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Toxicology

EXPERIMENTAL METHEMOGLOBINEMIA IN RAINBOW TROUT

EKSPERYMENTALNA METHEMOGLOBINEMIA PSTRĄGÓW TĘCZOWYCH

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In the experimental way methemoglobinemia was caused to occur in rainbow trout, *Salmo gairdneri* Rich., 1836 by keeping the fishes for 11 weeks in $Ca(NO_3)_2$ and KNO_3 solutions, the NO_3 doses in which being 26.2 mg/l and 30.6 mg/l, respectively.

At the same time measurements of the hepatic tissue respiration rate, histopathologic studies of liver as well as examination of the peripheral blood and hematopoietic organs were carried out.

Marked changes which eventually could be lethal were found out.

INTRODUCTION

More and more frequent reports on the deleterious effect of nitrates contained in drinkable water on the man's health (**Schaefer**, 1970 and others) turned our attention to a possible relation of the problem to fish. Thus an experiment on rainbow trout, *Salmo gairdneri* Rich., 1836 was planned and carried out; the results are presented herein.

The whole experiment was based on 50 mg/l nitrate dose corresponding with 26.2 mg NO_3/l for $Ca/NO_3/_2 \cdot 4H_20 = 236.16$ and 30.6 mg NO_3/l for $KNO_3 = 101.1$. The WHO – allowed NO_3 dose in drinkable water is 50 mg/l (**Schaefer**, 1970).

The following authors are responsible for the present report: E. Grabda, general principles and the histopathologic studies; T. Einszporn-Orecka, hematology; C. Felińska, the methemoglobin level determinations; R. Zbanyszek, the hepatic tissue respiration metabolism.

METHODS

60 two-years old individuals of rainbow trout measuring 18/20 – 23/26 cm (L.c./L.t.) and weighing 73–155 g (only a few of them of weight below 90 g) taken from the same hatchery were subject to experiments.

The experiments were carried out simultaneously in 3 tanks: two of 400 l effective capacity each, the remaining one of 230 l. The fishes were divided into 3 groups of 20 individuals per tank. Tank „A" (400 l) filled with clean tap water was a control. 50 mg/l of calcium nitrate c.p., $Ca/NO_3/_2 \cdot 4H_2 0$ and pure potassium nitrate, KNO_3 were added to water of tanks „B" (4001) and „C" (230 l). respectively. The three tanks contained normal drinkable tap water. To ensure possibly equal nitrate concentrations the water was changed every 1–2 days (in exceptional cases every third – in the final stage of experiments) instead of applying the water flow. Usually such a change was accomplished after feeding. The fishes were fed on frozen cod supplemented, when necessary, with a granulated food normally used in the hatchery. The artificial food was given only exceptionally and could not influence the course of experiments. The water was constantly aerated with an electric aerator, the action being doubled in the smaller tank to compensate for losses resulting from a greater fish density there.

Doses of 50 mg/l pure salts were used, the fresh solution being prepared after each water change in the experimental tanks. To adapt the fish organisms the doses were gradually increased during the first three days.

At the beginning of the experiment, water temperature was approximately 15°C, decreasing to 11°C on completing. The oxygen content in the tanks evaluated just before the water change ranged within 3.1–7.84 mg/l, usually 4.8.–6.6. mg/l, while that of the tap water was about 6.76 mg/l; pH values of the experimental and tap water were 6.76–7.04 and ca 6.9, respectively.

The experiment was started on November 5, 1972 and proceeded till January 20, 1973. For each series of the experiment 4 fish individuals were taken out of each group while all the data were analysed for each individual separately. Because of limitations of the equipment (the Warburg apparatus capacity) the examinations of each series took two subsequent days. The first examination was carried out one month after the experiment had been started; dates of each are as follows: I, 5 – 6.12.1972; II, 18–19.12.1972; III, 3–4.01.1973; IV, 17–20.01.1973 (V, 19–20.01.1973 was treated separately only in the tissue respiration studies). The last group comprised a double number of fishes. Further on, the examinations will be denoted following the above indications.

The blood for morphologic studies and the methemoglobin content evaluation was taken from the caudal vein (*vena caudalis*) of living specimens; the after a decapitation the livers were taken out. The hepatic tissue samples were collected for both the oxygen consumption measurements and histologic studies, materials for the latter being fixed with the Bouin fluid. Simultaneously, kidney and spleen squeeze mounts were prepared.

The first drops of blood served to made the following quantitative evaluations: 1. leukocyte, erythrocyte, and thrombocyte numbers in the Bürker chamber according to generally accepted principles; 2. the hemoglobin level assessed photocolorimetrically with

the Green and Teal modified Drabkin method; 3. the hematocrit value estimated with heparin micropipettes. Additionally, the percentage blood composition was determined from the May-Grünwald and Giemza panoptically stained smears. Changes in the appearance of blood cells were followed and squeeze mounts from the blood-producing organs were examined.

The methemoglobin content was calculated according to the formula:

$$\%\text{MetHb in the blood} = \frac{\text{g MetHb/100 ml blood}}{\text{g Hb/100 ml}} \cdot 100$$

using m/60 phosphate buffer, 10% sodium cyanide neutralized with 12% acetic acid plus 5% potassium ferrocyanide. The results were read on the „Specol" photometer at 635 mm (acc. to **Antczak** and **Gross**, 1956).

The hepatic tissue respiration metabolism was directly examined with the Warburg method (**Kleinzeller**, 1965; **Umbreit** et al., 1957). The liver segments rinsed with the glacial Ringer fluid of pH 7.5 adapted to fish organisms (**Raquignot**, 1964) were prepared as sections of 100 mg moist tissue and placed in Warburg vessels surrounded with ice. The filter paper moistened with 0.2 ml 15% KOH served as a CO_2 absorber, while the atmospheric air made up a gaseous phase. The vessels were swayed at a rate of 116–120/min. in a 25°C water bath. The pre-incubation period took 15 minutes. Readings were taken at 20 – min. intervals. Respiration processes intensity was expressed in $\mu l O_2$ and calculated on 1 g of wet tissue per hour.

OBSERVATIONS ON LIVING FISHES

During the whole period of the experiments the fishes behaved in their normal way, were very lively and showed a good appetite. However, the rainbow trouts influenced by nitrates were observed to gather eagerly around the air supply inlets, particularly so in Tank „C" with KNO_3; sometimes the fishes „stood" vertically directly within the air bubbles zone. Later on they were observed to behave in this way even more eagerly. Although their natural in-bred feature of an upstream orientation may be taken into account when explaining reasons of this behaviour, some difficulties in their respiration cannot be excluded, the phenomenon not being observed in the control fishes.

HISTOPATHOLOGICAL CHANGES

$Ca(NO_3)_2$

Within the first period slight changes of the hepatic cells, protoplasm appearance were observed. The borders of individual cells were distinct, only slightly enlarged intertrabecular spaces could evidence a more intense accumulation of the body fluids.

In the II period, the hepatic cells' plasm tended to be more vacuolized; a few fine necrobiotic centres occurred.

In the III period, the intertrabecular spaces were distinctly seen while the hepatic cells mostly lost their trabeculae and formed a homogenous syncytial mass with only the nuclei visible. The protoplasm itself had a rather even mat shade. The nuclei were usually poor in chromatin appearing only in the peripheral zone.

Rather considerable changes were noted in the blood system. Part of large vessels exhibited features of a poor patency. In these spots the leukocytes occurred almost exclusively within the vessels, accompanied by serum visible as a denatured coagulate. Moreover, outside these vessels significant lymphocyte infiltrations appeared as extensed centres in the surrounding tissue. The erythrocytes occurring in some parts were highly hypochromatic. They frequently appeared as shades with their framings hardly visible. The blood cell nuclei, considerably changed, stained poorly with hematoxylin.

On the other hand, the occurrence of intra-tissue gaseous blisters was quite a nevelty. They were of various sizes and completely devoided of their own walls, no coagulate being found inside (Fig. 1). Their formation presumably had taken place intravitally because the samples were taken immediately after having killed a fish. They are very likely to result from a fracture of smaller blood vessels induced by gas concentrated within the blood. The surrounding tissue cells showed no regressive changes at all.

It the IV period, a very strong vacuolization of the hepatic cells protoplasm took place. The protoplasm itself occurred only in the nearest vicinity of the nuclei thus giving a mosaic-like appearance to the whole mount. Considerable changes were observed in the hepatic cells' nuclei which looked like empty bubbles because of their chromatin granulation losses. In general, they were only poorly distinguishable from the protoplasm background. The nuclei with small grains of chromatin were very rare.

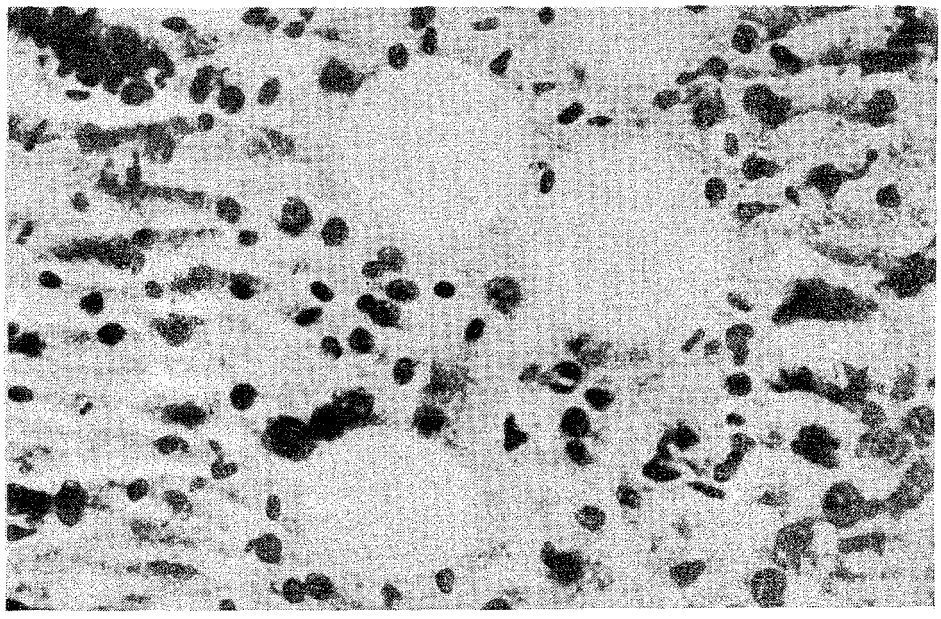


Fig. 1. A necrotic centre in the hepatic tissue; four gas-filled blisters visible

Generally poor chromasia of the tissue was very frequent, the intertrabecular spaces, however, were markedly visible (Fig. 2). The stasis phenomena in the blood vessels,

described previously, became more significant, a strong swelling of cells in the superficial layer corresponding with the membrane covering the liver being very prominent. Protoplasms of neighbouring cells tended to fuse in places, constituting a homogenous one-layer syncytium with only nuclei occurring. The nuclei of these hepatic cells were definitely different than the normal ones; they were small, with a compact chromatin, and resembled leukocytes.

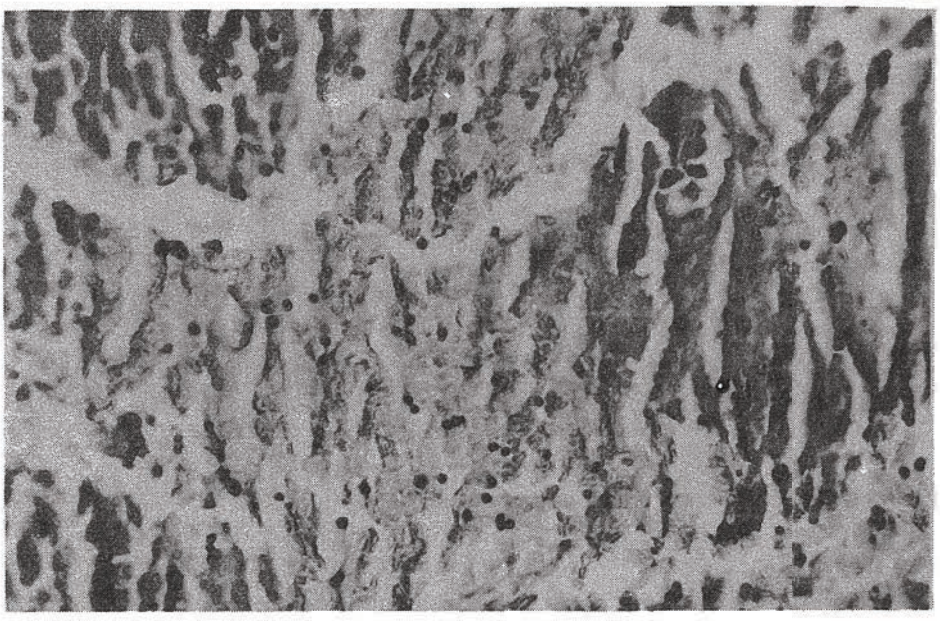


Fig. 2. A necrobiotic centre passing into necrosis; a trabecular structure still visible; trabeculae partly separated by serum

Also, the blood stasis in larger vessels was clearly seen, the erythrocytes not being eosin-stained in spite of good staining properties of the tissue cells. They looked like a homogenous mass with only the nuclei visible, but poorly stained as well (Fig. 3). Large intra-tissue effusions were present, occassionally even fragments of the liver being found there. It is of interest that the necrobiosis occurred more often within the surface layers of the liver, particularly within those directly adjoining the fat tissue surrounding the organ. However, the fat tissue is not frequently found in rainbow trout.

Beginning with the III period, hemosiderin accumulation in the hepatic tissue was noted.

KNO_3

In the histologic picture a strikingly strong vacuolization of the hepatic cells was observed from the beginning (the I period), the protoplasm being thus restricted only to a small amount around the nucleus and the whole mount having a mosaic-like look

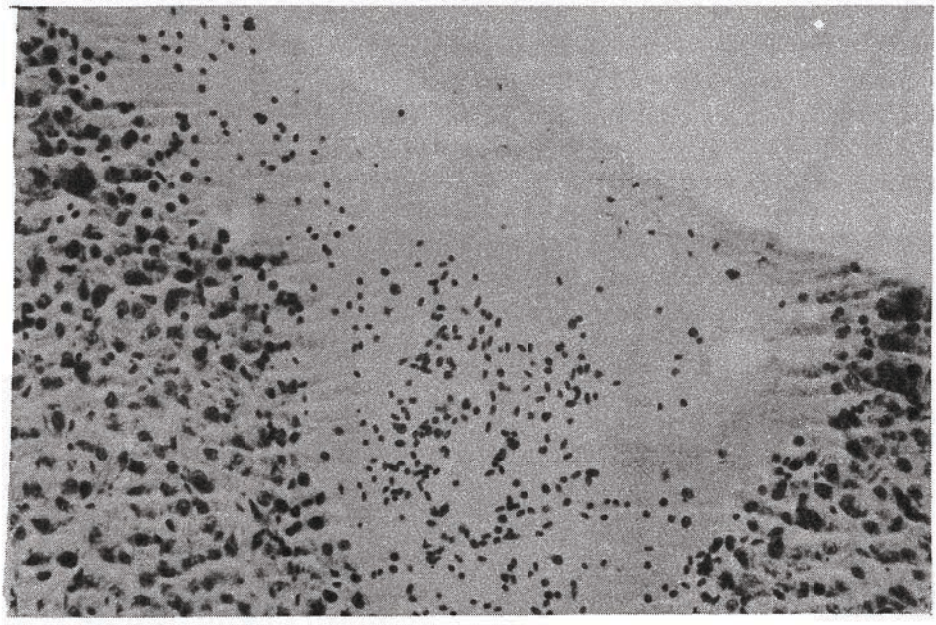


Fig. 3. A complete hepatic tissue lysis induced by $Ca(NO_3)_2$

(Fig. 4). In spite of a normal tissue chromasia, small necrobiotic centres were found. The blood vessels were clearly visible, considerably filled with blood without any regressive lesions.

A similar picture is noted for the II period with only small intratissue effusions from the pre-capillar type small vessels being present mostly. These are the early effusions since no regressive lesions of the adjoining tissue and the blood cells themselves were observed.

Beginning with the III period, the tissue picture changed markedly. The hepatic cells only seldom exhibited the previously described vacuolization. The protoplasm, dim and containing granulations, filled the entire cell; this stage could be defined as a dim degeneration. The hepatic cells' nuclei showed no changes at first, but later on they degenerated as discussed below.

Poorer tissue chromasia centres of a possible early necrobiotic nature occurred in this time. Additionally, small lytic centres, sometimes occupying a rather vast area were also present. In the extremal cases, considerable intra-tissue spaces were filled with a denatured coagulate.

On the other hand, rather large intra-tissue spaces were found empty, regularly spheric with no trace of any coagulate. This probably resulted from a smaller destruction of the blood vessels as well as from liberation of gases from the blood into surrounding tissues what had previously been suggested in case of similar structures $Ca(NO_3)_2$ – induced (Fig. 5). Undoubtedly, they had been formed in the living fish since the tissue fixation always followed killing of the fish. Those blisters show no trace of their own walls and are surrounded by the hepatic cells with no pathologic changes.

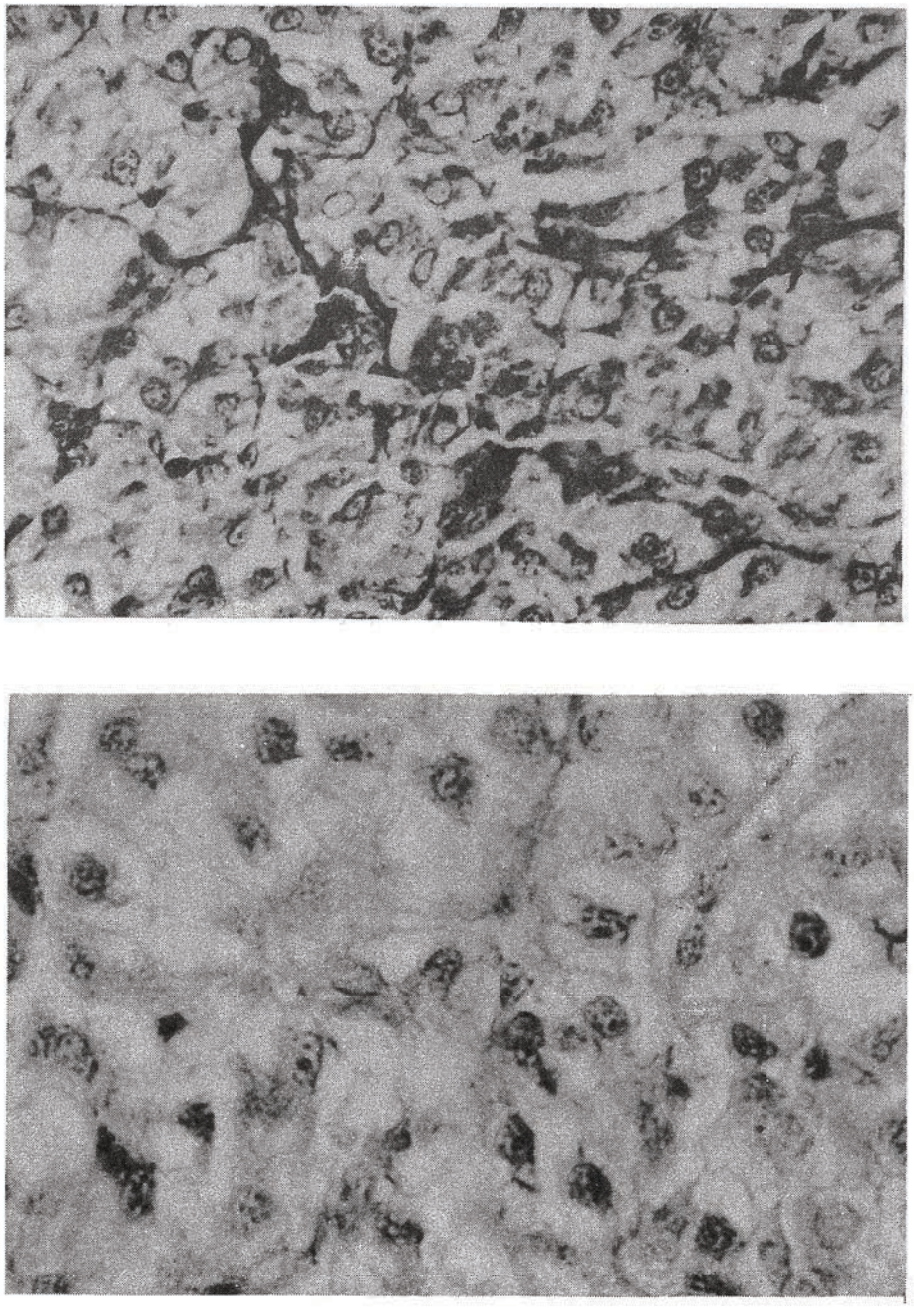


Fig. 4. A mosaic-like appearance of the hepatic tissue resulting from a hepatic cells vacuolization induced by KNO_3. B – under a greater magnification

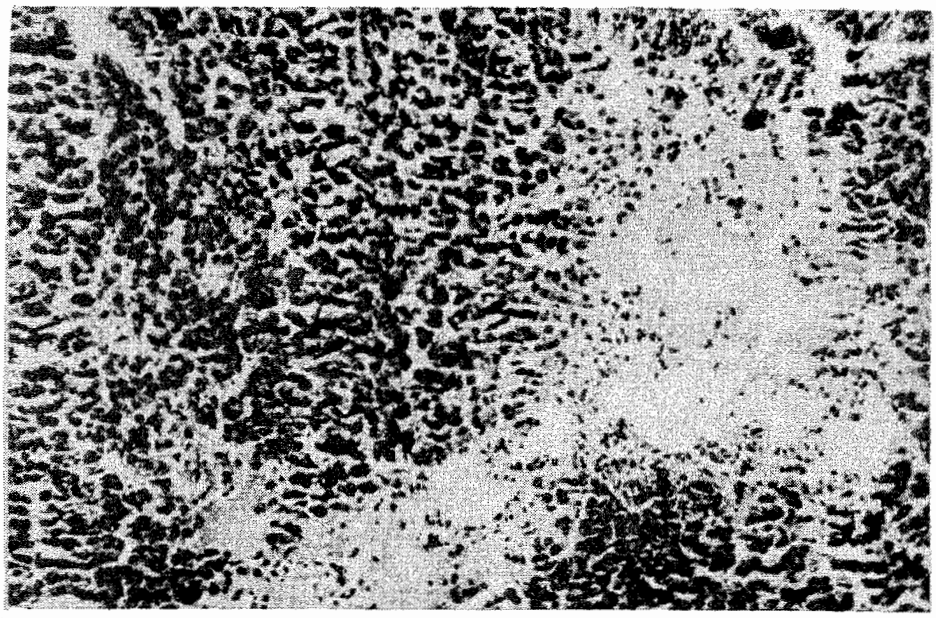


Fig. 5. An extensive KNO_3 – induced necrotic centre in the hepatic tissue; inside visible numerous blisters filled with gas, partially fused

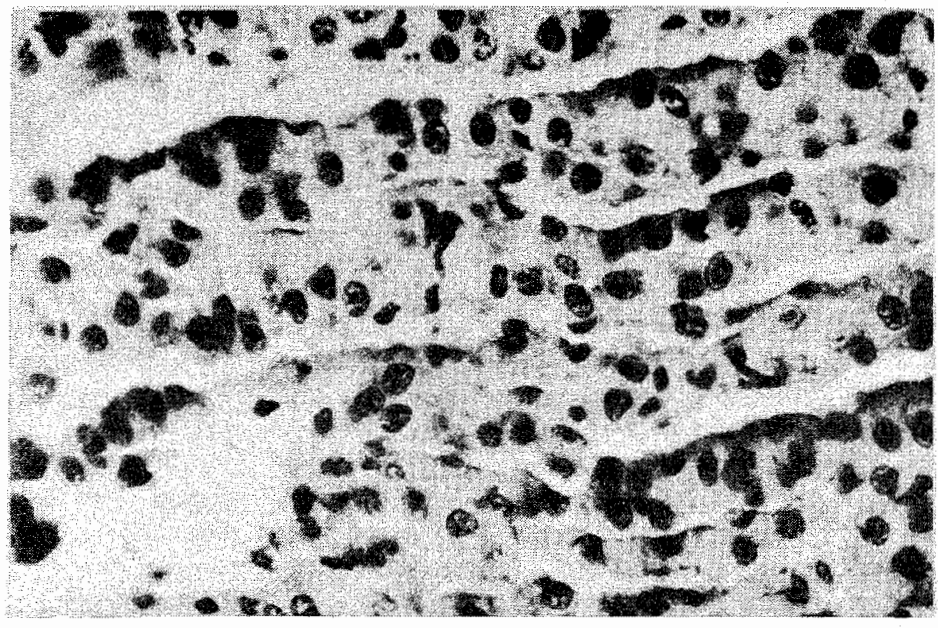


Fig. 6. A KNO_3-induced necrobiosis of the hepatic tissue. A trabecular structure still remains, but with a complete obliteration of the cells within the trabeculae. Lytic processes strong by pronounced in places

In the IV period, the changes within the hepatic cells' protoplasm proceeded further on. The protoplasm became more dim with a „dirty" shade, of various staining properties: in spite of the same procedure the shade was more blue (basic) or red (acidic) (Fig. 6). The cell nuclei changed markedly too. They lost their staining properties completely, their chromatin granulations vanished. The nuclei got a form of empty blisters poorly distinguishable from the protoplasm.

The hepatic trabeculae spacing became slightly greater, but never to an extent as in case of $Ca(NO_3)_2$.

Beginning with the II period, the hepatic tissue contained cells clearly distinguishable by their dark colour, with narrow, mostly falciform nuclei. Presumably they were capillar endothelium cells, perhaps the Kupffer ones of the reticulum-endothelium system. A strong irritation could have activated the resulting in their visibility not observed under normal conditions.

As a rule, the erythrocytes did not show the changes described in the $Ca(NO_3)_2$ section. Most vessels were abundantly filled with normally-stained blood cells. Only exceptionally the stasis parts were encountered to a small extent a well as less advanced changes in the blood cells.

Hemosiderin granulations were present to a considerably smaller scale than it was the case with $Ca(NO_3)_2$.

HEMATOLOGIC CHANGES

Peripheral blood

The erythrocyte number in the rainbow trout peripheral blood decreases under the influence of both, potassium and calcium nitrates. The range of changes as compared to the control level averages from 0.2–0.4 million per mm^3 (Table 1, Fig. 7A). The fishes kept in the potassium nitrate-treated water showed a decrease in their erythrocyte numbers after 50 days of the experiments, while those in calcium nitrate after 30 days. A low erythrocyte level remains in both groups during the rest of the experiment time.

The hemoglobin value (determined together with methemoglobin) remains on an almost even level ranging from 4 g% to 7 g% during the whole experiment; in one case only it decreased down to 3 g% with a marked drop in the erythrocyte number. The hemoglobin amount of the control fishes ranged within 6–7 % (Table 1, Fig. 7B), the hemoglobin capable of oxygen assimilation and transport being present in significantly smaller quantities in the experimental fishes.

The hematocrit index comparative analysis gives no unequivocal result (Table 1, column B). Both groups of fishes, in the II period (after 50 days) showed a discernible increase in the blood cell volumes reaching up to 10% of the control value in case of $Ca(NO_3)_2$. Later, in the III and IV periods, the volumes decreased to 9% (KNO_3) and 10.17% ($Ca(NO_3)_2$).

The leukocyte number in the experimental rainbow trout' blood is usually within the limits of the minimum value for the control fishes (about 8,000–10,000/mm^3) (Table 1,

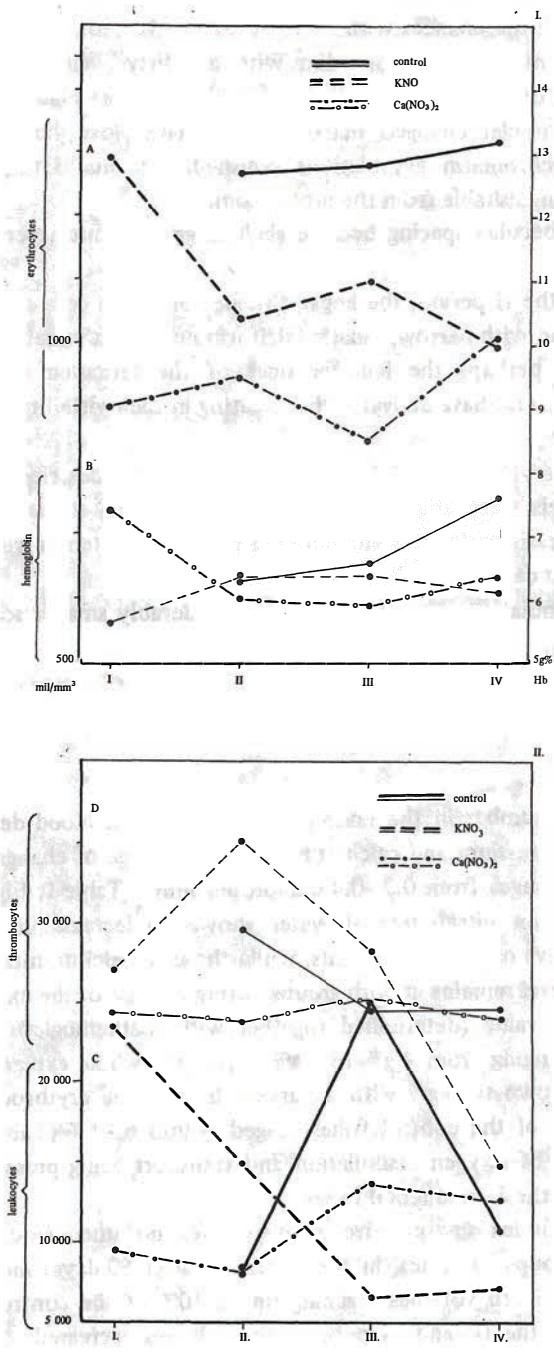


Fig. 7. Differences between the erythrocyte numbers (arithmetic means) (A), hemoglobin content (B), leukocyte (C) and thrombocyte (D) numbers in the peripheral blood of rainbow trout kept in the KNO_3 – and $Ca(NO_3)_2$ – treated water

Table 1

KNO_3 – and $Ca(NO_3)_2$ – induced differences in hematologic indices of the rainbow trout peripheral blood

Period of experiment	Number of fishes	erythrocytes million/mm^3	hematocrit %	hemoglobin g%	leukocytes thousand/mm^3	thrombocytes thousand/mm^3
		a	b	c	d	e
I control	–	–	–	–	–	–
KNO_3	4	1.29	50	5.64	23.4	27.0
		1.32–1.27	47–55	5.15–6.64	23.2–34.8	23.2–34.8
$Ca(NO_3)_2$	4	0.90	49	7.37	9.5	24.4
		0.60–1.29	47–52	7.25–7.49	4.6–14.4	24.4–24.4
II control	4	1.27	47	6.32	8.4	29.6
		1.10–1.48	30–48	5.50–7.35	5.20–11.6	28.0–30.8
KNO_3	4	1.04	57	6.32	14.9	35.3
		0.89–1.25	48–68	4.85–7.58	12.8–18.8	30.0–43.2
$Ca(NO_3)_2$	4	0.94	51	6.04	8.3	23.8
		0.72–1.17	48–55	4.3 –6.52	4.8–10.8	16.4–38.0
III control	3	1.28	59	6.56	25.0	24.7
		1.10–1.39	58–60	6.26–6.76	19.6–34.8	20.0–23.2
KNO_3	4	1.10	49	6.39	6.7	28.3
		0.92–1.10	48–51	5.25–7.35	4.8–9.2	25.6–29.6
$Ca(NO_3)_2$	3	0.85	42	5.93	13.7	25.0
		0.74–0.97	36–51	5.05–6.81	12.0–16.4	18.8–30.4
IV control	8	1.32	54	7.62	10.8	24.7
		1.15–1.51	40–59	6.69–8.13	6.4– 15.6	20.0–28.0
KNO_3	8	0.99	45	6.23	7.1	14.8
		0.44–1.23	40–49	3.04–7.94	2.8– 17.6	1.4–23.0
$Ca(NO_3)_2$	8	1.01	45	6.32	12.6	24.2
		0.78–1.09	36–54	4.26–7.71	6.8–19.3	19.2–28.0

Table 2

Numerical characteristics of the peripheral blood

Cell type	Peripheral blood composition									
	w %					1 mm^3				
	Control	I	II	III	IV	Control	I	II	III	IV
KNO_3 Lymphocytes	91 83–97	83 81–87	77 62–82	78 58–93	63 42–87	13.428	19.422	11.473	5.226	4.473
Young granulocytes	5 2–7	13 9–12	10 7–13	8 2–13	9 3–18	737	3.042	1.490	536	639
Segmented granulocytes	4 1–12	4 1–7	13 7–25	14 5–31	28 5–50	590	939	1.937	938	1.988
$Ca(NO_3)_2$ Lymphocytes		63 53–85	77 69–91	89 84–91	62 18–86		5.985	6.391	12.193	7.812
Young granulocytes		13 7–22	12 3–15	5 2–7	13 2–28		1.235	996	685	1.638
Segmented granulocytes		24 8–35	11 6–17	6 4–9	25 12–54		2.280	913	822	3.150

column d; Fig. 7C). Potassium nitrate gradually decreases the leukocyte number down to 6,700/mm^3 while with the calcium salt it is constantly low, in average 13,700–8,300/mm^3.

A similar pattern of numerical values is typical of the peripheral blood thrombocytes. Under the influence of KNO_3, beginning with the II period their number drops to about 14,800/mm^3, while with $Ca(NO_3)_2$ it remains all the time on the control level, i.e., 23,800–25,000/mm^3 (Table 1, column e; Fig. 7D).

The peripheral blood composition changes within the both groups in different way depending upon a kind of nitrate in the water (Table 2). Potassium nitrate gradually decreases the lymphocyte number down to 63%, i.e., 4,473 cells per mm^3, the control level being about 13,000/mm^3. The granulocyte number is altered, too: in general, the segmented granulocytes are more abundant (by 28%), periodically the young stages of these cells grow in number, especially so after the I and II periods.

The blood of the rainbow trouts kept in the calcium nitrate-treated water contains always a smaller amount of lymphocytes than that of the control fishes, the lowest number being noted in the I and II periods. Moreover, both the young and mature segmented granulocytes increase their numbers; a higher amount of these cells is maintained during the whole experiment, the highest values being recorded in the IV period (up to 1,638 young and 3,150 segmented granulocytes per mm^3, the control values being 737 and 590/mm^3, respectively).

Apart from the quantitative changes, the morphologic alterations of the blood cells were also observed, dependant upon the kind of nitrate used. In the peripheral blood of fishes kept in the potassium nitrate-treated water a mass appearance of early erythrocyte forms in various developmental stages including basophilic erythroblasts was evident after 30 days (Fig. 8). In the blood smears the polychromatic erythrocytes prevail showing a clear size-differentiation and poikilocytosis symptoms. Macrocytes are relatively abundant among them; also the mature erythrocytes with strongly basophilic protoplasm occur (Fig. 9A). The properly staining erythrocytes are scarce (a few in each field of view), while microcytes with improperly situated nuclei predominate. Both amitotic and mitotic divisions of the basophilic erythroblasts are encountered as well.

Polychromatophilia is typical of the erythrocytes; most blood cells have grey protoplasm with numerous vacuoles. The younger stages, the basophilic erythroblasts in particular have their protoplasm unevenly concentrated or poorly staining, showing spotted bright intervals usually around the nuclei.

A significant percentage of the erythrocytes shows necrobiotic lesions of a considerable intensity; in the pre-disintegration stages the nuclei structures are coarsely granulated, loosened or completely obliterated and „washed away". The other blood elements, particularly the granulocytes are fine and show the nuclei pyknosis in various stages of development as well as a poorly staining vacuolized protoplasm. Many cells, especially the thrombocytes are deformed, certain elements even disintegrate.

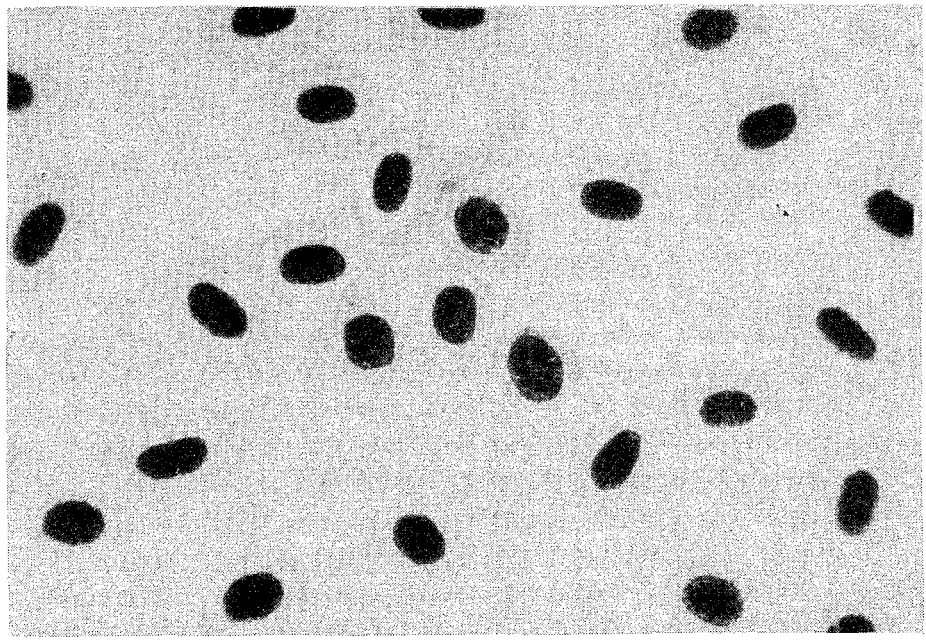


Fig. 8. Irregularly-staining early erythrocyte forms with basophilic erythroblasts prevailing in the peripheral blood of rainbow trout kept in the KNO_3 – treated water

The blood smears show almost exclusively the polychromatic and basophilic erythrocytes after 50 and 66 days (the II and III periods, respectively), the erythroblastic reaction and mature well-staining erythrocytes are missing. Anisomicrocytosis symptoms and in some of the samples also those of poikilocytosis are sharpened. The number of macrocytes is generally high. The regressive lesions are also marked, appearing either as swellings and looseness of the chromatin reticulum or as hypochromatosis and pyknosis of the nuclei.

The disintegration processes become intensified also in the granulocytes and, to a smaller extent, in the lympho- and thrombocytes, cells of various stages of degeneration, necrosis and pyknosis being found. The young blood cells (granuloblasts and progranulocytes) as well as the mature ones with slight regressive lesions appear in the blood of some fishes in the III period of the experiment.

In its final stage, after 80 days, pyknosis is even more pronounced, aniso- and poikilocytosis as well as the erythrocyte chromatophilia being more acute, too. The polychromatic erythrocytes still prevail while the erythroblasts are found sporadically. All the forms are altered due to an advanced degeneration. The necrobiotic process affects also the other blood elements, the lymphocytes included. The normal granulocytes are rare while the strongly deformed ones with their protoplasm poorly or non-staining and pyknotic nuclei predominate.

With the quantitative indices of the peripheral blood considerably decreased (a single case in the IV period of the investigation), pathologic lesions of the morphologic blood

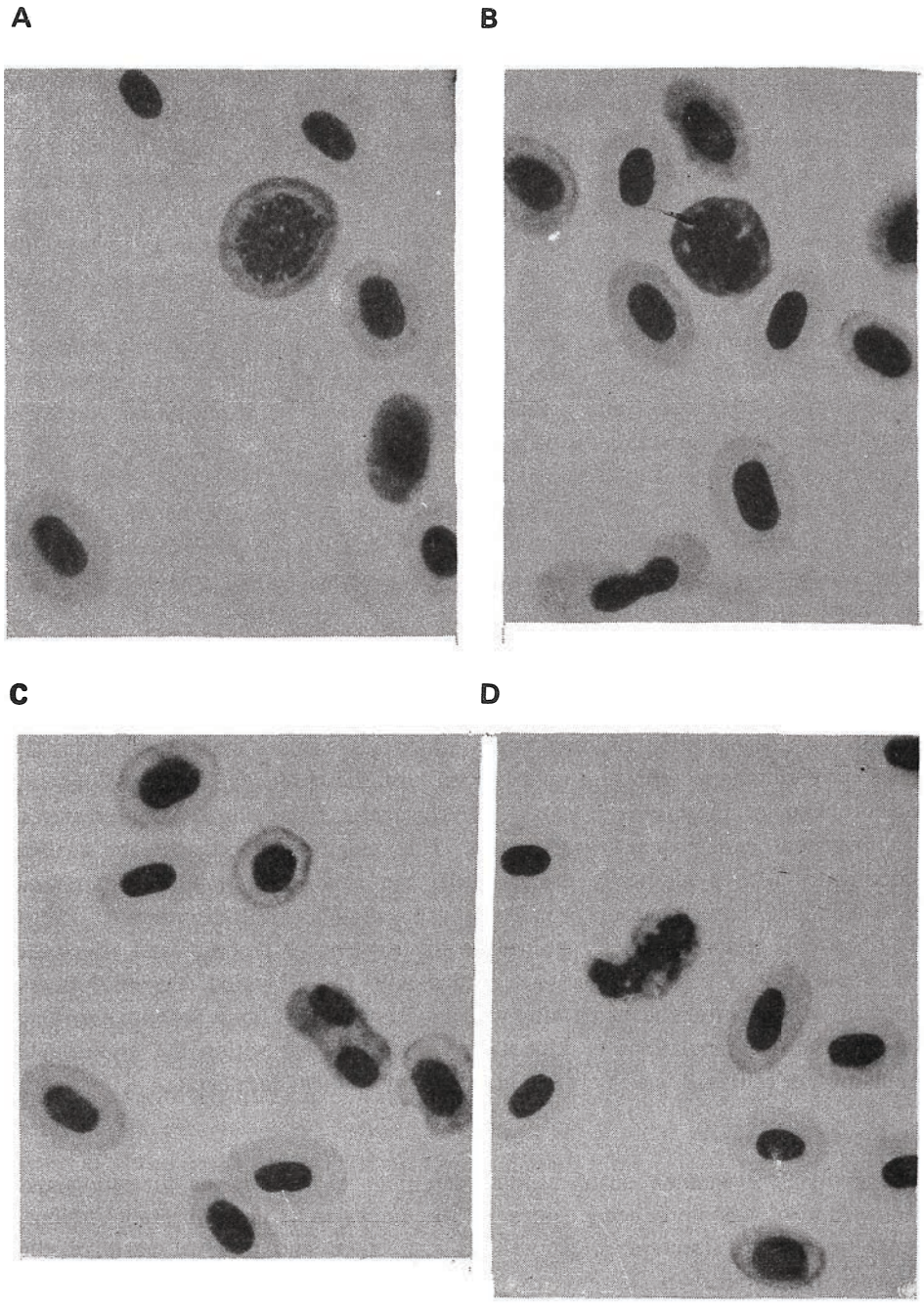


Fig. 9. The peripheral blood of rainbow trout kept in the KNO_3-treated water: A) a macrocyte-type basophilic erythroblast; B) a basophilic erythroblast (macrocyte) in a degenerated form together with basophilic and polychromatic erythroblasts; a single blood cell in amitosis; C) a basophilic erythroblast in mitosis; D) a mitotic division of a degenerating basophilic erythroblast

picture become more acute and lead to an extremal form: hyperchromatic, early basophilic macrocyte- and poikilocyte-type erythrocytes are in majority among the blood cells. Non-typical forms with large deformed nuclei and abnormal chromatin patterns are discernible, the cells resembling paraerythroblasts. Also the proerythroblasts and mitotic figures of various stages of chromosome translocation are abudant. Amitotic divisions of the basophilic and polychromatic erythrocytes are more common, the schizocytes appearing in numbers (Fig. 9A, B, C, D).

A changed protoplasm of the polychromatophilic erythrocytes contains basophilic granulations while that of the other cells is non-homogenous, finely granulated with small bright intervals appearing as minute vacuoles. The nucleic chromatin in the erythrocytes is usually coarsely granulated, irregularly displaced, densed, and showing some tendency to pyknosis. Degenerative lesions affect the other forms as well, a compensating reaction of young granulocyte stages and granuloblasts being evident. Single phagocytes with a greenish pigment appear within the protoplasm.

As far as the experiment with calcium nitrate is concerned, the changes in the blood picture are more intense and homogenous from the beginning. After 30 days only a moderate reaction, involving basophilic erythroblasts occurs; the blood cells' size differentiation is poor, micro- and macrocytosis being weak. Single erythrocytes are encountered during the amitotic division.

Advanced regressive lesions of both the erythrocytes and leukocytes reflect more profound disturbations. A considerable amount of the blood elements shows obliterated structures at the pre-disintegration stage. The erythrocytes display this phenomenon essentially as a degeneration of their nuclei which are swollen with loosened, partly washed away chromatin reticulum or deformed with the chromatin dense and opaque. The protoplasm of these cells is usually polychromatic, basophilic at younger stages, non-homogenous with patched bright spots. The remaining blood cells are smaller, predominantly with pyknotically densed nuclei and abnormally staining protoplasm. Most cells exhibit blurred contours or indistinct cell walls. Many cells disintegrate.

The pyknotic lesions evidently deepen as the experiment proceeds and affect the whole population of the blood cells. Beginning with the III period, the erythrocyte protoplasm is hypochromatic and hyaline with greyish-yellow or, more seldom, grey-blue shades. Basophilic granulations or blue-stained needles appear within the erythrocyte protoplasm. The cells walls are finely crenulated; many of them disintegrate.

Hematopoietic organs

The cytologic evaluation enabled us to determine changes in the formation and differentiation of blood-producing centres of the kidney in its three different sections, i.e., the pronephros (section 1), as well as the initial and terminal parts of the mesonephros (section 2 and 3, respectively). Distempers of the hematopoietic action altered with time of the experiment and differed in the intensity of changes.

An intensified proliferation of the erythrocytes in the pronephros and the initial part of the mesophephros (Fig. 10) was found after 30 days of potassium nitrate influence. Within the intra-tissue parenchyma mainly the hemocytoblasts and proerythroblasts

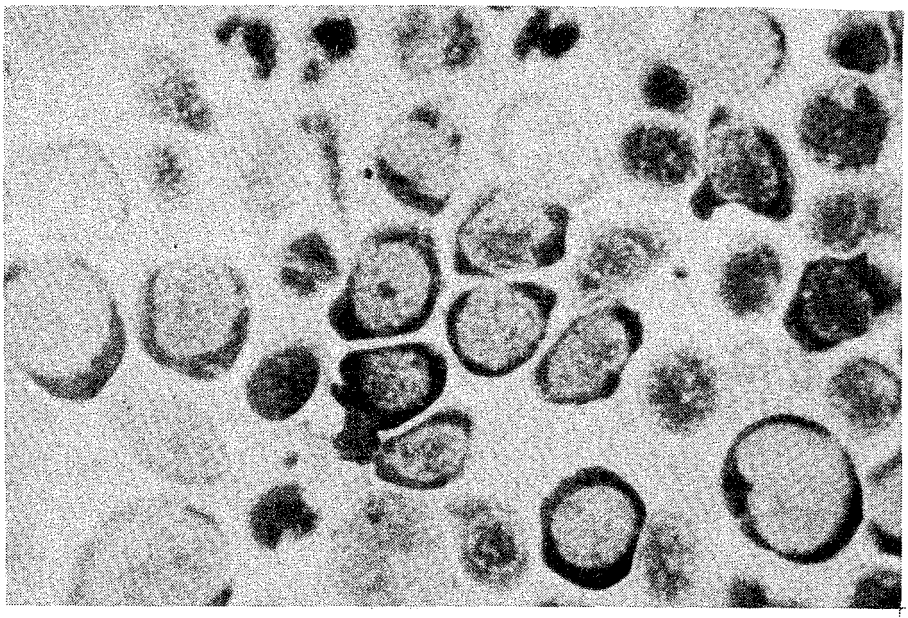


Fig. 10. An intensified proliferation of the erythrocyte-order cells in the kidney of rainbow trout kept in the KNO_3-treated water (the I period)

occurred, less so the basophilic erythroblasts in various stages of maturity (earlier and later ones). On the other hand, only a few polychromatic erythrocytes were found, those completely mature with an acidophilic protoplasm occurring in the lowest numbers. The erythroblastic system distempers involve a retardation and distortion of the maturity process along with proceeding regressive lesions. In the initial period, the maternal cells show small deviations from the normal state, containing a non-homogenously stained and coarsely striped nucleus chromatin. Greater changes take place in the younger stages of basophilic erythroblasts, hyperchromatic macrocytes with unevenly densed nucleus chromatin being found most frequently. Further stages of the basophilic erythrocytes are small (microcytes), deformed (schizocytes), with their protoplasm abnormally staining and streaky. More mature forms of the polychromatic erythrocytes are strongly degenerated with enlarged nuclei which are poorly stained and obliterated or pyknotically deformed. In the grey non-homogenous protoplasm of these cells yellowish vacuoles appear as inclusions. Pathologic mitoses are numerous.

Relatively small is the number of granulo- and lymphopoietic centres. Free cells of the reticulum and phagocytal forms, i.e., the so-called phagocytal cells of the reticulum are abundant showing yellow- or blue-grey vacuoles in their protoplasm. The other macrocytes found in the greatest quantity within the kidney section 2 are large cells filled with green pigment grains.

In the terminal part of the mesonephros (section 3), vast necrotic areas with degenerated cells are observed. The erythrocytes with pyknotic nuclei separated from the

protoplasm prevail. Loosely distributed naked nuclei as well as, small papules of thickened chromatin and plasm of yellow- or blue-grey colour occur in masses. The phagocytal cells of the reticulum are very abundant. The amount of the forms mentioned increases with disintegration processes.

After 50 days of the experiment, the kidney parenchyma in the sections 2 and 3 still contains various cells, the original centres, particularly those of erythropoiesis, prevailing. However, secondary disintegration processes become acute and a stronger destruction of the blood cells occur, especially around the kidney canalliculi necrotically changed. The picture of the tissue is changing; apart from regularly differentiated cell groups the hypochromatic zones are extended containing cells with nuclei of obliterated structures. Simultaneously, centres with intensified pyknotic lesions are encountered, degenerated erythrocytes prevailing in them. Within such smears naked nuclei and huge protoplasm clots, most often yellow-grey, are observed. Some blisterous forms resemble nuclei-less erythrocytes with granulated basophilic incisions. The phagocytosis processes are intensified; much more reticulum cells with grey-yellow vacuoles are present.

The disintegration processes in the kidney section 3 are more pronounced; the kidney parenchyma consists almost exclusively of free nuclei, clots of protoplasm, phagocytal cells, and decaying blood elements. Also the maternal cells in the hematopoietic centres are subject to necrosis.

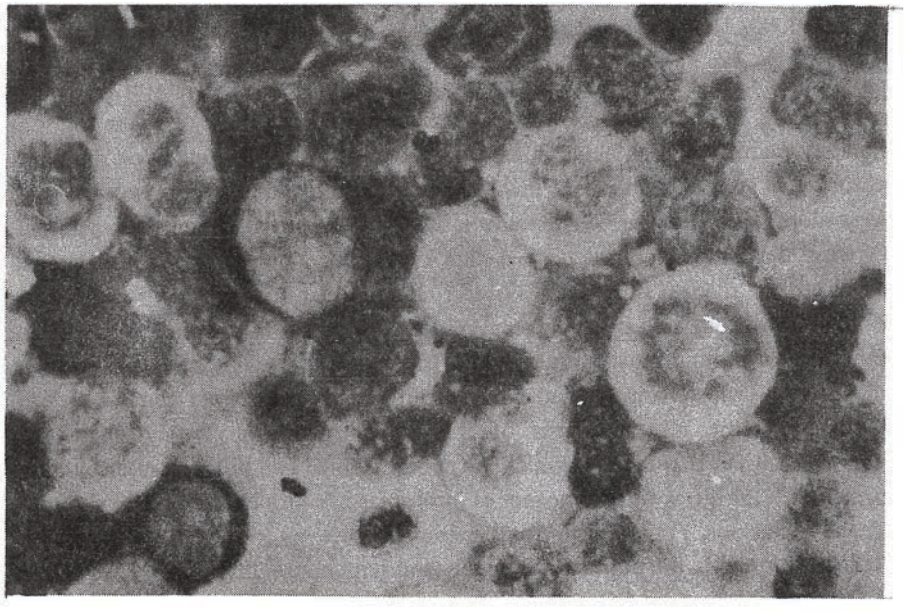
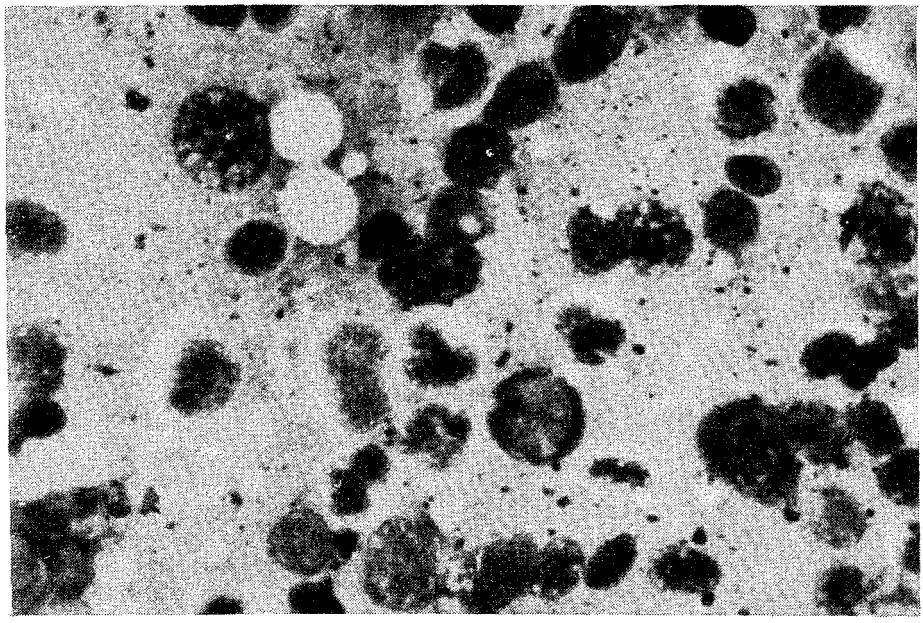


Fig. 11. An intensified differentiation process in the erythrocyte, – and leukocyte-orders of cells in the III period of the experiment with KNO_3

After 66 days, the changes and kidney blood composition are slightly different than the above description. The original cells, mainly the granulocytes, appear in reproductive centres (Fig. 11). Regular mitotic divisions of the early cells of this order are frequent.

A



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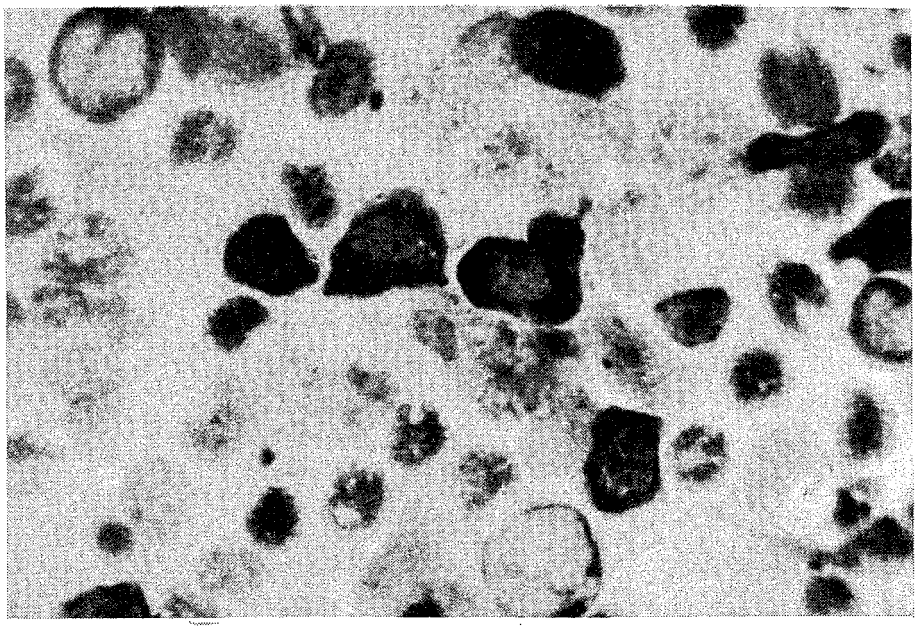

Fig. 12. The kidney parenchyma cells' degeneration in the IV period of the experiment with KNO_3: A) small amounts of degenerating granulocytes in the pronephros, B) single erythroblasts in the mesonephros

A moderate granulopoiesis takes place also within the terminal section of the mesonephros along with necrobiotic and necrotic processes proceeding at their strongest within that part of the kidney.

In the final stage of the experiment, after 80 days, the whole kidney parenchyma exhibits a domination of necrobiotic and necrotic processes. Even the maternal forms of the blood- producing systems degenerate (Fig. 12A, B). The giant forms of hemocyto-, proerythro- and granuloblasts as well as the reticulum cells appear in two cases within the hematopoietic centres.

The regressive changes taking place in the calcium nitrate-treated water are of a greater range and intensity from the beginning of the experiment. In the I period, either moderate or excessive granulopoiesis centres occur with segmented forms prevailing together with disintegration zones. Mitoses of the cells of this order are numerous while the erythrocyte differentiation process is poorly developed. The later-stage basophilic erythrocytes are most abundant, clearly polychromatic mature ones being generally in the minority. The erythrocyte population degeneration is stronger, too, in some places occurring in the maternal forms. The intensity of changes within these cells in comparable to that of the IV period with potassium nitrate applied.

The prolonged duration of the experiment results in more deep necrotic lesions which can affect substantial parts of the kidney. Its parenchyma is non-homogenous; at the same time areas of various intensities od degeneration occur, containing hypochromatic

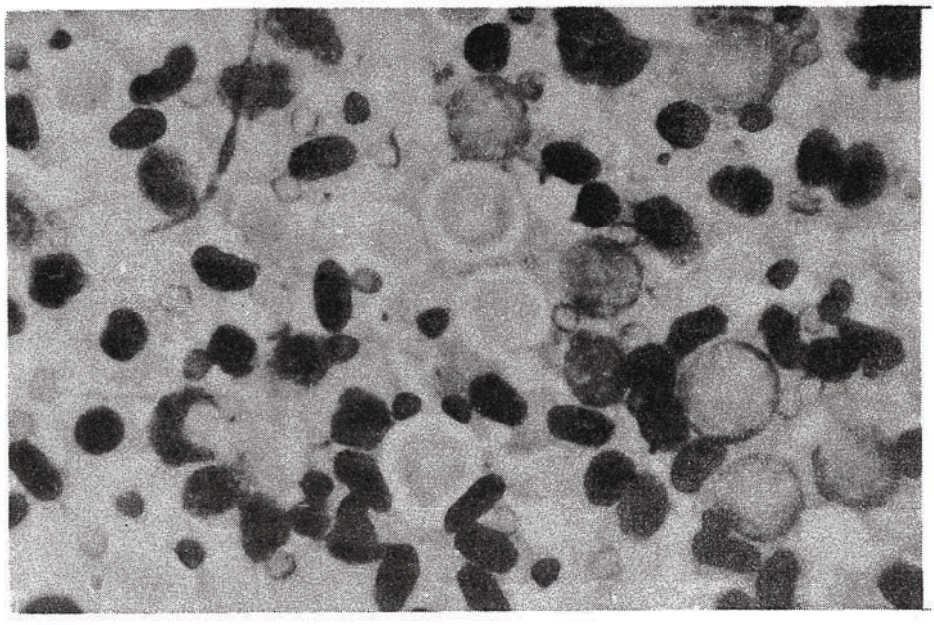

Fig. 13. A cell disintegration within the spleen parenchyma; numerous erythrocyte nuclei devoided of their protoplasm as well as a poorly staining granulocytes and other cells

cells disintegrating during the chromato- and karyolysis as well as hyperchromatic ones pyknotically deformed. Within the destructed tissue mixed with an amorphous mass and granulations, individual aggregations of properly differentiated cells remain. Phagocytal processes decrease with time of the experiment, many cells with green pigment grains occuring, however. Generally speaking, the pronephros is more damaged in this series of the experiments whereas the cell proliferation in the terminal part of the mesonephros is recorded more frequently. These centres could possibly be of a compensating reaction nature.

In both the experiments, the spleen shows an intensified destruction of the blood cells, particularly the erythrocytes. The spleen structure is almost completely devoided of any cell elements; naked nuclei, parts of chromatin, loose clots of protoplasm, and poorly staining lymphocytes occur in small amounts. The erythrocytes in a disintegration stage are noted only sporadically. Slightly more cell elements remain in spleens of the fishes kept in the calcium nitrate-treated water. All cells, however, show signs of degeneration (Fig. 13).

Recapitulations

Generally speaking, nitrate cause the hypochromatic anemia with a clear anisomicrocytosis, poikilocytosis, and polychromatophilia. Besides, the erythrocyte population is most often mixed with hyperchromatic macrocytes, basophilic erythroblasts prevailing. In a single case of a strong insufficiency, a typical macrocyte hyperchromatic anemia occur.

At the same time, a moderate leucocytopenia takes place periodically, with alterations in the peripheral blood compositions involving, an increase in the number of mature or younger granulocytes (granulocytosis). The amount of thrombocytes decreases (thrombocytopenia) in the blood of the fishes kept in potassium nitrate-treated water.

The symptoms of anemia result from distempers in the blood cells production within the hematopoietic centres, caused, by the iron defficiency and abnormal hem synthesis. An excess of immature erythroblasts and erythrocytes containing no hemoglobin or its lowe amount (oligosideremia) is typical of these changes. In the kidney hematopoietic centres, a retardation in the blood cells' maturing process occurs at the stage of basophilic erythroblasts. At this stage their abnormal differentiation as well as degeneration begins. As a result, an intensified proliferation of cells of this order in the hematopoietic centres accompanied by changes in the peripheral blood picture takes place. The erythrocyte population together with renewed erythroblasts shows pathologic features and gradually undergo the destruction (erythrocytopenia). In the course of experiments, the necrobiotic and necrotic processes in the hematopoietic centres become more intensified apart from a stronger phagocytosis. These processes are accompanied by a hyperfunction of the spleen (*hypersplenismus*) caused by an excessive destruction of the cellular blood components.

METHEMOGLOBIN OCCURRENCE IN BLOOD

Parallel with the morphotic examination, the peripheral blood methemoglobin level was measured. Table 3 demonstrates the methemoglobin contents in the blood of fishes examined.

The control fishes' methemoglobin content of $\bar{x} = 0.81$ was assumed as a referrence point for the subsequent determinations. Mean values ranged from $\bar{x} = 14.97$ and $\bar{x} = 25.47$ at the beginning of the experiment to $\bar{x} = 21.15$ and $\bar{x} = 26.93$ at the end for $Ca(NO_3)_2$ and KNO_3, respectively.

As the data summarized in Table 3 evidence, a kind of a nitrate as well as duration of its influence on the fish organisms affect the methemoglobin percentage in the blood.

Moreover, the individuals examined have a different MetHb content in each experimental period since the methemoglobin-producing action of the compounds applied depends on metabolic processes taking place simultaneously.

The methemoglobinogenic compounds act towards an oxidation of the two valent iron hemoglobin to a three-valent iron one. Thus the normal hemoglobin function of the oxygen transport becomes hindered. The iron porphirin compound oxidized to a ferric form binds no oxygen and becomes useless for respiration (**Williams**, 1947).

In mammals suffering from strong methemoglobinemia, with the blood MetHb content exceeding 40–50%, the oxygen transport and release in tissues is handicapped (**Hłyńczak** and **Sysa**, 1968). The histopathologic changes as well as the hepatic tissue respiration rate indicate an earlier occurrence (20–25% MetHb) of the process in the fishes (rainbow trout).

CHANGES IN THE HEPATIC TISSUE RESPIRATION RATE

The metabolism of the whole organism reflects the processes occurring in particular organs. It has been assumed purposeful to measure the liver respiration metabolism of rainbow trout affected by calcium and potassium nitrates in order to determine changes in the hepatic tissue oxygen requirement under defined experimental conditions.

Effect of calcium nitrate $Ca(NO_3)_2$

In the first period of exposing the fishes to unfavourable conditions, the hepatic tissue respiration intensity dropped by 18%, the value being maintained throughout the entire second period. Beginning with the third period, the oxygen consumption increased only for a very short time. In the next period, the respiration rate was more and more decreasing and towards the end of the V period reached a value almost four times smaller than the initial one*. The course of changes in the respiration metabolism as affected by calcium nitrate is almost parallel to adaptation changes in the control fishes, which partly explains a temporary increase in the oxygen consumption recorder in the III period (Fig. 14).

*Because of significant differences in fish tissue respiration rates, the last two days of the fourth period were separated and treated as the V period.

Table 3

Methemoglobin percentage in the blood of rainbow trout, *Salmo gairdneri*

	Number of fishes	5.XII.1972	Number of fishes	18.XII.1972	Number of fishes	3.I.1973	Number of fishes	17.XII.1973
Normal level	4	$\bar{x}$ = 0.79±0.61	4	$\bar{x}$ = 1.01±0.59	3	$\bar{x}$ = 0.93±0.56	8	$\bar{x}$ = 1.07±0.42
$Ca(NO_3)_2$	4	$\bar{x}$ = 14.97±5.78	4	$\bar{x}$ = 16.88±1.36	4	$\bar{x}$ = 20.49±3.49	8	$\bar{x}$ = 21.15±6.72
KNO_3	4	$\bar{x}$ = 25.47±5.78	4	$\bar{x}$ = 27.10± 3.58	4	$\bar{x}$ = 27.95±5.95	7	$\bar{x}$ = 26.93±5.11

$\bar{x}$ – mean values from n determinations

± – standard deviation

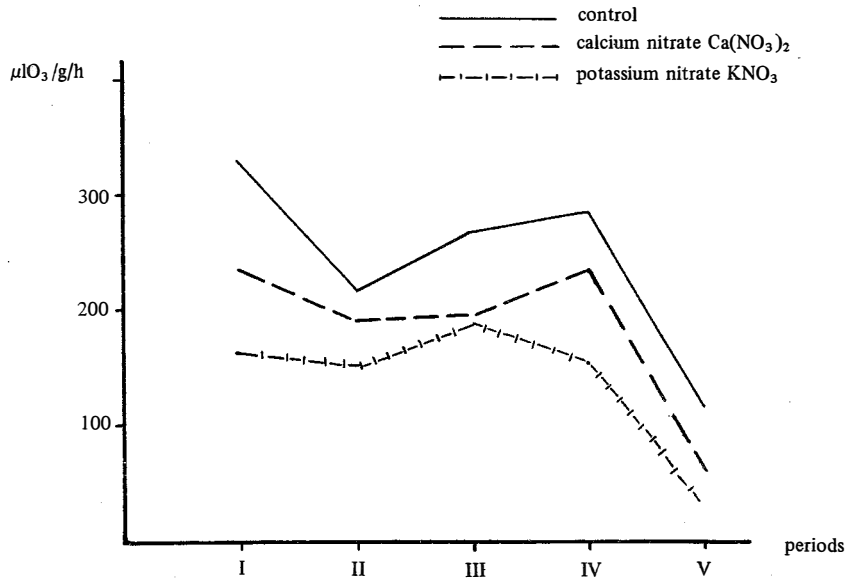

Fig. 14. The influence of KNO_3 and $Ca(NO_3)_2$ on the hepatic tissue respiration rate expressed as the oxygen consumption in μlO_2/g/h

Compared to the control group, the oxygen consumption intensity in the fishes influenced by calcium nitrate decreased by 28% in the first period, while at the end of the fifth the decreased was much more pronounced, amounting to 48%.

Effect of potassium nitrate KNO_3

Similarly to the $Ca(CO_3)_2$ effect, a decrease in the oxygen consumption amounting to 9% was observed in the first period. The next one produced a slight oxygen consumption increase for a short time, then beginning with the III a more and more evident decrease in the respiration rate appeared reaching at the end of the V period a value smaller by almost 76%, i.e., 5 times greater as compared to the original one.

When the respiration rate of the potassium nitrate-affected fishes was compared to that of the control ones, very significant differences were found, particularly in the I period: a decrease in the respiration rate by 48% being evident then. The smallest differences appeared within the II period, a decrease amounting to ca. 27%. Presumably the fishes adapted themselves to a certain extent to the altered conditions of life. In the later phase of the IV period the respiration rate is four times slower than the initial value (Table 4).

The present studies suggest a strong adverse effect of nitrate on the hepatic tissue respiration rate in rainbow trout; the rate is considerably decreased at the end of the experiment leading to an almost negligible oxygen consumption in the organ and consequently towards an agony.

Table 4

Oxygen consumption in livers of nitrate-affected rainbow trout
(expressed as $\mu l\ O_2/g$ wet weight/h)

Period	Control		$Ca(NO_3)_2$		KNO_3	
	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ
I	328	15	235	19	166	10
II	216	12	193	15	151	11
III	265	20	194	10	192	9
IV	283	17	220	12	153	7
V	115	11	61	8	22	5
	254		180		140	

$\bar{x}$ – arithmetic mean
σ – standard deviation

DISCUSSION OF THE RESULTS

The experiments described were designed to ascertain the influence of nitrates upon methemoglobinemia in the peripheral blood of fish, rainbow trout (*Salmo gairdneri*) in particular. Relatively low concentrations of $Ca(NO_3)_2$ and KNO_3 were applied, 26.2 and 30.6 mg/l, respectively. The differences in the NO_3 concentrations, in our opinion rather an insignificant factor, resulted fortuitously from disregarding the chemical composition of both salts and taking the equal amounts (50 mg/l) of each. The K and Ca salts, however, were used deliberately in order to establish possible differences in their effects.

Both salts turned out to be highly toxic for rainbow trout and eventually strongly damaged the fish organisms, their death being the final result. Since the experiments had been planned to assess the methemoglobinemia – producing influence of nitrates and the fishes were still very active in the last period, they were left alive on the outset of the experiment. The last examination (V) was combined with the IV one without a fortnight interval as in the previous cases. As the elaboration of the results revealed, the fishes examined in the V run appeared to be in a condition that probably would not permit them to survive the last two weeks, the histopathologic changes as well as the peripheral blood hematology and hematopoietic organs being taken into account together with the hepatic tissue redox potential.

Both nitrates appeared to cause profound and irreversible changes tending to destruct the fish organism. The differences between the two salts are found only in the dynamics of their action, not in the final result.

At the first place a profound effect on the blood system occur. It is improbable that only a partial elimination of hemoglobin through its inactivation in the form of

methemoglobin would be the sole cause of the observed changes resulting from keeping the fishes in an environment of a wider nitrate spectrum. Undoubtedly a direct toxic effect on the blood system takes place regarding both the peripheral blood and the hematopoietic organs.

With the cells' degeneration found in the peripheral blood, significant anemia symptoms were observed accompanied by those of anisomicrocytosis, poikilocytosis, and polychromatophilia. Moderate leukocytopenia and thrombocytopenia occurred periodically; the blood composition changed towards either mature or young granulocytes' predominance.

Anemia is tightly connected with distempers in the blood production process in the kidney hematopoietic centres. An increased requirement to compensate for the oxygen defficiency in the organism caused an intensified proliferation of the erythroblast-order cells followed by their irregular differentiation and degeneration. It seems difficult to establish an extent to which the changes were caused by an excessive demand for the blood cells met by introducing immature elements to the peripheral blood and by a direct toxic influence on the blood-producing organs. An irregular differentiation and degeneration occurred in a rather short time. In the course of the experiment necrobiotic and necrotic processes were intensifying within the hematopoietic centres; they were accompanied by an excessive destruction of the blood components.

This state of affairs could not be indifferent to the hepatic tissue respiration ability, which was particularly well evident when compared to the control fishes. While with calcium nitrate a decrease in the tissue respiration ability was of a progressive nature (a gradual redox potential decrease, except for the first blow) a potassium nitrate-caused drop was very marked from the beginning and remained so (altering slightly) till the end. Thus we can speak of a stronger and more toxic influence of potassium nitrate.

Also the histopathologic picture reflected the above conditions. A strong tissue reaction, i.e., a very pronounced hepatic cell protoplasm vacuolization is observed in the I period for the potassium nitrate whereas similar changes occurred with calcium nitrate in the final part of the experiments. It should be borne in mind, however, that only the vacuolization is taken into account now, the other regressive lesions being noted there from the beginning as well.

In the case of calcium nitrate, initially a tissue swelling and a rather wide fluid-caused separation of the hepatic tissue trabeculae predominated (the I period) followed then by damages in the protoplasm, mainly an obliteration of its structure. The tissue became more and more opaque, intercellular borderlines being weakened. Not until the final stage (the IV and V periods) a strong vacuolization in the hepatic tissue cells occurred, although earlier (the II and III periods) single cases of similar conditions had been noted, but never in the first period as it was the case with KNO_3. With $Ca(NO_3)_2$, an increase in the necrotic centres abundance was marked in the IV period.

The nitrate influence upon the liver blood system was quite different. With KNO_3, the blood vessels were generally well-filled with blood and no changes such as those induced by $Ca(NO_3)_2$ were observed. The vessels in general were clearly eosin-stained, however,

a significant effect of $Ca(NO_3)_2$ on both the blood vessels and the blood itself was established. The vessels were often excessively dilatated and in some places exceptionally strong dilatations seemed to have been formed. The vessels were abundantly filled with denatured serum with rather numerous leukocytes whereas the erythrocytes were less abundant and as a rule showed a significant hypochromasia, occurring even as shades.

A similar difference in the course of the blood methemoglobin formation process rate is noted depending upon the kind of a nitrogen salt used. With calcium nitrate the methemoglobin percentage increases gradually, while with potassium salt it attains its final value at the very beginning and maintains it till the end of the experiment.

One more aspect of the experiment should be emphasized, though only indirectly related to the studies'aim, nevertheless rather a significant one as far as a sanitary point of view is concerned. The sanitary conditions of water used in the settlements situated down the rivers as a rule are strongly impaired owing to various chemical compounds. The water is treated in filter stations, but as we experience it ourselves it is of a distinct colour, changed taste, and even deposits additional precipitates apart from usual boiler incrustations. Obviously the chlorination process plays a part here.

The whole experiment discussed was carried on using tap water; the chlorination is supposed to be of negligible importance here as the water was changed at intervals.

On the other hand, the histopathologic pictures of the control fish livers left rather much to be desired. Even if we assumed certain changes occurring due to abnormal conditions of life the fishes had been kept under, fine intra-tissue necrotic centres as well as other lesions indicated to an additional effect of some toxic factors difficult to define but affecting the health of fish and probably that of the people, too.

The changes occurring in the other fishes and their extent do not shake the validity of results obtained from the experiments discussed regarding toxicity of nitrates.

CONCLUSIONS

1. Calcium and potassium nitrates, even in relatively small doses (25–30 mg NO_3/l) induce methemoglobinemia in rainbow trout *Salmo gairdneri* Rich.
2. A prolonged period of their influence results in deep toxic changes appearing as damages in the peripheral blood and hematopoietic centres as well as serious imparing of the hepatic tissue (cyto- and karyoplasm degeneration, necrotic centres, redox potential decline).
3. An influence of the two salts, $Ca(NO_3)_2$ and KNO_3 leads to the same final effect, the differences lying only in the rate and details of their action. In general, the potassium salt influence is of a more rapid nature, sharper changes being evident from the beginning, while a gradually intensifying action is observed for calcium nitrate.
4. A harmful effect of nitrogen compounds abundantly outflowing from the fertilized cultivated areas on fish should be taken into account.

ACKNOWLEDGEMENT

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Translated: mgr Teresa Radziejewska

EKSPERYMENTALNA METHEMOGLOBINEMIA PSTRĄGÓW TĘCZOWYCH

Streszczenie

Coraz częściej pojawiające się w literaturze naukowej doniesienia o szkodliwości w wodzie pitnej jonów NO_3 dla zdrowia ludzi a zwłaszcza dzieci zwróciły naszą uwagę na wpływ tych jonów na zdrowie ryb. Zwłaszcza, że coraz intensywniejsze nawożenie związkami azotowymi naszych pól powoduje spłukiwanie soli azotowych do wód. Dopuszczalna dawka jonów azotowych NO_3 wynosi wg World Health Organization 50 mg/l. W naszym doświadczeniu użyliśmy dawki po 50 mg/l KNO_3 i $Ca(NO_3)_2$, co wynosi 30,6 mg/l i 26,2 mg/l NO_3. Doświadczenia przeprowadzono na 60 sztukach 2-letnich pstrągów tęczowych o długości 20–26 cm l.t. Pstrągi podzielono na 3 grupy po 20 osobników, pozostawiając jedną grupę jako kontrolę. Pstrągi przetrzymywano w wodzie wodociągowej. Doświadczenie trwało 11 tygodni. Po miesiącu przystąpiono do pierwszego badania, przeprowadzając dalsze co dwa tygodnie. Do badania pobierano każdorazowo po 4 pstrągi z każdej grupy.

Przebadano poziom hemoglobiny i methemoglobiny, obraz krwi obwodowej i w narządach krwiotwórczych oraz zmiany histopatologiczne wątroby oraz jej potencjał oksydo-redukcyjny metodą Warburga.

Badania wykazały daleko idące zmiany. Hemoglobina w około 27% przy KNO_3 i 20% przy $Ca(NO_3)_2$ została zablokowana w methemoglobinę. We krwi obwodowej w obu przypadkach wystąpiło znaczne unieczynnienie erytrocytów. Wystąpiły różne postacie ich uwstecznienia. Wzmożone zapotrzebowanie na elementy krwi odbiło się w sposób nader widoczny na pracy narządów krwiotwórczych. Przedni hemopoetyczny odcinek nerki nie nadążał z produkcją ciałek krwi i wypuszczał do krwioobiegu niedojrzałe erytroblasty. Te z kolei nie były w stanie spełnić swojej roli fizjologicznej i ginęły, zwykle przed okresem wytworzenia hemu. W wyniku tego potencjał oksydo-redukcyjny tkanki wątrobowej zmalał w końcowym okresie prawie do 0. Pociągnęło to za sobą głębokie zmiany histopatologiczne w wątrobie. Powstały liczne ogniska martwicze śródtkankowe jak też szereg zmian degeneratywnych w komórkach wątrobowych. Wpływ obu użytych do doświadczenia soli był w końcowym efekcie identyczny, różnice zaznaczyły się jedynie w szczegółach przebiegu procesu. Zmiany te musiałyby doprowadzić w ostateczności do śmierci ryb.

ЭКСПЕРИМЕНТАЛЬНЫЙ МЕТГЕМОГЛОБИН РАДУЖНОЙ ФОРЕЛИ

Р е з ю м е

Всё чаще появляющиеся в научной литературе сообщения о вреде ионов NO_3, содержащихся в питьевой воде, для здоровья людей, а особенно детей, обратили наше внимание на влияние этих ионов на здоровье рыб. И прежде всего потому, что всё более интенсивное удобрение наших полей азотными соединениями приводит к смыванию азотных солей в водоёмы. Допускаемая доза азотных ионов NO_3 составляет по данным World Health Organization 50 мг/л. В нашем опыте мы применили дозы по 50 мг/л KNO_3 и $Ca(NO_3)_2$, что составляет соответственно 30,6 мг/л и 26,2 мг/л NO_3. Опыт проводился на 60 экземплярях двухгодовиков радужной форели длиной 20-26 см. Форель разделили на 3 группы по 20 штук, оставив одну группу в качестве контрольной. Форель содержали в водопроводной воде. Опыт продолжался II недель. Через месяц приступили к первому исследованию, дальнейшие проводили через каждые две недели. Для исследования брали каждый раз по 4 форели из каждой группы.

Исследовали уровень гемоглобина и метгемоглобина, картину периферической крови и крови в кровообразовательных органах, а также гистопатологические изменения печени и её окислительно-восстановительный потенциал по методу Варбурга.

Исследования выявили далеко идущие изменения. Гемоглобин приблизительно на 27% при KNO_3 и на 20% при $Ca(NO_3)_2$ был заблокирован в метгемоглобине. В периферической крови в обоих случаях произошло значительное инактирование эритроцитов. Появились различные формы их регрессии. Усиленная потребность в элементах крови весьма заметным образом отразилась на работе кровообразовательных органов. Передний гемопоэтический участок почки не успевал производить кровяные тельца и выпускал в кровеносную систему несозревшие эритробласти. Последние в свою очередь были не в состоянии выполнить свою физиологическую роль и погибали обычно до наступления периода образования гема. В результате этого окислительно-восстановительный потенциал ткани печени снизился в конечном периоде почти до 0. Это привело к глубоким гистопатологическим изменениям в печени. Образовались многочисленные внутритканевые некрозные очаги, а также ряд дегенеративных изменений в клетках печени. Влияние обеих использованных в опыте солей было в конечном итоге идентичным, различия проявились лишь в деталях протекания процесса. Эти изменения должны были бы привести в конечном результате к смерти рыб.

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