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SELECTED INDICES OF IMMUNOLOGIC RESISTANCE  
IN RAINBOW TROUT (*SALMO GAIARDNERI* RICH.)  
DURING PROLONGED, SUBACUTE PHENOLIC INTOXICATION

WYBRANE WSKAŹNIKI OPORNOŚCI IMMUNOLOGICZNEJ  
PSTRĄGA TĘCZOWEGO (*SALMO GAIARDNERI* RICH.)  
W PRZEDŁUŻONEJ PODOSTREJ INTOKSYKACJI FENOLOWEJ

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Blastic transformation of the lymphocytes in peripheral blood and spleen, analysis of immunocompetent cells in the blood and haematopoietic organs of rainbow trout after phenolic intoxication (2 mg/dm<sup>3</sup>, 2 months, winter).

INTRODUCTION

As a result of phenolic intoxication of rainbow trout, several hypobiotic changes were observed (Własow, 1985b). At the same time, however, such reactions occurred as: reactive granulocytosis in the blood, intensified lymphopoiesis in the head kidney, and relative physiological activity of the lymphocytal system, reflected in the presence of activated forms and increased resistance to the effect of phenol. These processes, aimed at preserving the homeostasis, are somehow clarified by the present paper which describes some immunologic responses in rainbow trout exposed to phenol.

## MATERIAL AND METHOD

The materials consisted of rainbow trouts intoxicated with phenol (2 mg/dm<sup>3</sup> of phenol, 2 months) in the experiments described earlier (Własow, 1985a).

Blood samples were taken from the heart by means of 1 ccm syringe, using intradermal needles. Blood smears and reprints of the organs were made (Własow, 1985a) for quantitative analyses of the immunocompetent cells.

Cell resistance was determined by means of a test of blastic transformation of lymphocytes, with modification for fishes (Etlinger et al., 1976). The lymphocytes were cultured with phytohaemagglutinin (PHA). Intensity of blastic transformation of the lymphocytes was analysed in peripheral blood and lymphoid spleen cells in the experimental (30 indiv.) and control (30 indiv.) fishes. 3 cultures were made for each fish. The cultures (2 ml of Parker liquid, produced by the Factory of Sera and Vaccines in Lublin, plus antibiotics, 10 mg streptomycin and 10000 units of penicillin per 100 ml of the liquid, plus about 1 million of the lymphocytes, plus 0.4 ml of homogenic serum, plus 0.1 ml PHA — M. Difco) were incubated for 3 days in 20°C.

Survival of lymphocytes in the culture was controlled with 0.05% solution of trypan blue. Intensity of transformation was measured basing on 6 — <sup>3</sup>H thymidine incorporation (spec. act. 15000 mCi/m Mol, Czechoslovakia). Thymidine was applied 48 h. after the incubation, at a dose of 0.2 µCi per culture. Radioactivity was determined in Beckmann apparatus LS 100 C for liquid scintillation. The results are presented as the transformation index (Etlinger et al., 1976).

Physiological activity of the granulocytes was assessed taking advantage of the average reaction index — ARI (Astaldi, Verga, 1957) of peroxidase content, analysing blood smears stained with Graham's method.

Moreover, desoxyribonucleic acid (DNA) was determined in spleen cells with Feulgen method; the scraps were preserved in Carnoy liquid and immersed in paraffin.

## RESULTS

Ability of the lymphocytes for blastic transformation in the peripheral blood of rainbow trout intoxicated with phenol decreased insignificantly (Tab. 1). It could not reflect any lowering of immunological response of the cell type, connected with lymphocytes in the experimental trouts.

The results of the transformation test, carried out on the lymphoid cells of trout spleen, revealed an increase of the transformation index in the intoxicated fishes, by 35% compared to the control (Tab. 1). This increase reflected considerable stimulation of the immunological response of the cell type, and was highly significant.

During the studies on immunocompetent cells, carried out on permanent slides, cells with an ability of phagocytosis were distinguished — macrophages and heterophilic granulocytes, lymphocytes and reticular cells.

Table 1

Comparisons of transformation indices of lymphoid cells  
from blood and spleen of rainbow trout (*S. gairdneri* Rich.)  
after 2-month exposure to 2 mg/dm<sup>3</sup> of phenol

Transformation indices of cells from:	$\bar{x}$		$S_{\bar{x}}$		Significant + N. significant – differences
	E	C	E	C	
blood	0.89	0.99	0.65	0.74	– p = 0.05
spleen	1.35	1.00	0.26	0.48	+ p < 0.01

E – experimental, C – control

Differences in the frequency of macrophags in the xperimental and control fishes were noticeable in the spleen. In the intoxicated fishes macrophags were by 400% more frequent.

Heterophilic granulocytes were less numerous in the haematopoietic organs of the experimental fishes compared to the control, while their percentage in the peripheral blood increased to 115.7%. Granulocytosis in the blood of intoxicated fishes should be regarded as an intensification of the immunological reaction. At the same time, small but significant lowering of the histochemical index (ARI) of peroxidase content was observed in the granulocytes (Tab. 2).

Percentage of the lymphoid cells responsible for the production of antibodies decreased in the blood of intoxicated trout by 6%. This referred to the lymphocytes. A significant increase of the lymphoblast percentage was observed in kidneys, by 148%, as well as of prolymphocytes – by 156%. On the other hand, lymphoblast percentage in spleen decreased considerably (by 54.5%).

Table 2

Average reaction index (A.R.I.) for granulocytic peroxidase  
from blood of rainbow trout (*S. gairdneri* Rich.) after 2-month  
exposure to 2 mg/dm<sup>3</sup> of phenol

A.R.I.	$\bar{x}$		$S_{\bar{x}}$		Significant + n. significant – differences
	E	C	E	C	
	1.95	2.03	0.10	0.20	+ 0.01 < p < 0.05

E – experimental, C – control.

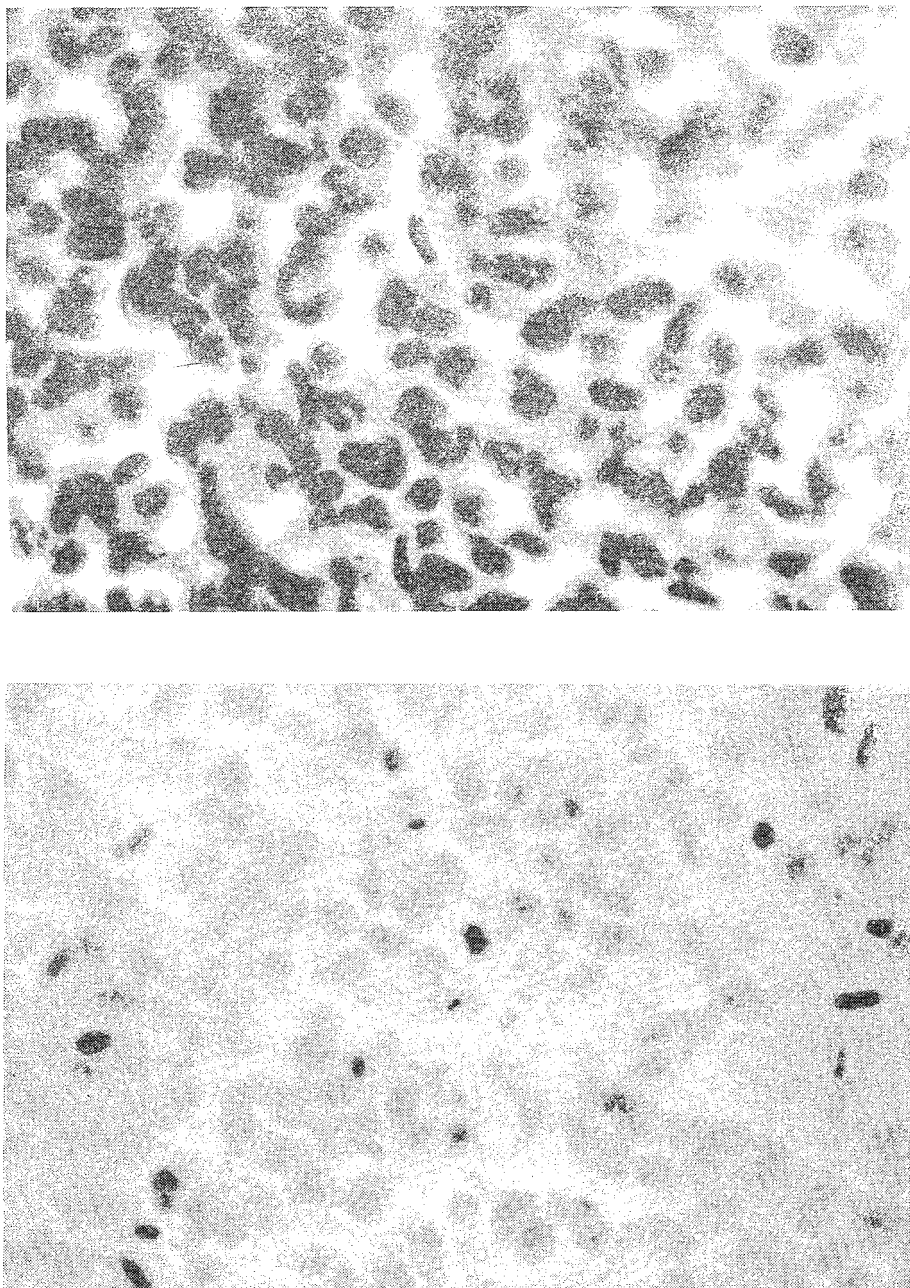


Fig. 1. The spleen of rainbow trout (*S. gairdneri* Rich.): a – after 2 month exposure to 2 mg/dm<sup>3</sup> of phenol, b – control. Positive Feulgen's reaction in a, negative – in b. Im. x 1250

Phot. C. Nagieć

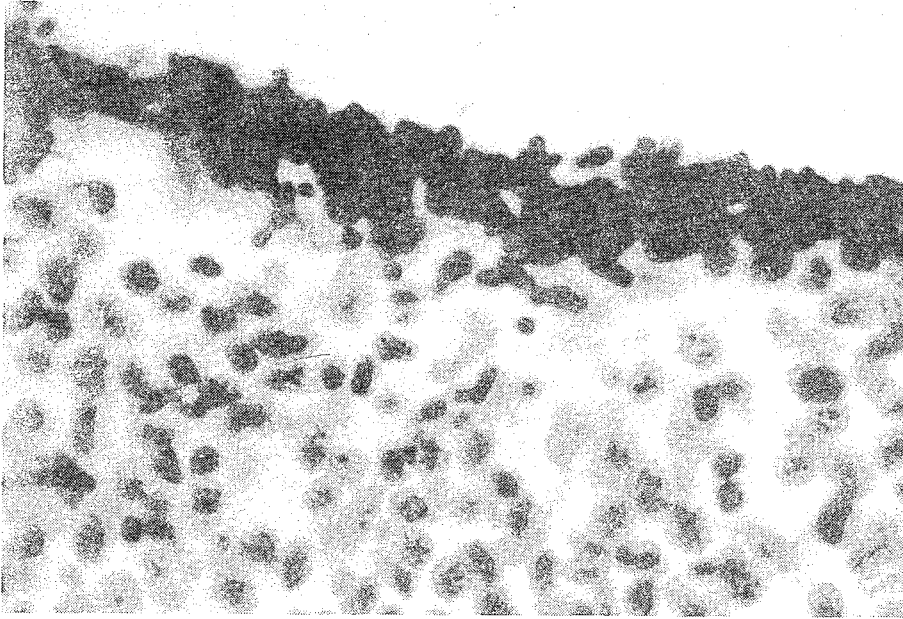


Fig. 2. The spleen of rainbow trout (*S. gairdneri* Rich.) after 2 month exposure to  $2 \text{ mg/dm}^3$  of phenol. Positive Feulgen's reaction. Note subcapsular region. Im. x 1250.

Phot. C. Nagi c

Nuclei of lymphoid cells in spleen of trout intoxicated with phenol (Fig. 1a) had higher DNA content than in the control (Fig. 1b). This was especially well noticeable in the cells close to the blood vessels, and under the sac (Fig. 2). Under-sac aggregates of the lymphoid cells with nuclei rich in DNA were three times more frequent in the intoxicated fishes than in the control ones.

## DISCUSSION

Cell resistance of rainbow trout was assessed with the test of blastic transformation of the lymphocytes, which so far has rarely been used for fishes (Cassey et al., 1976, Etlinger et al., 1976, Sakai, 1981). The test showed that the lymphocytes in peripheral blood of the intoxicated fishes were characterized by an unchanged cell resistance compared to the control. Increased cell resistance was observed in spleen of the experimental fishes. Contrarily to blood, this organ contains considerable amounts of "T" lymphocytes (Etlinger et al., 1976), i.e. PHA-reactive. Moreover, presence of reticular cells and macrophags could have stimulated transformation (Szewczyk, 1970), and these cells were not present in the blood. There were, however, no differences in the occurrence of these cells between the intoxicated and the control fishes.

Increased *in vitro* reaction of the "T" lymphocytes from spleen of the fishes intoxicated with phenol was confirmed by the histochemical reaction of Feulgen. Increased DNA synthesis noted in the histological preparates, which usually precedes transformation, suggests stimulation of this process, and stronger response of the cell type in trout intoxicated with phenol compared to the control.

Non-specific cell response to phenol was reflected in granulocytosis, observed in blood of the experimental fishes. However, lowering of the ARI index of peroxidase content in the granulocytes of these fishes reflects low intensity of this response. An increase of ARI of peroxidase in carps with septicaemia, considered to be a non-specific immunological reaction (Afanasyev, Kolot, 1971), was probably similar to the stimulation of myeloperoxidase activity, observed during phagocytosis in mammals (Tchórzewski, 1978). Hence, phagocytal activity of these cells in the intoxicated trout seems to be weakened, notwithstanding reactive granulocytosis in the blood.

Intensification of phagocytosis in fishes exposed to phenol took place in kidneys, in which a noticeable predomination of the macrophags was observed compared to the control fishes.

Percentages of cells production antibodies in trout blood and spleen were lower in the fishes exposed to phenol. Inhibiting effect of low phenol concentrations upon antibody production was observed also in carp (Goncharov and Mikryakov 1970). According to Mikryakov et al. (1974) humoral resistance decreased in fishes with chronic phenol poisoning. On the other hand, cell responses were intensified, such as macrophag production (Waluga 1975), reactive granulocytosis (Mikryakov and Flerov 1971, Waluga 1975).

In mammals cell resistance becomes noticeable mostly when the humoral reactions are not sufficient to protect the organism from a disease (Report of WHO 1973). In case of fishes protective cell responses (Corbel 1975, Finn and Nielson 1971) are probably similarly important.

## CONCLUSIONS

1. Non-specific immunological response to phenol consisted of an increase of cell resistance connected with "T" lymphocytes in spleen of rainbow trout.
2. Increased cell resistance was accompanied by intensified DNA synthesis in nuclei of the lymphoid spleen cells.
3. Reactive granulocytosis of blood in trout exposed to phenol took place at decreased non-specific immunological reaction of these cells, connected with the presence of peroxidases.

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STRESZCZENIE

Badano wybrane, niespecyficzne reakcje obronne pstrąga tęczowego po intoksykacji fenolowej ( $2 \text{ mg/dm}^3$  2 miesiące).

Przeprowadzony test transformacji blastycznej (TTB) limfocytów (metodą inkorporacji z  $6 - {}^3\text{H}$  tymidyną) wykazał nie zmienioną w stosunku do kontroli odporność komórkową związaną z limfocytami „T” w krwi obwodowej oraz wzrost tej odporności w śledzionie ryb doświadczalnych.

Potwierdzeniem wyników TTB był wzrost syntezy DNA (reakcja Feulgena) w śledzionie pstrągów narażonych na działanie fenolu.

W krwi ryb doświadczalnych stwierdzono istotną odczynową granulocytozę. Jednakże badania histochemiczne ujawniły osłabienie nieswoistej reakcji obronnej granulocytów.

W nerce pstrągów zatrutowanych fenolem notowano wzrost udziału komórek produkujących przeciwciała i wzmożenie fagocytozy.

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ИЗБРАННЫЕ ПОКАЗАТЕЛИ ИММУНОЛОГИЧЕСКОЙ  
УСТОЙЧИВОСТИ РАДУЖНОЙ ФОРЕЛИ (*SALMO  
GAIRDNERI* RICH.) В ПЕРИОД ПРОДЛЕННОЙ  
ПОДОСТРОЙ ФЕНОЛЬНОЙ ИНТОКСИКАЦИИ

Р е з ю м е

Исследовали выборочно неспецифические защитные реакции радужной форели после фенольной интоксикации ( $2 \text{ мг/дм}^3$  2 месяца).

Проведённый тест бластической трансформации (ТБТ) лимфоцитов (методом инкорпорации с 6 - 3Н тимидином) показал неизменённую по отношению к контрольным образцам клетчатую устойчивость связанную с лимфоцитами „Т” в периферийной крови и рост этой реакции в селезёнке подопытных рыб.

Подтверждением результатов ТБТ был рост синтеза ДНА (реакция Фюельгена) в селезёнке форели подвергаемой действию фенола.

В крови подопытных рыб обнаружили существенный реактивный гранулоцитоз. Однако гистохимические исследования обнаружили ослабление несвойственной защитной реакции гранулоцитов.

В почке форели, отравленной фенолом, отмечали рост количества клеток, производящих антитела и усиление фагоцитоза.

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