESULTS

Peripheral blood

The leukocyte count in the experimental and control fish peripheral blood averaged $6.7 \times 10^3 \text{ mm}^{-3}$ and $5.3 \times 10^3 \text{ mm}^{-3}$, respectively, the difference between the two means being non-significant ($p = 0.05$). On the other hand, significant was the increase, by 197.5%, in the experimental fish granulocyte count (Table 1) relative to the control.

Table 1

White cells in the blood of rainbow trout (*S. gairdneri* Rich.)
after 2 – month exposure to 2 mg/dm^3 of phenol

Parameters	\bar{x}		$S_{\bar{x}}$		Significant + non-signif. – differences	
	E	C	E	C		
Granulocytes/ mm^3	848	285	783	387	+	$P < 0.01$
Granulocytes %	11.0	5.1	5.1	4.8	+	$P < 0.01$
Lymphocytes %	87.9	93.5	6.6	5.2	+	$P < 0.01$

E – experimental C – control

The experimental fish leukocyte system composition showed a significant decrease in the lymphocyte percentage, while the proportion of granulocytes increased by as much as 115.7% (Table 1). The phenol-intoxicated fish leukogram (Fig. 1) revealed heterophile granulocytes with semsegmented and rod-like nuclei as well as juvenile granulocytes to occur more frequently than in the control.

The experimental fish blood smears displayed the presence of changed leukocyte system cells, the changes proceeding with a varying intensity. Heterophile granulocytes turned out to be most susceptible to phenol. Senescent and irregular forms containing basophilous granulations located usually on the margins of the cells were encountered (Fig. 2).

The cytoplasm of most damaged heterophile granulocytes underwent vacuolar degeneration (Fig. 2). Their nuclei were more seldom vacuolar (Fig. 2); however, they were affected by necrobiotic changes, from hypochromasia to lysis.

Stimulated lymphocytes with cytoplasm protrusions (Fig. 3) were frequently encountered in the experimental fish blood. Regressive changes in lymphocytes occurred more seldom than in granulocytes. Lymphocytes with unevenly thickened cytoplasm

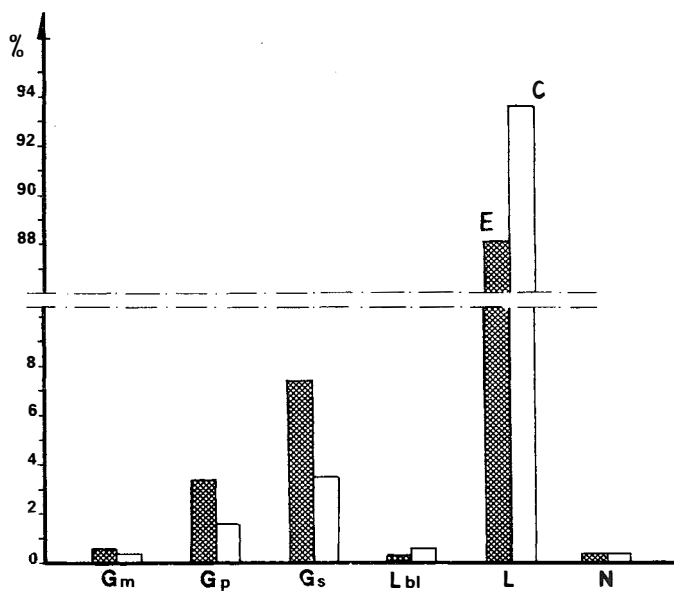


Fig. 1. The leucogramme of rainbow trout (*S. gairdneri* Rich.) after 2 – month exposure to 2 mg/dm³ of phenol. E – experimental, C – control. G_m – neutrophils – juvenile form, G_p – neutrophils – band form, G_s – neutrophils – segmented form, L_{bl} – lymphoblastes, L – lymphocytes, N – undifferentiated forms

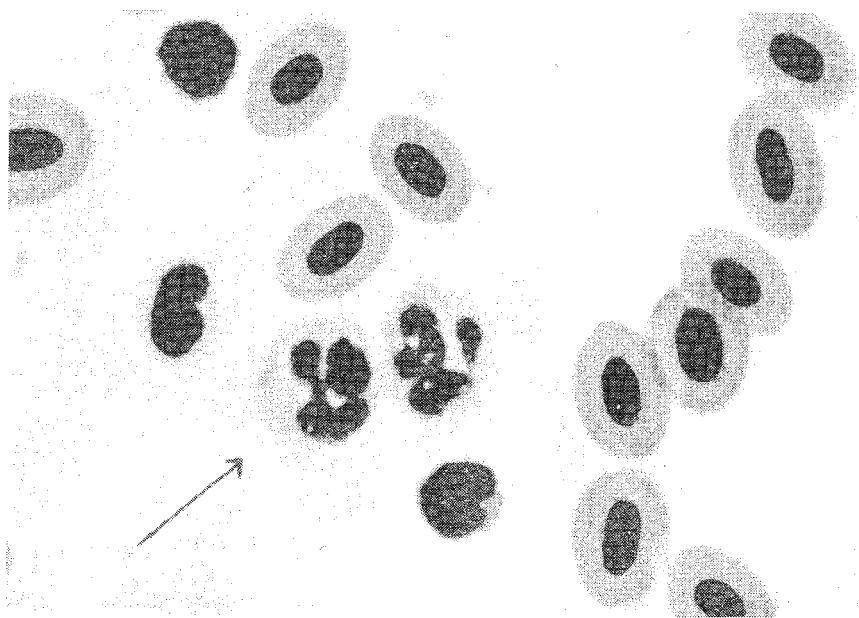


Fig. 2. Neutrophils – vacuole degeneration in nuclei. MGG x 1250.

Phot. C. Nagieć

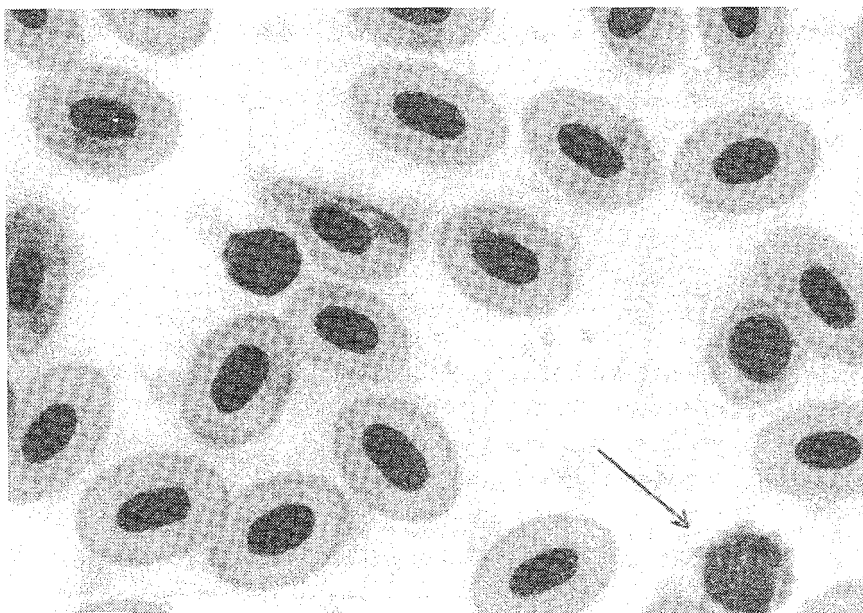


Fig. 3. The blood. Lymphocyt – active form. MGG x 1250.

Phot. C. Namięć

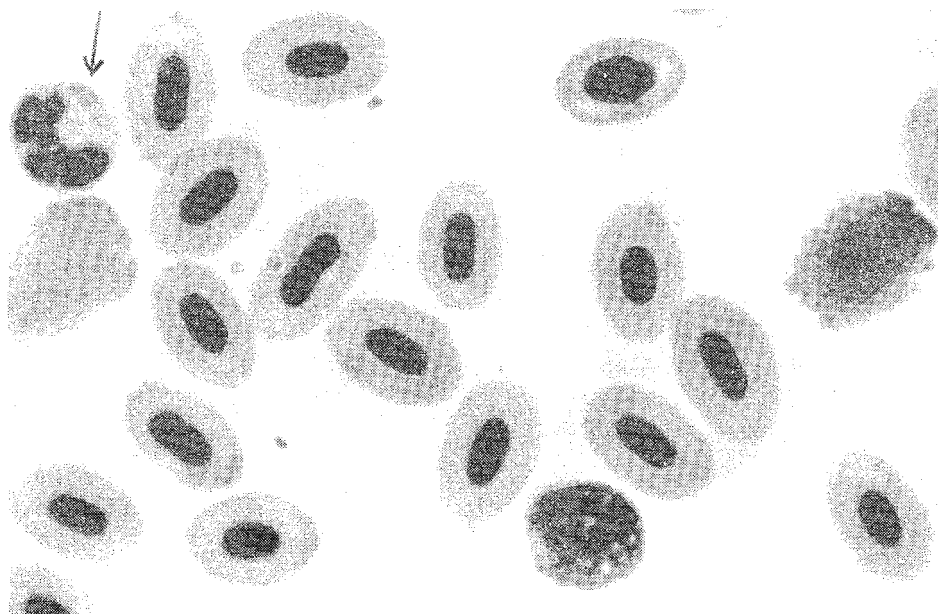


Fig. 4. The blood. Neutrophil with toxic granularity. Vacuole degeneration of lymphocyte. MGG x 1250.

Phot. C. Namięć

were observed; on occasion, the vacuolar degeneration of cytoplasm and/or nuclei (Fig. 4) was observed. Additionally, some lymphocytes in about 25% of the fish had their nuclei kidney-shaped, lobate, or divided. They resembled pathologic Rieder lymphocytes occurring in mammals (Ławkowicz and Krzemińska-Ławkowiczowa, 1973).

Hemopoietic organs

The phenol-exposed fish spleen showed significant decreases, by 40% and 75% relative to the control, in granuloblasts and juvenile granulocytes, respectively (Table 2). The percentage of segmented heterophile granulocytes was also, albeit slightly, lower, which would support a tendency to reduce the granulocyte percentage in the phenol-exposed rainbow trout. Among the qualitative changes in cells of the system described, vacuolar degeneration of cytoplasm and nuclei occurred most frequently (Fig. 5). Granuloblasts, often non-typical elongated ones, were particularly affected. On the other hand, granulocytes were observed to contain eosinophilous toxic granulations.

The lymphocyte system, similarly to the granulocyte one, showed a 54.5% decrease in the maternal cells (lymphoblasts) percentage relative to the control. The lymphocyte

Table 2

White blood cells in the spleen of rainbow trout (*S. gairdneri* Rich.)
after 2 – month exposure to 2 mg/dm³ of phenol.
(In percentage of all cells)

Cells	\bar{X}		$S_{\bar{x}}$		Significant + n. significant – differences	
	E	C	E	C		
Granulocytoblasts	0.6	1.0	0.5	0.6	+	P < 0.01
Neutrophils:						
juvenile form	0.06	0.24	0.2	0.3	+	P < 0.01
band form	2.7	2.4	1.4	1.4	–	P = 0.05
segmented f.	4.3	5.3	2.8	2.7	–	P = 0.05
Lymphoblasts	0.5	1.1	0.5	0.7	+	P < 0.01
Lymphocytes	57.8	54.6	8.5	10.5	–	P = 0.05
Reticular cells	2.3	1.9	2.0	1.5	–	P = 0.05
Macrophages	0.1	0.1	0.1	0.2	–	P = 0.05

E – experimental

C – control

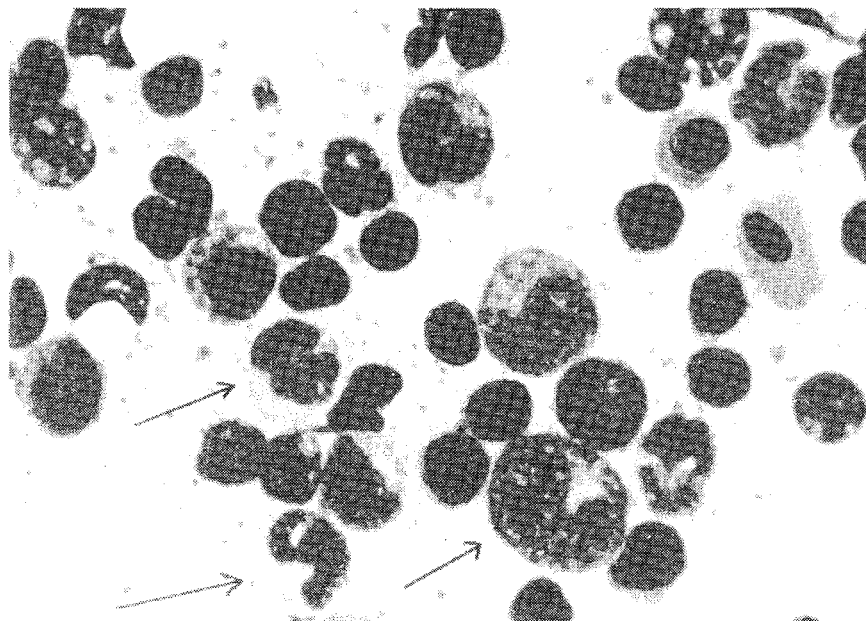


Fig. 5. The spleen. Granulocytic cells with vacuolized cytoplasm. MGG x 1250.

Phot. C. Nagieć

percentage was slightly elevated in the experimental fish. Degenerative changes, resulting in hypochromasia of nuclei, their swelling and obliteration of cell outlines, prevailed in lymphoblasts and lymphocytes. They, too, contained vacuoles (Fig. 5).

The percentage of reticular cells proper and macrophages in the experimental fish spleen and in the control was rather variable (Table 2), the higher values in the phenol-affected fish being non-significant, compared to the control. However, the phenol-exposed fish reticulum cells had less basophilous cytoplasm, evenly stained or containing highly basophilous areas. The intoxicated fish spleen possessed fewer fat cells, macrophages containing alien eosinophilous elements.

The phenol-exposed fish kidney showed, as was the case with the spleen, a significant reduction in granuloblasts and juvenile granulocytes percentages, by 49% and 48,5 %, respectively. Quantitative changes in heterophile granulocytes with segmented and rod-like nuclei were non-significant (Table 3).

The kidney of the experimental fish displayed qualitative changes similar to those occurring in the spleen. Vacuolar degeneration of cytoplasm and granuloblasts (Fig. 6) and juvenile granulocytes (Fig. 7) prevailed. The changes were more intensive and occurred more frequently than those in the spleen.

The kidney lymphocyte system in the experimental rainbow trout exhibited a significant increase in the percentages of lymphoblasts and prolymphocytes, which could have pointed out to a higher resistance of young lymphocytes compared to granulocytes.

Table 3

White blood cells in the kidney of rainbow trout (*S. gairdneri* Rich.)
after 2-month exposure to 2 mg/dm³ of phenol
(In percentage of all cells)

Cells	\bar{x}		$S_{\bar{x}}$		Significant + n. significant – differences
	E	C	E	C	
Granulocytoblasts	1.4	2.3	0.7	1.1	+ P < 0.01
Neutrophils:					
juvenile form	0.8	1.3	0.6	1.1	+ 0.01 < P < 0.05
band form	8.5	9.3	3.5	4.7	– P = 0.05
segmented f.	8.1	7.7	4.3	3.7	– P = 0.05
Lymphoblasts	10.4	4.2	11.6	1.7	+ 0.01 < P < 0.05
Prolymphocytes	2.3	0.9	2.1	0.9	+ P < 0.01
Lymphocytes	45.3	48.9	14.7	8.7	– P = 0.05
Reticular cells	3.0	1.4	3.9	1.4	– P = 0.05
Macrophages	0.5	0.1	0.8	0.2	+ 0.01 < P < 0.05

E – experimental

C – control

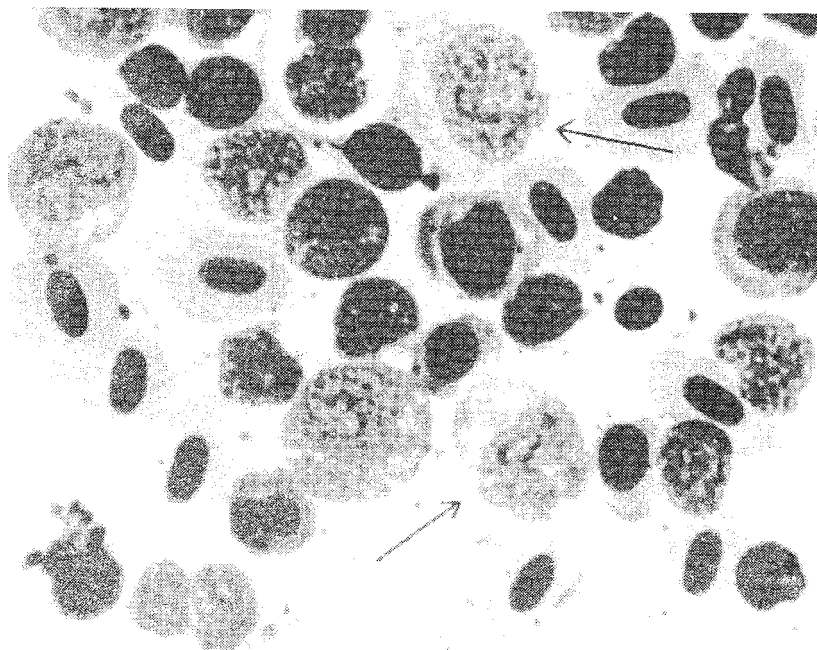


Fig. 6. The kidney. Vacuole degeneration in juvenile forms of leucocytes. MGG x 1250.

Phot. C. Napięć

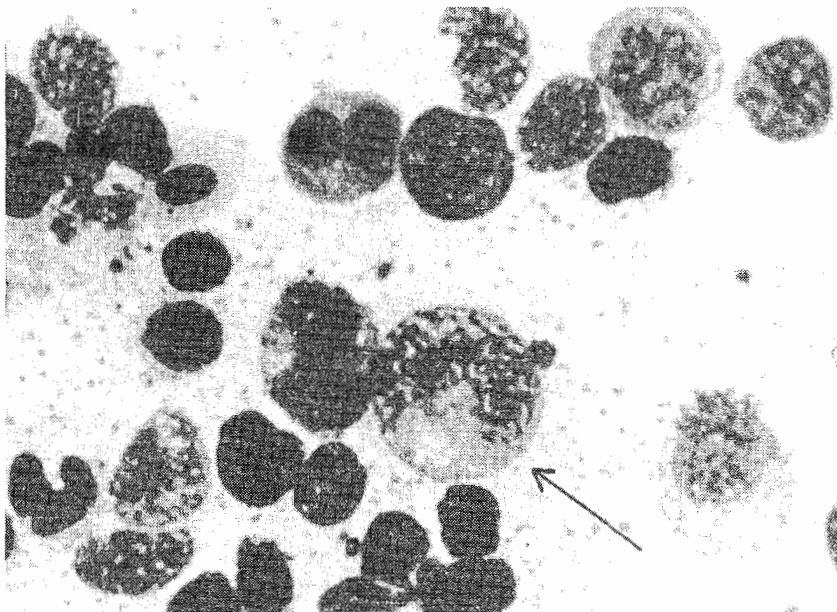


Fig. 7. The kidney. Juvenile form of neutrophil with vacuole changes in cytoplasm and nuclei. MGG x 1250.

Phot. C. Nagięd

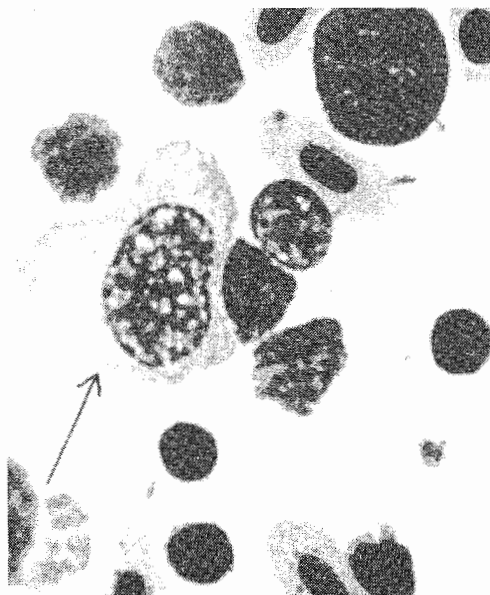


Fig. 8. The kidney. Reticular cell with vacuole degeneration in nuclei. MGG x 1250.

Phot. C. Nagięd

Changes in lymphocytes were not clear-cut. No stimulated lymphocytes whatsoever were found, either.

Macrophages occurred in the phenol-affected fish kidney more often than in the control. Changes in reticular cells in the kidney were similar to those in the spleen, degenerative changes in nuclei prevailing (Fig. 8).

DISCUSSION

The prolonged subacute phenol intoxication of the rainbow trout was found to bring about a mild leukocytosis in the fish affected. Similar phenomena were observed in tinch (Reichenbach-Klinke, 1965) and bream (Waluga, 1975). In some cases, however, leukopenia was observed in fish phenol intoxication (Mikryakov and Flerov, 1971; Waluga, 1966). A typical feature observed in the fish exposed to phenol, in both leukocytosis and leukopenia, was the increase in heterophile granulocytes (Mikryakov and Flerov, 1971; Waluga, 1975; Własow, 1979).

In the experiment discussed, the increase in the peripheral blood heterophile granulocytes was significant; it could be taken as an evidence of a defensive response of a phenol-exposed organism. At the same time, however, the peripheral blood of the rainbow trout tested showed degenerative and necrobiotic changes in rod and segmented heterophils. Those cells were more sensitive to changes than lymphocytes were. Similar changes in leukocytes were found in bream exposed to, i.a., phenol (Waluga, 1975).

The peripheral blood granulocytosis in the rainbow trout was accompanied by retarded, at the maternal cell level, granulopoiesis in the blood-producing organs. The process was retarded more strongly in the kidney than in the spleen. As, however, fish splenograms and „nephrograms” are very seldom made and analysed, it is difficult to compare the data obtained with other authors' findings. It can be supposed that the peripheral blood granulocytosis, present in the phenol-intoxicated fish, would not be permanent, as granulopoiesis was significantly retarded in the blood-producing organs. Moreover, the process was accompanied by destructive changes in granulocytes.

The lymphocyte system in the phenol-exposed rainbow trout showed a significant lymphopenia in the blood and spleen. At the same time, however, the „nephrogram” showed an increase in lymphoblasts and prolymphocytes in the kidney. Besides, the cells of the system showed an increased physiological activity, evident as an increased contribution of large and stimulated lymphocytes. Degenerative changes in the system, in both the peripheral blood and hemopoietic organs, were not as intensive and as common as those occurring in granulocytes.

The intensified lymphopoiesis in the kidney and the physiological activation of the peripheral blood leukocytes could be regarded as homeostatic processes in the phenol-exposed rainbow trout. Some explanation of the process will be arrived at after the analysis is made of the lymphocyte blastic transformation and other non-specific defensive mechanisms operative in the rainbow trout phenol intoxication (Własow, b).

CONCLUSIONS

1. The phenol-intoxicated rainbow trout leukocytes showed hypobiotic as well as compensative reactions; the latter occurred as:
 - blood reactive granulocytosis
 - intensified lymphopoiesis in the kidney.
2. Among the rainbow trout leukocytes, lymphocytes were more resistant to phenol and showed a higher physiological activity.
3. The splenogram and „nephrogram” show a strong retardation in granulopoiesis in the blood-producing organs and suggest the lack of permanence in granulocytosis of the phenol-exposed rainbow trout peripheral blood.

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UKŁAD BIAŁOKRWINKOWY PSTRAŻA TĘCZOWEGO *SALMO GAIRDNERI* RICH.
W PRZEDŁUŻONEJ PODOSTREJ INTOKSYKACJI FENOLOWEJ

STRESZCZENIE

Przeprowadzono analizę ilościową (analiza wariancji Fishera – test F) i jakościową krwinek białych krwi obwodowej i narządów krwiotwórczych pstrąga tęczowego po intoksykacji fenolowej (2 mg/dm^3 , 2 miesiące).

U pstrągów narażonych na działanie fenolu wystąpiła nieistotna leukocytoza przy znacznej granulocytozie odczynowej w krwi. Analiza „splenogramu” i „nefrogramu” wykazała znaczne zahamowanie granulopoezy w narządach krwiotwórczych trutych pstrągów.

W układzie limfocytów stwierdzono względną limfopenię w krwi, zahamowanie limfopoezy w śledzionie oraz jej pobudzenie w nerce głowowej.

Komórki siateczki o właściwościach żernych były częstsze w nerce ryb eksponowanych na fenol.

W krwinkach białych krwi i w narządach krwiotwórczych występowały zmiany hipobiotyczne i degeneratywne. Najbardziej podatne na te zmiany były komórki szeregu granulocytarnego.

Тереса Власов

СИСТЕМА БЕЛЫХ КРОВЯНЫХ ТЕЛЕЦ РАДУЖНОЙ ФОРЕЛИ
(*SALMO GAIRDNERI* RICH.) ПРИ ДЛИТЕЛЬНОМ ПОДОСТРОМ
ФЕНОЛЬНОМ ИНТОКСИКАЦИИ

Р е з ю м е

Проведён количественный анализ (анализ вариации Фишера – тест F) и качественный анализ белых кровяных телец периферийной крови и кроветворных органов радужной форели после фенольной интоксикации (2 мг/дм^3 , 2 месяца).

У форели, подвергнутой действию фенола, наблюдался несущественный лейкоцитоз при значительном гранулоцитозе в крови. Анализы „спленограммы” и

„нефрограммы” показали значительное торможение гранулопоза в кроветворных органах отравляемых форелей.

В системе лимфоцитов обнаружили относительную лимфопению в крови, торможение лимфопозы в селезёнке, а также её возбуждение в головной почке.

Клетки-сетки с пожерающими свойствами выступали чаще в почке рыб, подвергнутых действию фенола.

В белых тельцах крови и в кроветворных органах наблюдались гипобиотические и дегенеративные изменения. Наиболее податливыми на эти изменения были клетки гранулоцитарного ряда.

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Received: 22 II 1984