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Pathology

**HEMATOLOGICAL AND HISTOPATHOLOGICAL CHANGES
IN LEUKEMIA OF BREAM – *ABRAMIS BRAMA* (L.)**

**ZMIANY HEMATO- I HISTOPATOLOGICZNE PRZY
LEUKEMII LESZCZA – *ABRAMIS BRAMA* (L.)**

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The nature of anatomo-pathological and histopathological changes as well as the quantitative and qualitative evaluation of the peripheral blood and the hemopoiesis centres allowed to detect symptoms of leukemia in a bream female. The picture of changes indicates to a certain similarity between this disease and a form of granulocytic leukemia occurring in animal, particularly in hens. Since the literature available provides no description of any similar disease in fishes had been found, we have attempted to give a detail characteristics of these changes.

MATERIAL AND METHODS

A bream female (8+) of 46.5/56.6 cm (l.c./l.t., respectively) size and of 2.500 g weight together with 10 control bream individuals of similar size and weight, caught from the Dąbie Lake near the River Odra outlet, were subject to our observations.

Immediately, when the blood from the caudal vein had been taken, and the decapitation made, the smears were prepared and the tissue sections were taken for a histological examination. The tissues were fixed with Zenker, Susa, Stive, and Bouin fluids, and with

haematoxylin according to Delafield with eosin by the Mallory, Passini and Dominici method.

The morphotic elements in the blood taken were counted, i.e., the numbers of erythrocytes and leukocytes per 1 mm^3 of the peripheral blood were estimated in the Bürker chamber according to generally accepted procedures; the hemoglobin level was determined photocolorimetrically by the Drabkin method.

The smears of the peripheral blood and organs were stained with May-Grünwald and Giemsa. A blood percentage composition was determined from 500 subsequent blood cells. An anisocytosis curve from 2000 erythrocytes was also prepared. A hepatogram was established from the morphological features of 500 liver parenchymal cells. The differences in blood cell appearance were followed, and a cytopathological evaluation of the hematopoietic organ smears was carried out.

Any effect resulting from a parasitic invasion was excluded after a detail examination of skin, gills, muscles, peritoneal cavity and internal organs. A parasitological section of the female displaying leukemia gave the following results: on gills – numerous trematodes *Gyrodactylus elegans* Nordmann, 1832 (about 40 specimens under a cover slide), rare *Diplozoon paradoxum* Nordmann, 1832, and a few cysts of *Myxobolus rotundus* Nemezcsek, 1911. Besides, in fins, sub-gill membrane and muscles metacercariae of *Para-coenogonimus ovatus* Katsurada, 1914 were present. They occurred abundantly in fins (e.g., about 20 specimens in a membrane between the rays), while in the outer layers of dorsal muscles only individual specimens were detected. The other parasites were: *Posthodiplostomum cuticola* (Nordmann, 1832), *Asymphylogora imitans* (Mühling, 1898), and *Piscicola geometra* (L.).

RESULTS

Clinical and anatomo-pathological changes

Externally a characteristic swelling of the fish (*ascites*) is seen, the abdominal integuments being strongly protruded and scales perked up. (Figs. 1, 2). The swelling covers also the head muscles and the operculum, and gives rise to an eyeballs exophthalmosis. The anus is also protruding and congested (Fig. 2). The muscles and skin are infiltrated with an aqueous liquid which fills also the peritoneal cavity as a colourless effusion.

The external lamina of peritoneum as well as the abdominal membrane are both pale, unevenly thickened, accreted in places with the adjoining internal organs. Connective tissue adhesions link the liver lobes with the intestinal loop serous membrane. The liver itself is strongly enlarged, brown, of compact consistency with numerous conspicuously distinguished whitish nodules in the parenchyma. The stasis hyperemia, visible through the blood vessels dilatation, indicates to disturbances in this organ operation. An infiltration of brown pigment, hemosiderin, is present in the liver parenchyma.

As far as the other organs are concerned, mainly the kidney is affected, being greatly enlarged, deformed, grayish in colour, of a poor compactness. Out of its three parts, especially the pronephros is excessively overgrown, swollen and displaced into the peri-

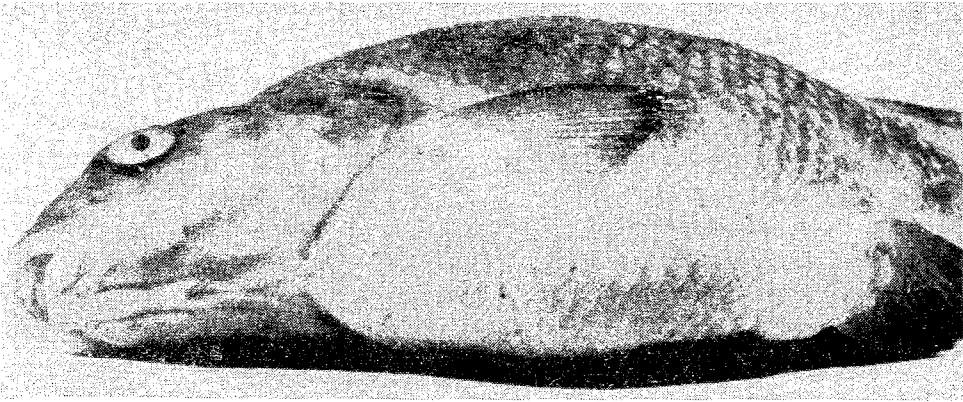


Fig. 1. Swelling of fish with leukemia

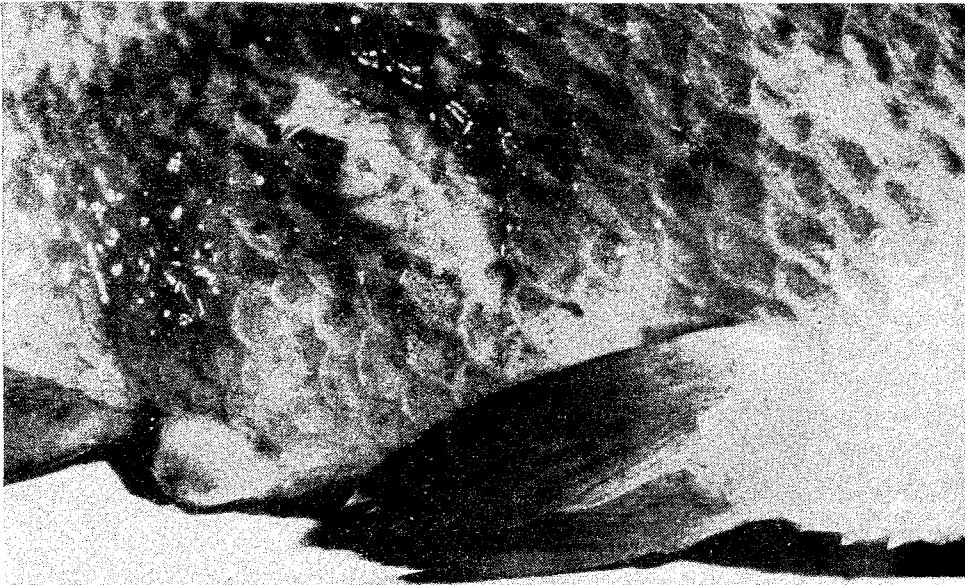


Fig. 2. Strongly protruded scales contour

toneal cavity as additional lobes. The spleen is relatively weakly enlarged and possesses a hard, unevenly dark-red parenchyma without any sanguineous liquid. Numerous aggregates of hemosiderin are visible within the tissue.

The disease described is accompanied by the acute catarrhal enteritis with edema and ecchymoses in the mucous and submucous membranes. The intestinal mucous membrane surface is covered with a dense, yellowish secretion mixed with an abundant effusion.

Hematological changes

Changes within the peripheral blood. The analysis of the peripheral blood parameters of the bream female affected by leukemia revealed a decrease in the erythrocyte number by about 1.2 ml/mm^3 , while the hemoglobin level was only slightly lowered, i.e., by over 2 g%, (Table 1), indicating to the erythrocyte hyperchromasia. An average hemoglobin content per one erythrocyte of the control females amounts to $46.07 \gamma\gamma$ while that of the examined one $80.50 \gamma\gamma$ (as derived from the Wintrobe formula).

Table 1
Comparison between peripheral blood hematologic indices of control and examined breams

	Number of fishes	Erythrocytes (mill/mm ³)	Leukocytes (mm ³)	Hemoglobin (g%)
Control breams	Males	2.05	10.080	10.26
	5	1.81 – 2.28	4.800 – 13.600	8.85 – 11.38
	Females	2.01	8.360	9.39
	5	1.68 – 2.24	4.600 – 10.800	8.26 – 10.41
Bream with leukemia	Female 1	0.84	142.800	7.46

Furtermore, the disease discussed is characterized by a pronounced leukocytosis resulting in 142,800 leukocytes per mm^3 (Table 1). Exceptionally high cell reaction, rarely found in fishes, consists almost completely of mature heterophile leukocytes (95.5%), particularly the bi-segmented ones (56.0%). Non-typical forms, absent in ciprinids, with multi-segmented nuclei amount to relative high percentage (9.5%) (Table 2, Fig. 3a). The giant cells (Fig. 3b) with intensely staining, coarse granulation, classed into progranulocytes, granulocytes, and heterophile metagranulocytes are also present. The remaining forms, unlike the described ones, show a moderate hypochromasia; for instance, the cytoplasm of mature heterophils is finely granulated or with no granulation at all, regionally reactive and slightly blue-gray peripherally. The blood cells are generally deformed, with blunt outlines and plicate cell membranes. Vacuolized lesions within the cytoplasm and nucleus reflect the degeneration (Fig. 3a). A number of leukocytes disintegrate due to plasmolysis, the other ones exhibit some dissociation when maturing; as a rule the nucleus differentiates earlier than the cytoplasm, and it keeps its loose chromatin structure even within the finally mature cells.

A few basophile cells (0.5%, Table 2) differ in their morphology from the normal ones. The prevailing forms are younger than normal ones, being classified as progranulocyte and granulocyte stages, the latter possessing even multi-segmented nuclei (triple-segmented,

Table 2

Bream hemogram

Type of cell	Control breams	Bream with leukemia
granuloblasts	—	2.0
progranulocytes	0.7 0–3	2.0
granulocytes	—	0.5
metagranulocytes	8.2 5–13	0.5
heterophils: 1–segmented	11.0 5–15	27.0
2–segmented	—	56.0
3–segmented	—	8.0
4–segmented	—	1.5
basophile granulocytes	—	1.0
basophils	2.0 0–8	0.5
total:	21.9	99.0
lymphocytes	76.0 60–85	1.0
phagocytes	2.1 0–3	—
total:	78.1	1.0

Fig. 3c). Their cytoplasm is intensively basophile with a delicate granulations in vacuoles. Most basophils exhibit degenerative features with a pronounced karyopyknosis.

Out of the other peripheral blood cells, the lymphocytes are seldom encountered (1% in the female with leukemia compared with 76% in the control ones; Table 2); they normally show an obliterated nucleus structure and more basophile cytoplasm. The degenerative lesions are visible also within the thrombocytes which are most frequently deformed pyknotically. The absence of erythrophagous phagocytes is a significant difference within the whole picture of the blood (Table 2).

In addition, the leukocyte arrangement is accompanied by certain irregularities within the erythrocyte population. The erythrocytes deviate from the standard; non-typical forms with anisocytosis are relatively frequent (Fig. 4). The erythrocytes are most often deformed, almost rounded, finer (the cells of $10.4\ \mu\text{m}$ amount to 16%) or abnormally elongated (poikilocytes). Fragments of erythrocytes and schizocytes are frequently found. Nuclei of all the erythrocytes are deformed, showing the various stages of pyknosis as well as thickened chromatin granules distributed peripherally or centrally. The nuclei of these blood cells are displaced from their normal position towards the cell membranes which are usually finely plicated.

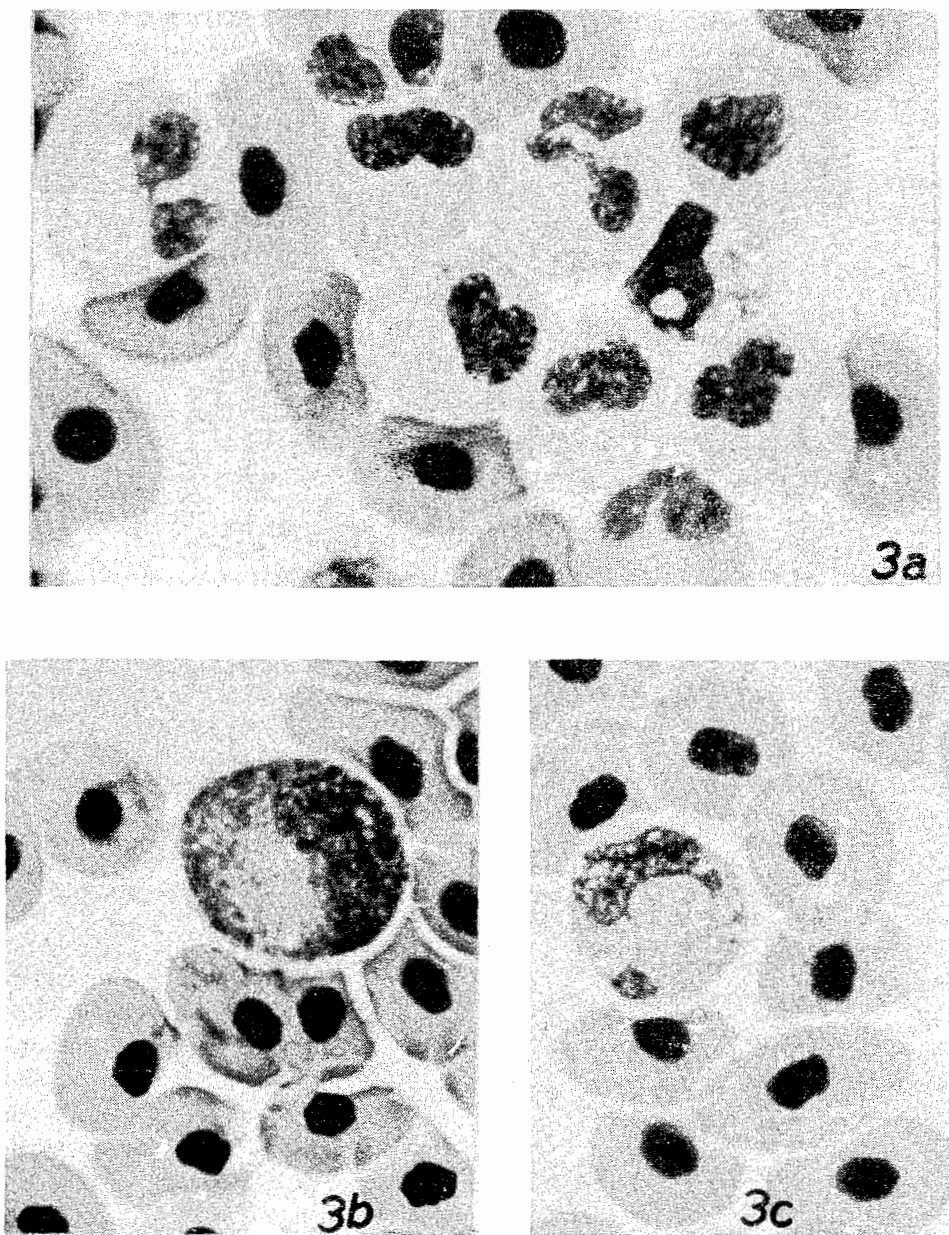


Fig. 3. Peripheral blood of the bream with leukemia: a. mature, single- and multi-segmented heterophils and changed progranulocyte, b. the giant heterophilic metagranulocyte, c. triple-segmented basophilic granulocyte MGGx1500

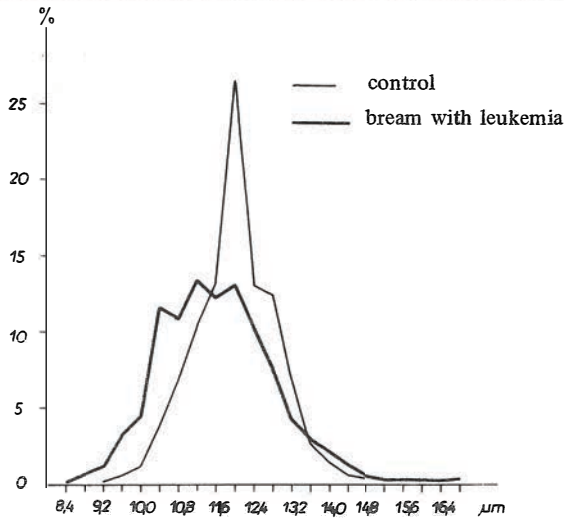


Fig. 4. Erythrocytes curve of the anisocytosis from peripheral blood of the bream

Histopathological changes

Liver. Within the inflammationally changed liver parenchyma, the leukemia infiltration centers are present, being poorly separated by fibroblasts. Most often they appear around the larger blood vessels (Fig. 5), bile ducts (Fig. 6), and endoparenchymally near the capillary vessels. These centres contain leukocytes in different developmental stages, from initially small and large hemocytoblasts, progranulocytes, and metagranulocytes to finally mature heterophils and basophils (Table 3, Figs. 7a, b).

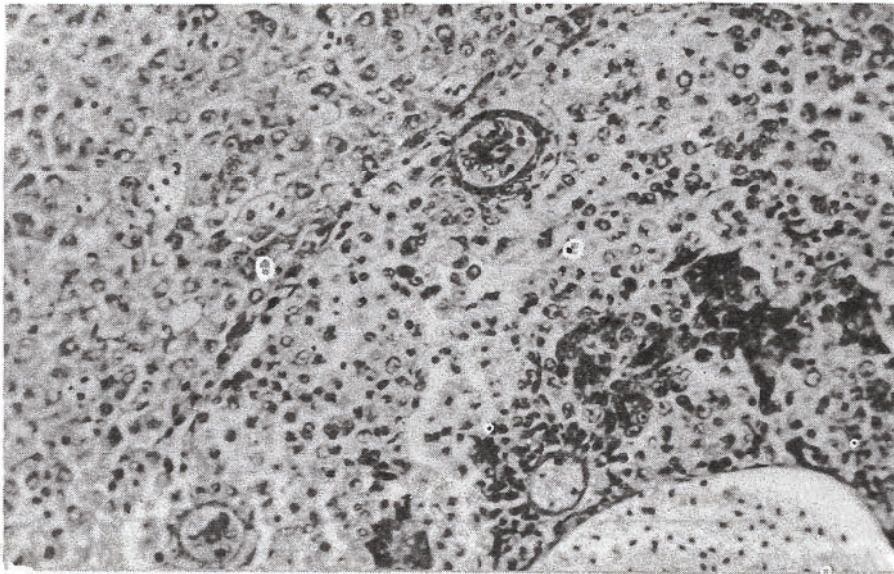


Fig. 5. Leukemia infiltration centres around the larger blood vessel within the liver parenchyma necrobiotically changed. H+Ex340

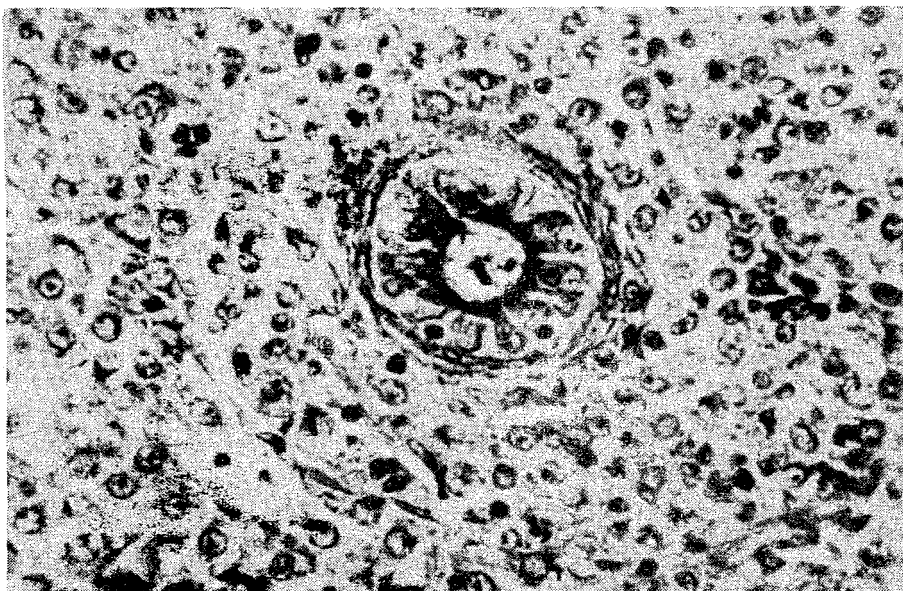


Fig. 6. Zone of the cells proliferation around of the bile duct parenchyma of the liver. H+Ex640

Table 3

The liver parenchyma hepatogram

Type of cell	%
large hemocytoblast	9
small hemocytoblast	36
heterophile order	
progranulocytes	7
metagranulocytes	9
heterophils: 1-segmented	12
2-segmented	7
3-segmented	2
4-segmented	1
basophile order	
progranulocytes	2
granulocytes	5
metagranulocytes	1
basophils	1
lymphocytes	8

Among the other things, an excessive cell proliferation effects in the liver trabecular structure destruction. The individual hepatocytes or larger tissue parts, because of pressing them down, undergo a degeneration leading to the necrosis. In places of mode-

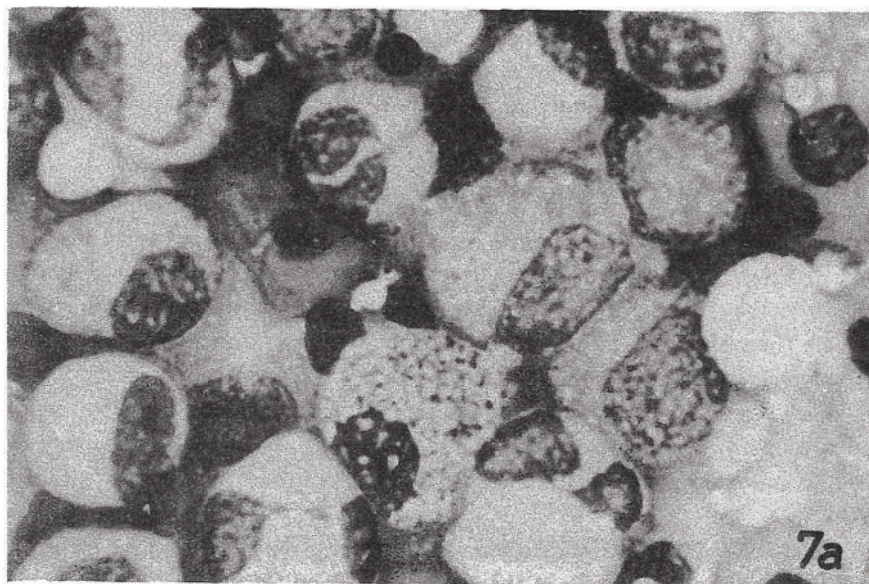


Fig. 7. Liver's parenchyma smear:

- a. numerous, pathologically changed cells of the heterophilic serie and single basophil. MGGx1400
- b. hemocytoblasts and other cells in the centre of the leukemia infiltration. MGGx1500

rate proliferation, the hepatocytes are loosely packed, and the intercellular spaces appear to be excessively extended. These parts exhibit more clearly swollen cells, the obscured parenchymal cytoplasm (parenchymatous degeneration), and vacuolized lesions. The phagocytes with brown pigmentary infiltrations within their vacuolized cytoplasm are more numerous.

Additionally, the venous hyperemia of the liver occurs; the blood vessels, particularly the capillary ones are excessively dilatated and densely filled with blood elements (Fig. 5). The erythrocytes in the lumen vessels usually exhibit the hypochromasis, granulated cytoplasm and pyknotically deformed nuclei. The endothelium cells of the capillary vessels are most often damaged, detached or swollen, eventually tending to proliferate. Then, they contain oval or almost rounded nuclei penetrating into the vessel itself. The nucleus number is sometimes considerable. The endothelium cells in the walls of the larger veins usually become excessively extended; their outer zone is overgrown. Numerous non-differentiated mesenchymal cells as well as fibroblasts and phagocytes tend to concentrate, together with the proper cells of the leukemia infiltration around the blood vessels.

The blood cells in the liver parenchyma smear exhibit much more pronounced regressive lesions than the similar cells in the peripheral blood (Figs. 7a,b). The heterophils, particularly their older forms, are blisterous, swollen, their cytoplasm and nucleus chromatin being poorly stained. In many cells the nuclei are bluntly outlined and the erythrocytes without cytoplasm prevail. The hepatocytes seldom are present in smears; a few of them possess a characteristic streaky cytoplasm with granules of a brown pigment. Only the vacuolized reticulum phagocytes or those filled with greenish and brown pigment granules occur in masses.

Kidney. Along the whole length of the kidney, the endoparenchymal tissue is overgrown (Fig. 8,9), embedding only a few nephrons with marked degenerative lesions. In the less damaged glomerules, a capillary loop proliferation is visible, the other ones having their capillary loops degenerated hyaline-like, and washed out externally into the capsule which is enlarged due to the agglomeration of an effusion. These lesions are accompanied by a simultaneous obliteration of the cell element structures and a considerable disintegration of nuclei during the chromatinorrhexis and karyorrhexis.

Owing to the glomerulonephritis, the kidney canaliculi are also injured; the canaliculus epithelium cells become enlarged and detached, the parenchyma cytoplasm obscured (parenchymatous degeneration), and the nuclei affected by swelling or pyknosis (Fig. 8). In an extremal case, especially within the truncal kidney part, the colliquative necrosis of canaliculi occurs. Degenerative lesions within the larger kidney ducts appear as the hyalinosis of an excessively overgrown mucous membrane connective tissue as well as the external muscular membrane fragmentation. The liquefacted epithelial cells, leukocytes and dead erythrocytes are collected within the strongly creased duct lumen.

The necrobiotic changes and necrosis occur almost within the entire intracanal tissue (Figs. 9,10). Only in the perivascular zones, near veins filled with blood cells, the band — like agglomeration of the initial blastic cells has remained. The blood elements analysis in

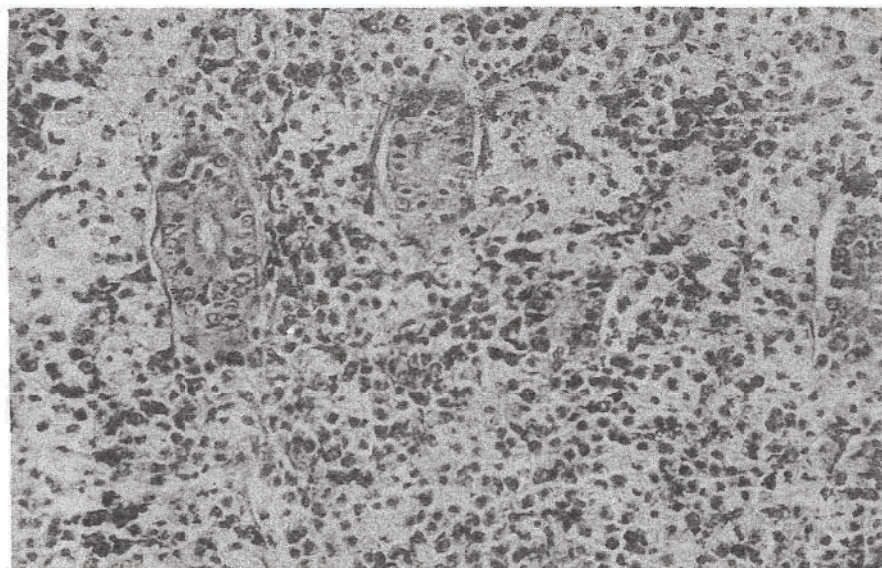


Fig. 8. Necrobiotic changed within the intercanal tissue and the kidney canalicules. H+Ex340

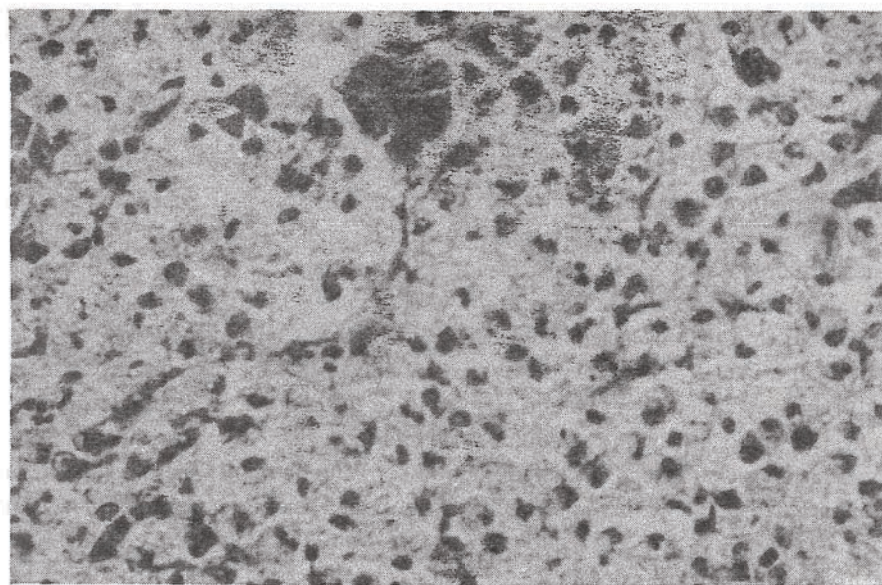


Fig. 9. Disintegration of the hematopoiesis centres in the kidney. H+Ex640

smears (stained with MGG) permits to determine more precisely the nature of changes in the hematopoietic cells. The kidney parenchyma in its cephalic part consists of dead, unstainable leukocytes, differentiated to a various degree. Among these vestiges of cells hemocytoblasts and the basophils have most often remained. The latter are very abundant within the whole kidney parenchyma.

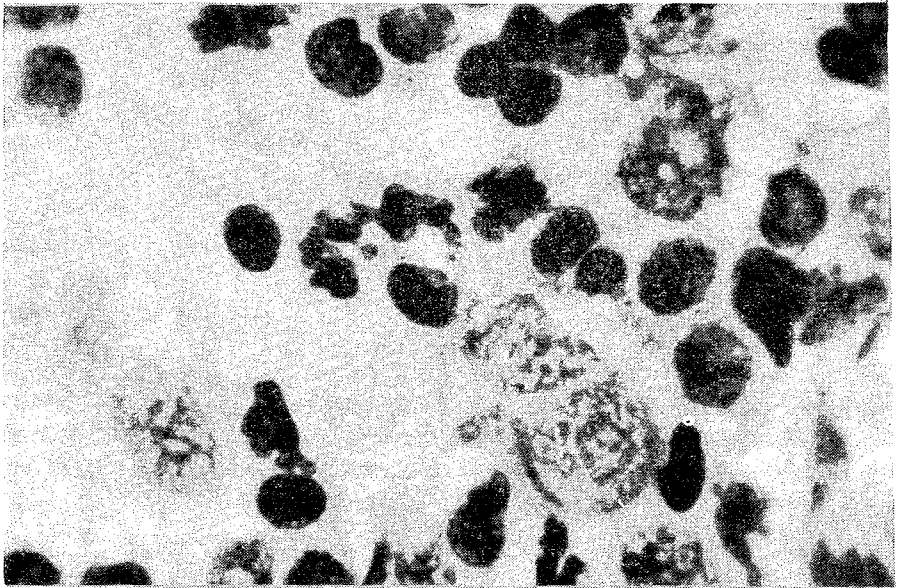


Fig. 10. A few hemocytoblasts and the bare pyknotical nuclei of other cells in the necrotic centre of the kidney. MGG x1400

The lytic processes are even more marked in the hematopoietic centres of the truncal kidney (Fig. 10). These processes result in producing bare, multi-lobed nuclei with pyknosis and loosely packed cytoplasm papules. Rarely, in contrary to the cephalic kidney part, vestiges of the entire cells are visible; individual small hemocytoblasts with their nucleus chromatin changed and unevenly stained are sporadically present.

Within the caudal part of the kidney, larger fragments of the parenchymal tissue, only weakly changed and with the blood elements slightly damage, have remained (Fig. 11). Among the blood cells, the reticulum, small and large hemocytoblast, heterophil (with cytoplasm less stainable and nuclei poorly outlined) and basophile ones prevail, the latter containing their nuclei with and excessive pyknosis and finely granulated, or non-granulated cytoplasm.

Spleen. The histological appearance of the spleen indicates, in the first place, to the intensified local destruction of the morphotic blood elements. Relatively large amounts of the erythrocytes are contained in the blood vessels and intracellularly. Their sensitivity to staining is, however, weak, the cells with coarsely granulated cytoplasm and

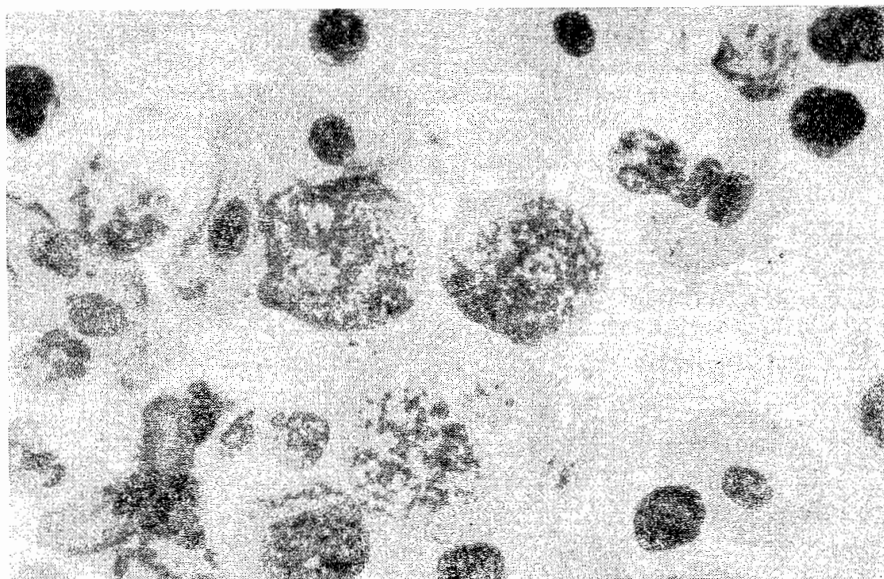


Fig. 11. Inconsiderably changed granuloblasts heterophils and other cells within the caudal part of the kidney. MMG $\times 1500$

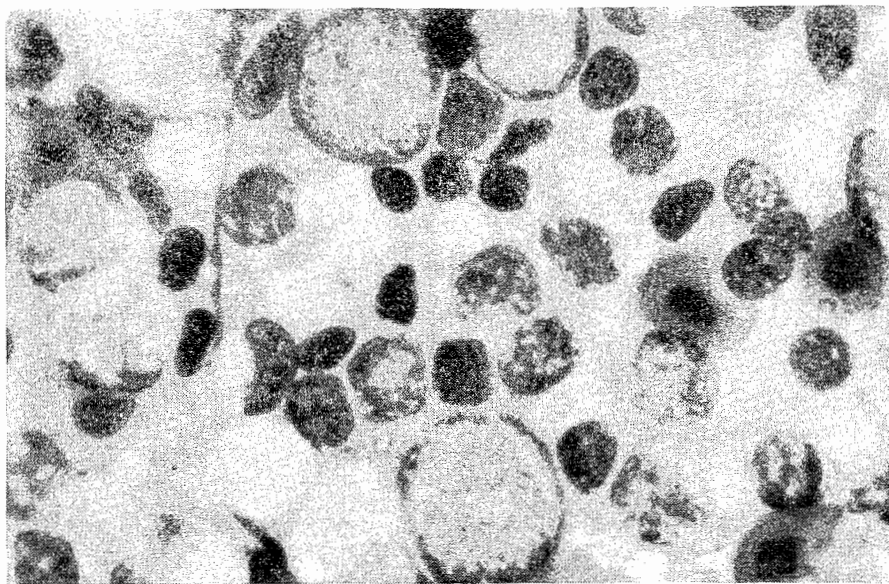


Fig. 12. A few hemocytoblasts in the spleen parenchyma necrobiotically changed. MGG $\times 1400$

pyknosis – shrunk nuclei prevail. The smears display also the deformation of these cells and their plasmolytic disintegration. Numerous bare erythrocyte nuclei with pyknosis are present (Fig. 12).

The other parenchymal cells are considerable diversified as to their types and developmental stages. The lymphopoiesis and granulopoiesis centres are irregularly distributed, without any clearly marked borders within the connective tissue network. Compact groups of differently shaped cells, including the lymphocytes and phagocytes, are placed most often around the blood vessels as branched bands. Such an arrangement is visible most clearly within larger veins and arteries despite strong signs of the wall degeneration (the fibroid degeneration, especially that to the central zone).

The necrobiotic lesions and necrosis encompass all the systems of the spleen parenchyma cells. The lymphocytes forming almost homogenous concentrations are less changed; the small and large hemocytoblasts possess a pale, abnormally stainable chromatin reticulum and a stripe – like concentrated, strongly basophile cytoplasm (Fig. 12). The splenogram shows a considerable preponderance of the heterophils, usually with multi-lobed, unevenly stained and coarsely striped nuclei and the cytoplasm without its specific granulation. The basophils are found sporadically. The phagocytes, however, with a blue – greenish or brown – black pigment, occur abundantly as well as the vacuolized and swollen reticulum cells. An intensified disintegration process is also evidenced by numerous nuclei without cytoplasm and the cytoplasm papules which are visible from the spleen smear (Fig. 12).



Fig. 13. Heart muscle degeneration: myofibrils swelling and abundant infiltration around the epicardium blood vessels. H+E x170

Out of the other organs, the heart muscle is subject to the degeneration, apart from the pericardial acquosity. The effusion gathers between the myocardium muscle bundles, resulting in the myofibril swellig, looseness and obliteration. Numerous centres of the colliquative necrosis appear, particularly in deeper layers of the myocardium. The epicardium connective tissue has its fibres loosely packed and obliterated zonally. The slits of the muscles of these layers are abundantly infiltrated by mainly the lymphocytes, phagocytes, granulocytes and their initial forms (Fig. 13). Apart from the infiltrating cells, the fibroid masses and denatured plasm precipitates are sedimented. Particularly abundant perivascular infiltrations have been observed around the epicardium blood vessels.

DISCUSSION

The case described in the previous sections show a good agreement with the clinical symptoms of leukemia. This disease is characterized by the hyperchromatic anemia and high peripheral leukocytosis with the domination of one group cells of the leukocyte system, most often they are mature heterophils. As to their differentiation, the leukocyte population is heterogenous, heterocellular, with clear signs of degenerative lesions.

At the same time, both in places of normal hematopoiesis and in the other organs (the case discussed relates to the liver, mainly round the blood vessels and bile ducts) the intensified proliferation processes take place. In the liver leukemia infiltration centres, the cells possessing properties of potential precursors of different maternal ones prevail apart from those of the heterophile order. Similar is the picture in the kidney, except for almost complete destruction of the hematopoietic centres caused by the colliquative necrosis. The proliferation centres have only partially remained with the hemocytoblasts in the spleen which has been changed owing to an intensified action of the hypersplenism as well as necrobiotic processes and necrosis.

Because of the accompanying phenomena such as the effusional inflammation (with the significant injuries in the blood vessels, swelling, softening and infiltration within the parenchymatous organs, and a concentration of the effusion liquid in the pericardial and peritoneal cavities), the discussed type of leukemia should be defined as acute. However, it could be rather difficult to determine it so strictly when the cytological criteria are taken into account, which is always being done under such circumstances. In the case considered, in contrary to higher animals, the peripheral blood is dominated by the mature blood cells of multi-lobed form, absent in the normal bream blood appearance.

It is quite obvious that not only the leukocytosis composition and extent are the decisive factors to identify the disease and to classify it as leukemia, but also an intensified activity of the hematopoiesis centres must be considered, particularly that occurring outside the places of the normal hematopoiesis. The normal appearance of tissues in all the organs is changed by an anomalous overgrowth obscuring their structure and resulting in the extinction of the normal structure of the organs themselves. Special attention should be paid to the activation of the reticulum – endothelium system which reveals its differentiating abilities developed to various extent within different organs.

It is generally suggested that leukemia is a compensating productive reaction to some toxic — infectious factors. The causes of this disease in bream are certainly various and difficult to explain, and they should be looked for, among the other things, in disturbances of the water environment balance.

Zmiany hemato- i histopatologiczne przy leukemii
leszcza *Abramis brama* (L.)

Streszczenie

Przypadek białaczki opisano u samicy leszcza (*Abramis brama* (L.)) odłowionej z jeziora Dąbie woj. szczecińskiego. Schorzenie to cechuje niedokrwistość nadbarwliwa oraz wysoka leukocytoza we krwi obwodowej. Odczyn komórkowy krwi obwodowej składa się prawie wyłącznie z dojrziałych granulocytów heterofilnych.

Zarówno w narządach o prawidłowej czynności krwiotwórczej jak i w innych np. w wątrobie, występują wzmożone procesy proliferacyjne komórek. Sekcyjnie w mięszu wątroby stwierdzono liczne białe guzki (nacieki białaczkowe), powstałe w wyniku rozplemu białych elementów krwi.

Obok procesów proliferacyjnych we wszystkich narządach zaznaczają się, w różnym stopniu nasilone zmiany degeneracyjne i martwicze.

ГЕМАТО- И ГИСТОПАТОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ ПРИ ЛЕУКЕМИИ ЛЕЩА
ABRAMIS BRAMA (L.)

Р е з ю м е

В настоящей работе описан случай заболевания лейкемией самки леща (*Abramis brama* (L.)) выловленной из озера Домбе в Щецинском воеводстве. Это заболевание характеризуется интенсивной анемией и высоким лейкоцитозом в периферической крови. Клеточная реакция периферической крови состоит почти исключительно из зрелых гетерофильных гранулоцитов.

Как в органах с правильным кровообразовательным функционированием, так и в других органах, например в печени, происходят усиленные пролиферативные процессы клеток. При вскрытии в паренхиме печени обнаружены многочисленные белые шišки (лейкозные опухоли), образовавшиеся в результате размножения белых элементов крови.

Наряду с пролиферативными процессами во всех органах отмечаются с разной степенью интенсификации дегенеративные и некротические изменения.

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