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Hematology

**CYTOLOGICAL EVALUATION OF THE PERIPHERAL BLOOD CELLS
AND HEMOPOIETIC ORGANS IN *PESTIS ANGUILLARUM* OF EELS***

**OCENA CYTOLOGICZNA OBRAZU KRWI OBWODOWEJ
I NARZĄDÓW KRWIOTWÓRCZYCH PRZY *PESTIS ANGUILLARUM* U WĘGORZY**

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The symptoms of *Pestis anguillarum* in eels were determined basing on clinical and anatomopathological changes, quantitative hematologic parameters and the cytological evaluation of the peripheral blood and hemopoietic organs. The cytological diagnosis indicates the necrotic processes domination over proliferation. The symptoms are typical of an acute form of the disease and give no successful prognosis for a recovery.

MATERIAL AND METHODS

In May and June 1974 and 1975 eels showing pest (*Pestis anguillarum*) symptoms were caught from the northern part of the Szczecin Firth. 20 fish specimens to be examined (50–80 cm length, 250–270 g weight) were supplied by the Fishery Station at Karsibór and Przytór. The control eels were selected from a large number of fishes (ca 300 individuals) since the systematic monitoring of the eel health state had been carried out over the annual cycles from 1970 till 1974 in the Szczecin Firth, Dąbie (Szczecin District) and Siecino (Koszalin District) Lakes. Clinical and anatomopathological changes, hematologic parameters, and parasitologic and bacteriologic examinations

* The present paper comprises a part of investigations carried out by the Inland Fisheries Institute, Olsztyn.

served as a basis for the evaluation. Inoculations of diseased eels' blood, internal organs and anabroses resulted in the *Aeromonas* growths. Only the protozoan parasites were found: from single cells to a mass occurrence of *Trypanosoma granulosum* Laveran et Mesnil, 1909, in 2 instances and from single cells to very numerous *Spironucleus* sp. in 9 eels. The protozoan presence was noted in the peripheral blood and visceral organs (organs infested with mass-occurring flagellates were not considered. Changes associated with the *Spironucleus* sp. invasion are planned to be described elsewhere).

Blood to be analyzed was taken from the caudal veins and hearts by a heparin Pasteur pipette. Quantitative determinations of the blood morphotic elements (number per 1 mm^3) were made by standard methods in Bürker chamber with the physiologic fluid 200-fold dilution, violet being added. Besides, the hemoglobin concentration was determined photocolorimetrically by the Drabkin method and the hematocrit value assessed using Wintrobe heparin micropipettes. The following erythrocyte indices were calculated: the hemoglobin average amount and concentration per one cell and the mean erythrocyte volume.

The blood squeezes were made for the peripheral blood, skin necrotically changed, and the parenchyma of internal organs: liver, spleen, kidney (pronephros, middle part, and dilated mesonephros). May-Grünwald, Giemsa and Wright methods were used for staining. The percentage blood composition was determined from 500 consecutive cells; the granulocyte-order cells' maturity stage was detected basing on morphology of 100 consecutive cells. Also the differences in the peripheral blood cells were analyzed and the parenchymal organs' smears evaluated.

RESULTS

Clinical and anatomopathological changes

Externally, the pest-diseased eels showed skin inflammation with clear scales' contours and small amount of mucus. Patchy congestions of skin were most frequently observed abdominally. Ecchymoses appear also in anal fin and more seldom in dorsal and caudal ones, the fins gaining an intensive red colour. Swelling and congestion of skin were observed in some fishes around the anus.

Over the whole skin cover, depigmented, irregularly shaped spots and considerable anabroses appearing as extensive ulcers occurs. Their number and size per one fish range from 1 to 8 and 1.5–6 cm, respectively. They are encountered most frequently over the body sides below the anterior part of dorsal fin, around the anus and sometimes dorsally.

Macroscopically, irregularly shaped flat anabroses can be detected; they show vast necrotic zones together with a layer of swollen, congested, desquamating skin with dark-stained parts determined by the melanine reaction. Muscles affected by putrefaction are exposed over several cms in the skin anabroses (Fig. 1). Another type of anabrosis looks like a hollow crater with edges congested, swollen and effusion-infiltrated. The anabrosis bottom is small, deeply penetrating into the muscle tissue. The anabroses are filled with a sanguineous, evil-smelling fluid (Fig. 2).

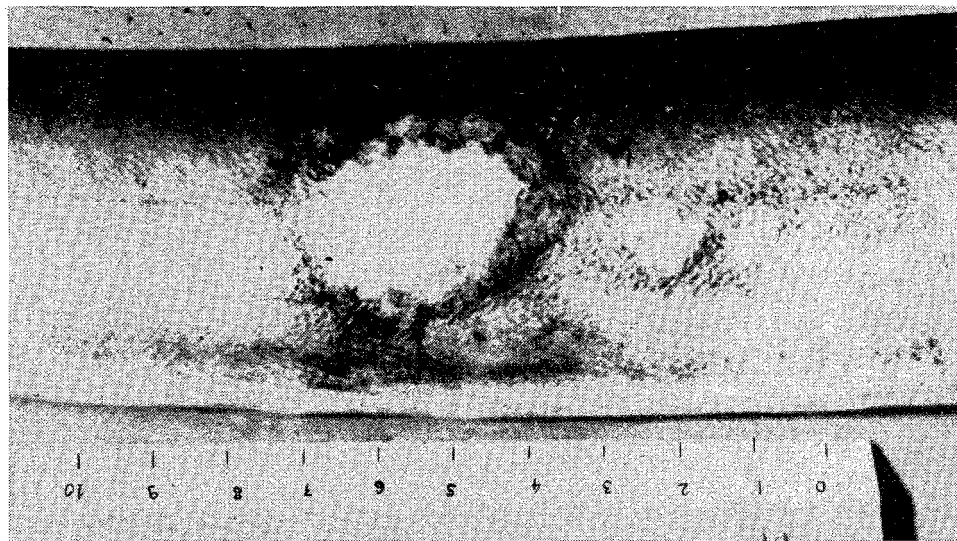


Fig. 1. Anabroses showing an excessive necrotic part, congested skin layer and exposed muscles

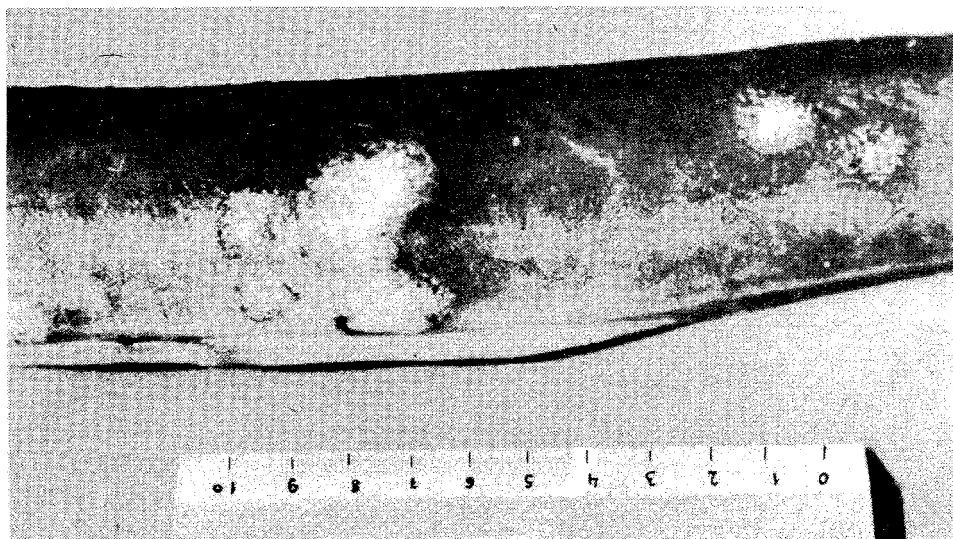


Fig. 2. Anabroses with strongly congested, swollen and effusion infiltrated edges

The section-disclosed symptoms indicate to a general inflammation and degeneration. The liver of fish affected is dark red, swollen, flaccid, spotted with ecchymoses and fine necrotic centres. Sporadic blood stasis restricted to the liver lobes was observed. All the

Table 1

Quantitative characterization of peripheral blood of eels with *Pestis anguillarum*

	Control eels	Eels with <i>Pestis anguillarum</i>
No of erythrocytes per 1 mm ³ (in millions)	1.45 1.34–1.71	0.83 0.79–1.18 (2.38)*
No of leukocytes per 1 mm ³ (in thousands)	21.9 14.4–33.6	62.9 48.7–78.8
Hemoglobin g%	10.05 8.90–11.23	6.39 4.61–11.93
Hematocrit index	38 32.0–40.0	27 33.0–21.0 (46.0)*

* values not included into the mean.

eels examined had their gall bladder at least doubled in size and filled with a light fluid. The bile deposits are connected with the gut insufficiency resulting from acute gut and stomach catarrh. The intestinal mucous membrane shows strong inflammational changes; it is swollen covered with ecchymoses and desquamated epithelium. Profound anabroses in the intestinal fold mucous layer were found out in the absorbing part of the gut.

Both the spleen and kidneys are mostly congested, increased in size, of a loose texture. In a few instances the organs mentioned were grey, their colouration indicating extensive necrotic lesions. Sporadically, the superficial spleen lipotrophy was noted.

The abdominal membrane is strongly congested with excessive vasodilatation. In the peritoneal cavity in 2 cases slightly sanguineous effusion fluid occurred abundantly.

Cytological diagnosis

Peripheral blood

A decrease in erythrocyte numbers per a volume unit and in hemoglobin content is one of the symptoms in *Pestis anguillarum* in eels (Table 1). The mean hemoglobin content per one cell is proportionally lowered down to 50.12γγ against the control value of 66.23γγ; the mean hemoglobin concentration per one erythrocyte decreases to 21.95%, the control value being 28.83%, whereas the average erythrocyte volume increases up to 277.3 μm³ (the control: 225.3 μm³).

An increase in the peripheral blood erythrocyte number, a hematocrit index, is a less typical reaction observed in two instances in association with an abundant effusion in peritoneal cavity. Of the hematocrit indices, in this case the average erythrocyte volume is increased up to 333.3 μm³.

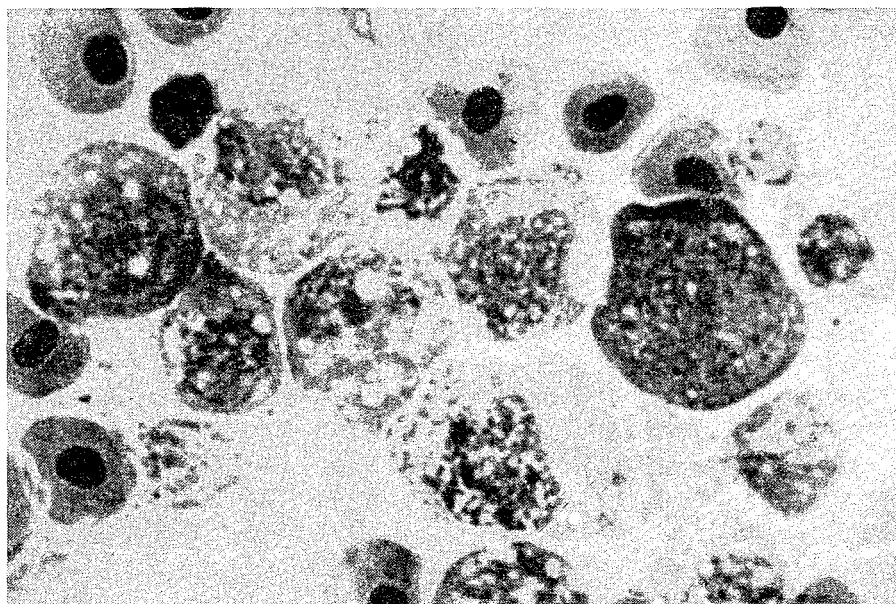


Fig. 3. Altered granulocytes of the peripheral blood in *Pestis anguillarum*

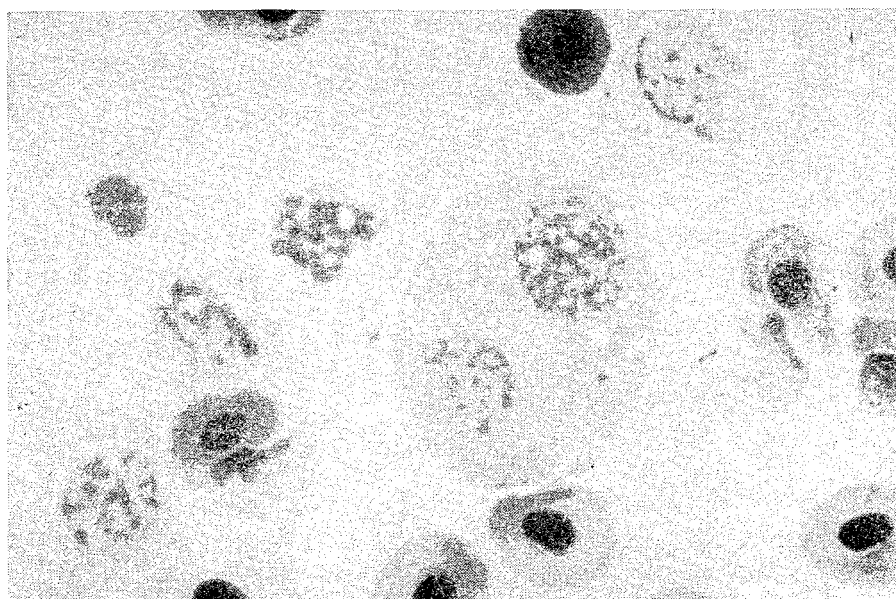


Fig. 4. A binuclear granulocyte and blurred cells of the peripheral blood

The erythrocyte picture of the peripheral blood smears is similar. The erythrocyte population is generally homogenous, consisting of mature and senile normoblasts. The erythrocytes show anisocytosis and considerable poikilocytosis; micro- and shizocytes are in abundance and there are many senile cells with fine, rounded pyknotic nuclei. Irregular amitotic divisions with fragments of nuclei or deformations typical of the so-called "gemmation" of a nucleus proceed in many cells. These nuclei have one, or more rarely few elongated processes. Besides, the erythrocyte nuclei show an atypical arrangement of the chromatin which is coarsely granulated, sharply outlined or concentrically aggregated. In most nuclei fragmentation of the chromatin and its partial or entire obliteration occur, the nucleus membrane being retained. Not infrequently the necrotic lesions of this type are observed in 70% of the blood cells' population. In extreme cases of intensification of disease symptoms, the pyknosis of 95% of the blood prevails.

The disease proceeds with an increased number of leukocytes and a granulocytes level lifted up to 40% (83% at most). The young forms in the progranulocyte, granulocyte, and, to a lesser extent, granuloblast stages predominate in the blood composition. Some forms resemble the necrotically changed non-differentiated reticulum cells.

In general, the granulocyte-order cells exhibit altered protoplasm staining properties as well as coarsely streaked or granulated nuclei. They are grain-less and finely vacuolized, because of the fatty degenerating. Swollen, poorly outlined and partly obliterated due to autolysis cells constitute a significant percentage in blood smears (Fig. 3). Also many "shadows" of necrotically changed granulocytes are found. Giant polynuclear (most frequently with 2 nuclei) cells are numerous, showing anisochromia and polymorphism (Fig. 4). Granulocytes in different stages of regular and atypical mitosis are present as well (Fig. 5).

In the caudal vein blood smears and most of all in those of the heart an enormous amount of rod-shaped bacteria were found scattered or associated in groups. Part of them is phagocytized by granulocytes which show various stages of autolysis-induced disintegration of protoplasm and nuclei. Apart from the bacteriophages, the erythrocytephagous cells (Fig. 6), lymphocytes (Fig. 7) and, to a lesser extent, thrombocytes constitute a numerous group (20% granulocytes per 100 cells). In some smears, particularly those made of the heart blood, an increased number of pigmentophages with pigment intrusions occurs along with lipophage-like cells.

Necrotic granulocytes with small pyknotic nuclei typical of microleukocytosis prevail in smears in the intensified pathologic lesions.

Of the other leukocyte system cells, lymphocytes show various stages of necrotic degeneration and necrosis. The changes are of an exceptionally wide scope affecting, in average, 80% of the cell population. In general, the lymphocytes are hyperchromatous in their protoplasm and nuclei; those latter show a coarsely streaked chromatin decomposition. In more than usually abundant cytoplasm a circumnuclear thinning and single vacuoles of considerable size appear. Hyperchromatous lymphocyte nuclei, typical of this disease, usually show irregular outlines; non-typical multilobate nuclei with deep incisions are formed. Many cells resemble, in their morphologic characters, the plasmatic ones with

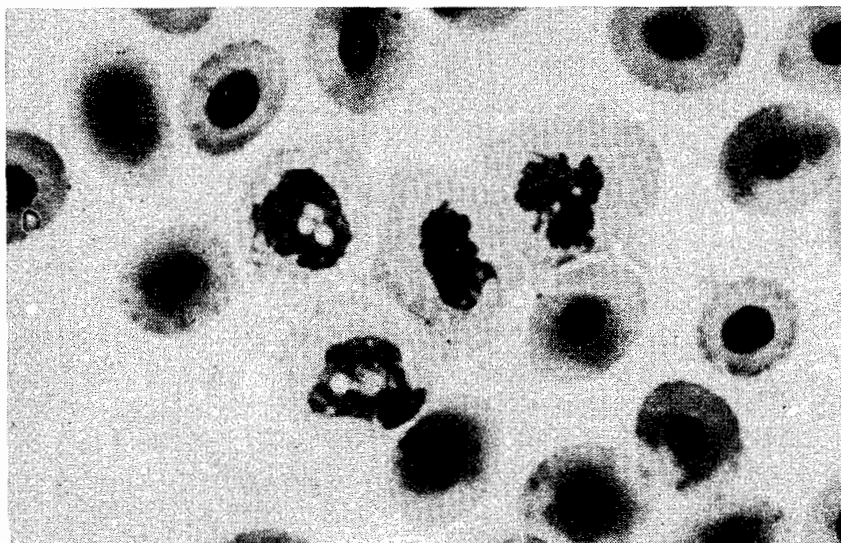


Fig. 5. The peripheral blood degenerated granulocytes in mitosis



Fig. 6. The peripheral blood phagocyte with an erythrocyte in its protoplasm

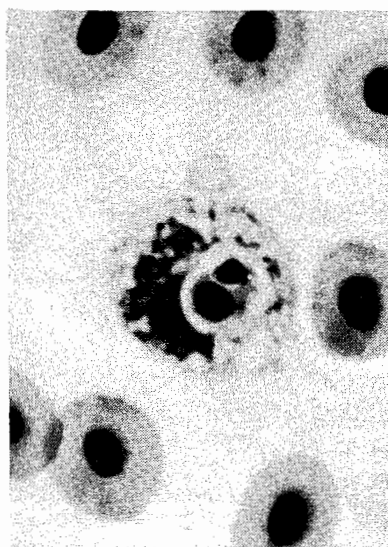


Fig. 7. The peripheral blood granulocyte with a phagocytosed lymphocyte in its protoplasm

a typical nuclear chromatin arrangement. Some of them are close in their appearance to small lymphoid reticulum cells, particularly so in smears of the heart blood taken intravitaly.

Acute pest is dominated by the necrotic processes. Most lymphocytes disintegrate during pyknosis, karyokinesis and cytolysis. Hypochromatous cells with delicate nuclei contours and chromatin fragments are typical; the "shadows" of these cells are numerous. The thrombocytes disintegrate more uniformly; they are, as a rule, hyperchromatous and necrotically changed due to different stages of pyknosis.

Hemopoietic organs

The tissue of the pronephros is in general rich in cells, the leukopoiesis centres being retained more or less regularly. One direction of the granulocyte differentiation prevails in smears, pro- and granulocytes being most numerous while granuloblasts and hemocytoblasts less so. No mature heterophile leukocytes are found. Poorly differentiated elements resembling granulocytes as well as some changed cells difficult to identify are abundant.

The quantitative cytologic picture of kidney is different in each case of the disease. In some, more moderately stimulated granulopoiesis with regular and atypical mitoses in pro- and granulocytes was observed (Fig. 8). At the same time a small number of this order precursors was recorded.

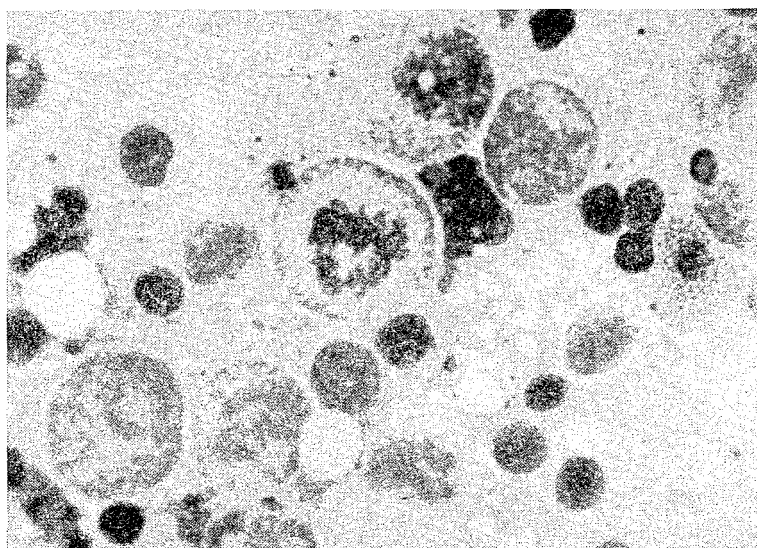


Fig. 8. An atypical mitosis in necrotically changed kidney

In all the cases examined, the degeneration and necrotic processes dominate over proliferation. In the intensive leukopoiesis centres the fatty degenerating is visible as early as in progranulocytes. Their cytoplasm is foamy or finely vacuolized, weakly basophile,

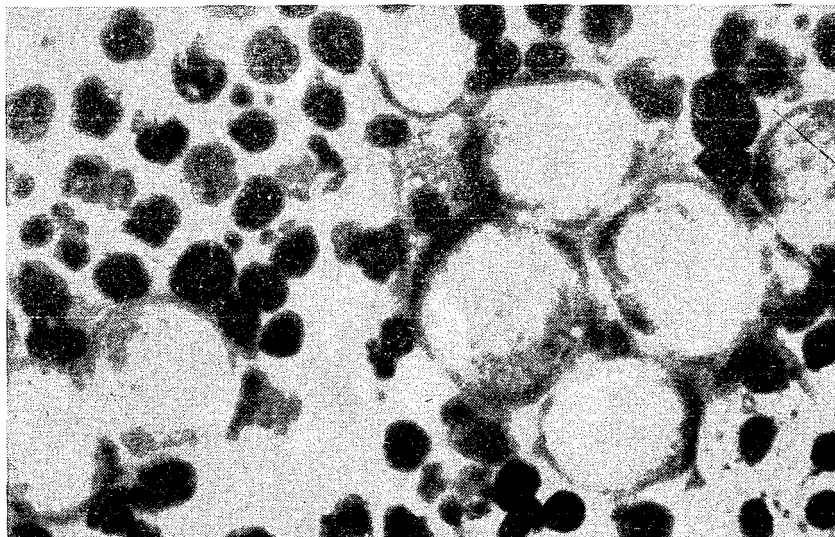


Fig. 9. Hypochromatous granulocyte-order cells and pyknotic lymphocytes in a hematopoiesis centre in kidney

with hypochromatous nucleus. Besides, the nuclei show excessive irregularities in their outlines, multilobateness occurring even in granuloblasts. Usually poorly staining, blisterous nuclei have loose, coarsely granulated or streaked chromatin with clear nucleoplasm thinnings. Such cells undergo a mass decomposition due to autolysis (Fig. 9).

More intense disintegration is evident within the lymphocyte population; in some kidneys the lymphopoietic centres differentiate weakly, forming only small groups of cells. In general, the lymphocytes show a clear anisocytosis as well as anisochromia and pyknosis. As a specific feature, lymphocytes with irregular, kidney-shaped or multilobate nuclei with deep incisions occur (Fig. 9). The cells showing irregular, hyperchromatous and most often pyknotic nuclei are most numerous in kidney as well as in the peripheral blood smears, comprising up to 60% of the lymphocyte population.

Vacuolized lymphocytes, particularly small hyperchromatous hemocytoblasts are the other unusual feature of degeneration; the protoplasm usually contains one or more seldom two vacuoles gradually filling up the plasm entirely and eventually rupturing the cell membrane. Because of that very high numbers of nuclei in karyo- and cytolysis without plasm (lympho- and thrombocytes) and necrotic cells with protoplasm outlines retained (most frequently granulocytes) are observed. Along with them a small amount of swollen, vacuolized phagocytes, sometimes containing intruded fragments of protoplasm or pigment grains occur. On the other hand, loose chromatin grains remaining from disintegrated cells as well as dead naked nuclei are very numerous. The kidney tissue is

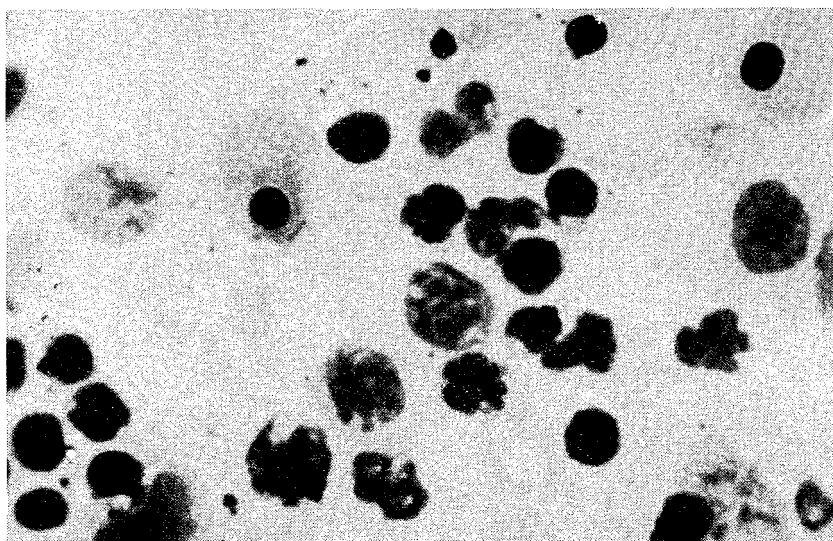


Fig. 10. Hyperchromatous multilobate lymphocytes in necrotically changed mesonephros

infiltrated with a big number of mature erythrocytes with poorly-staining deformed nuclei. There are many old forms with blurred outlines corresponding to dying cells.

The cytologic picture of the mesonephros initial part does not differ substantially in its cell composition and kinds of changes. Most abundant in the granulopoietic centres are granulocytes, the young forms together with mitotic divisions occurring less abundantly. The disintegration changes appear in zones; apart from regular cells, necrotic ones with hypochromatous nuclei predominate. Among lymphocytes, the forms with an excessive anisocytosis, hyperchromasia and pyknosis reach the 90% level. Also the amount of necrotic nuclei without protoplasm, those belonging to erythrocytes among the others, increases; the same concerns free protoplasm fragments and a necrotic matter (Fig. 10) not infrequently forming a thick layer in a smear.

In the terminal, broadend part of mesonephros, the picture of changes shows a marked variability. Granulocytes still differentiate with a considerable amount of hemocytoblasts. The other parts show the granulocyte numbers lower and all elements, those most primitive included, disintegrated necrotically. The most extensive necrotic areas were observed in this part of kidney; they were filled with numerous cells with hyperchromatous, homogenous nuclei of lympho- and erythrocytes as well as those of hypochromatous granulocytes. The nuclei without plasm are most densely spread. The lymphocytes disintegration reaches 100%; the erythrocyte number falls down, only single mature forms and sporadic polychromatous erythroblasts being retained.

The intensity of phagocytosis is also variable, usually it is limited, occasionally the erythrophagous cells and those resembling lipophagous ones as well as the pigmen-

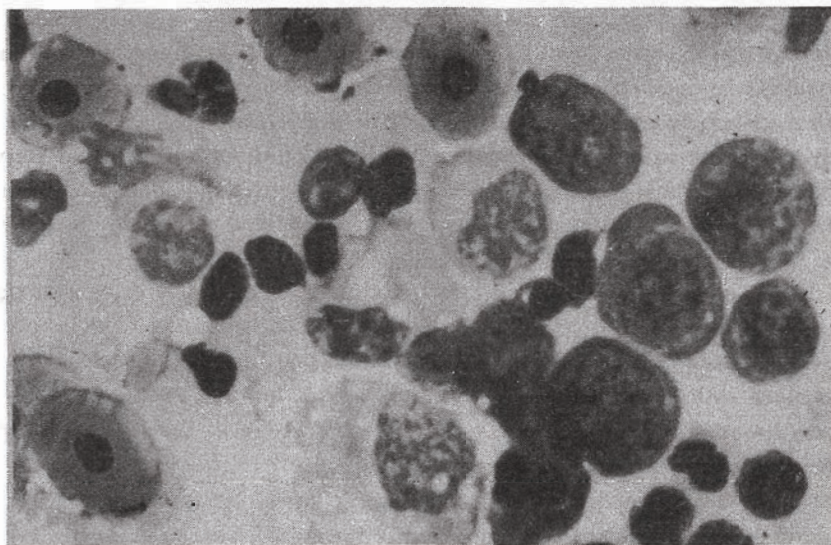


Fig. 11. Granulocyte-order primitive cells in spleen

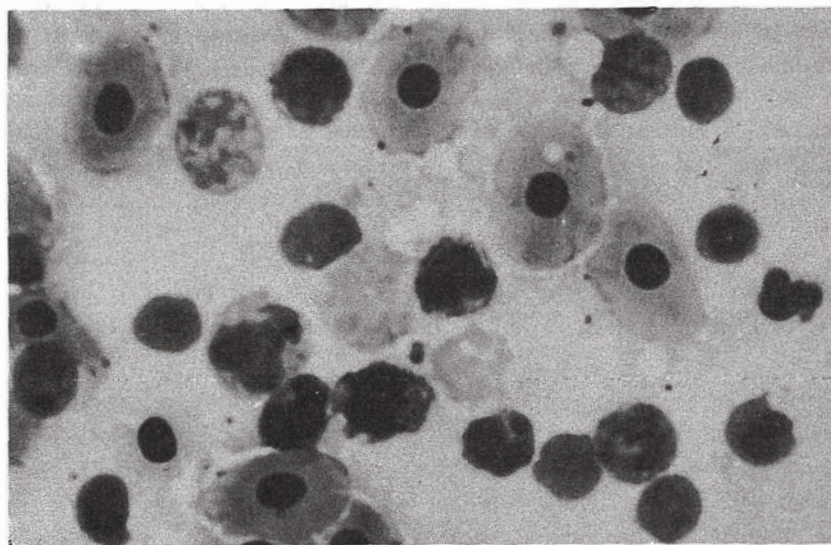


Fig. 12. Pyknotic lymphocyte in disintegrated spleen tissue

tophages being slightly more abundant. Those latter containing phagocytosed pigment grains are more numerous in the terminal part of the mesonephros.

The picture of spleen in *Pestis anguillarum* is variable showing a diverse composition of cells. More or less extensive granulopoietic centres occur with granulocytes prevailing. In some mounts, the numbers of small and large blastic cells are clearly increased (Fig. 11). The differentiation process is deformed by a parallel regressive and degenerative lesions. Similarly to the situation in kidney, the granulocytes disintegrate owing to autolysis, exhibiting a considerable swelling, changed staining properties and obliterated chromatin pattern. In the extremal cases the extensive centres are filled with many "shadows" of cells.

Destruction of erythrocytes in the spleen texture covers a wide area, greater than in the normal picture. Mature erythrocytes with deformed, obliterated nuclei occur in masses. Degenerated cells and numerous homogenous ones lacking the nucleoplasm form deposits along with denatured amorphous grey-blue matter. In some smears only single regular erythrocytes remain while the number of reticulum cells increases. Keeping pace with their proliferation, stimulated and phagocytosing cells grow in numbers retaining remains of erythro-, lympho- and thrombocytes in their plasm. Pigmentophages with hemosiderin deposits are increased in their numbers, too.

Also the pyknotically deformed lymphocytes disintegrate in excess; they constitute up to 60% of all lymphocytes (Fig. 12). The thrombocytes are necrotically changed as well; juvenile forms of lympho- and thrombocytes are completely lacking.

Of the other parenchymal organs, the liver hemopoiesis very often becomes stimulated. The granulocyte-order cells proliferate with all the transitional stages, hemocytoblasts included (Fig. 13); their numbers are variable. Similarly to the two previously

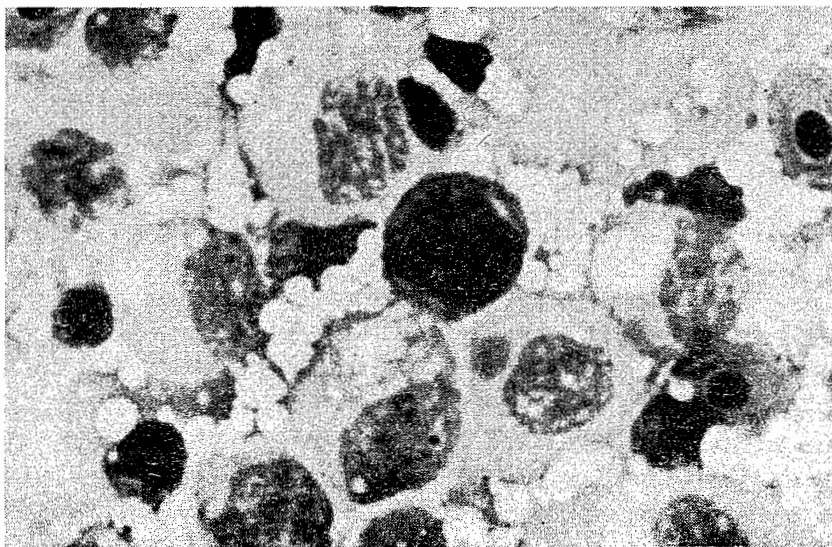


Fig. 13. A granuloblast, changed pro- and granulocytes in liver

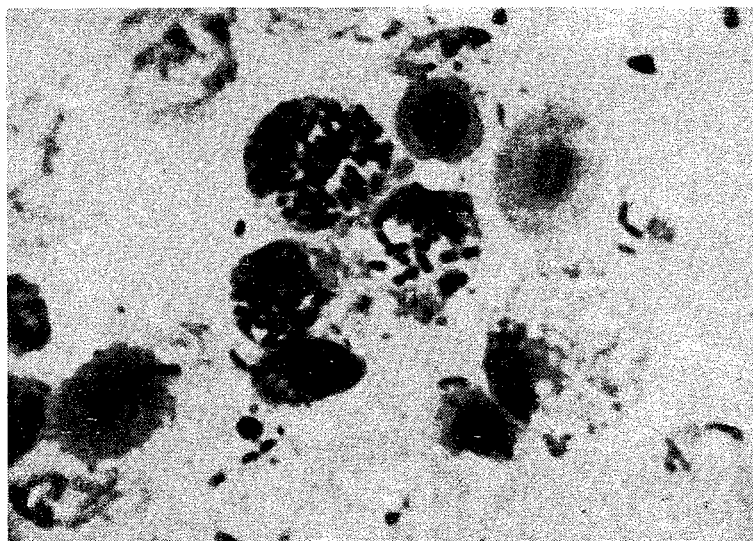


Fig. 14. Bacteriophages and a degenerated granulocyte in necrotically changed skin of eel

discussed organs, the necrotic lesions are strongly marked. The hepatic tissue is excessively infiltrated with erythrocytes, mostly necrotically changed. Hepatocytes are generally hyperchromatous, with cytoplasm strongly basophile and karyopyknosis; they disintegrate showing symptoms of the protoplasm fatty degenerating.

Smears taken from the skin anabroses show a mass occurrence of various developmental stages of juvenile granulocytes. Most of them die out with various pyknotic lesions. Considerable amount of granulocytes is stimulated towards phagocytosis, particularly on bacteria (Fig. 14). Pyknotic erythrocytes, lymphocytes and cells resembling plasmocytes are numerous.

DISCUSSION OF RESULTS

The acute form of *Pestis anguillarum* in eels shows, as its principal symptom, a hypochromatous anemia with some deviations as to the shape and size of erythrocytes (oligochromasia, aniso- and poikilocytosis), distempers in nuclei maturation and their divisions ("gemmation"). These processes are connected with a disturbed erythropoiesis, the balance between the erythrocyte production and destruction being lost. An excessive erythrocytolysis occurs in the peripheral blood, kidney, liver, being at its strongest in spleen. No substitutional compensating reaction renewing the erythroblasts takes place along with the disintegration of cells. Such a symptom is disadvantageous and is always accompanying the erythropoiesis retardation.

The disease discussed is characterized by a strong peripheral leukocytosis (or micro-leukocytosis), immature granulocytes or non-differentiated and atypical primitive cells

prevailing (parablastosis). An increase in the leukocytes-order juvenile forms indicates a shift to the left in the granulocyte picture and to quantitative changes in the course of the inflammatory reactions taking place. These processes are accompanied by an intensified proliferation of the granulocyte system in the normal hemopoietic organ i.e., kidney. The other parenchymal organs, however, are more strongly stimulated, spleen and liver in particular. Due to a disturbed blood cells' maturation, all these organs produce forms with irregular nuclei more mature than the protoplasm (progranulocytes, rod-shaped and lobate granulocytes). The stimulation is expressed also by numerous mitoses in various stages of the cell development and polynuclear hemocytes.

Because of the excessive proliferation of cells in the homopoietic centres, an increased size of the organs discussed can be noted during the clinical observations. In the course of the disease, degeneration and necrotic lesions develop while the compensating ability of the blood system deteriorates. In the late phase, the extensive necrotic lesions in the proliferation centres (e.g., in the terminal part of the mesonephros) can occur and the granulocyte precursors die out.

Under the pathologic circumstances, leukopoiesis is stimulated by disintegration products formed during inflammations accompanied by a necrosis. Enzymes released on granulocytes' disintegration take part in digesting the pathologically changed tissues. In the pest cases, an excessive lysis of granulocytes occurs mainly in the peripheral blood, necrotically changed skin, and the parenchymal organs, particularly in kidney. Simultaneously, the granulocytes are actively phagocytosing on bacteria (in the peripheral blood and skin anabroses) and necrotic hemocytes (erythrocytes, lymphocytes and others). An intensified phagocytosis observed in some cases is probably indicative of an earlier stage of the disease and reveals the still active defence of the organism. Also the intensified proliferation and the macrophagous cells' activity in spleen and kidney accompanied by the growth of reticulum cells indicate to the active resistance mechanisms.

A specific role in the immunity processes should be undoubtedly ascribed to lymphocytes although their reaction to pest is difficult to explain. On the one hand, the morphologic changes (nuclei segmentation, cytoplasm vacuolization, their appearance becoming similar to plasm cells) indicate to their differentiation ability, while on the other to an intensified cytolysis typical of acute pathologic states. The reaction of an apparent lymphopenia (almost complete disintegration of cells in the hemopoietic organs) deteriorates the cell functions and suggests a decreased role of these cells in the immunity processes.

The cytologic analysis of the hemopoietic organs shows the cells in only a part of the disease cases to maintain their characteristic reaction, necrotic lesions exceeding proliferation in the others. It should be presumed, then, that the further development of the disease would be unfavourable to an organism concerned.

OCENA CYTOLOGICZNA KRWI OBWODOWEJ I NARZĄDÓW KRWIOTWÓRCZYCH
PRZY *PESTIS ANGUILLARUM* U WĘGORZY

Streszczenie

Schorzenie *Pestis anguillarum* charakteryzuje się niedokrwistością niedobarwliwą oraz anizocytozą i poikilocytozą erytrocytów. Przebiega z wysoką leukocytozą (względnie mikroleukocytozą) z przewagą komórek niedojrzałych w stadium granulocytów, progranulocytów rzadziej granuloblastów oraz komórek niezróżnicowanych i atypowych (parablastoza).

Zarówno we krwi obwodowej jak i narządach krwiotwórczych występuje, różnie nasilona, cytoliza komórek krwi. Zmiany martwicze, poza erytrocytami, najsilniej obejmują limfocyty, zwłaszcza w ogniskach hematopoezy nerki i śledziony. Obok procesów rozpadu i degeneracji pobudzone są zjawiska proliferacji i fagocytozy. Aktywność żerną wykazują głównie komórki szeregu granulocytów (bakteriofagi, erytrofagi) we krwi obwodowej i martwiczej skórze oraz makrofagi w nerce i śledzionie.

Ogólny obraz cytologiczny oraz objawy nasilonej fagocytozy, w niewielu przypadkach chorobowych, wskazują na uruchomienie mechanizmów obronnych i odpornościowych. W innych daleko posunięte zmiany martwicze, obejmujące komórki pierwotne układu krwiotwórczego, nie rokuje korzystnego przebiegu schorzenia.

T. Ореца

ЦИТОЛОГИЧЕСКАЯ ОЦЕНКА КАРТИНЫ ПЕРИФЕРИЧЕСКОЙ КРОВИ
И КРОВЕТВОРНЫХ ОРГАНОВ ПРИ *PESTIS ANGUILLARUM* У УГРЯ

Р е з ю м е

Заболевание *Pestis anguillarum* характеризуется гипохромной анемией, анизоцитозом и пойкилоцитозом эритроцитов. Оно протекает одновременно с интенсивным лейкоцитозом (или же микролейкоцитозом) с преимуществом незрелых клеток в стадии гранулоцитов, програнулоцитов, реже – гранулобластов и однородных нетипичных клеток (парабластов).

Как в периферической крови, так и в кроветворных органах отмечается цитоллиз клеток крови, протекающий с разной интенсивностью. Некротические изменения вне эритроцитов наиболее интенсивно происходят в лимфоцитах, особенно в очагах гематопоеза почки и селезёнки. Наряду с процессами распада и дегенерации начинаются процессы пролиферации и фагоцитоза. Поглотительную активность проявляют главным образом клетки ряда гранулоцитов (бактериофagi, эритрофagi) в периферической крови и некротической коже, а также макрофagi в почке и в селезёнке.

Общая цитологическая картина, а также симптомы усиленного фагоцитоза, в некоторых болезненных случаях, указывают на активность защитных и иммунитетных механизмов. В других случаях значительно развитые некротические изменения, происходящие в первичных клетках кровообразовательной системы, не предвещают благополучного исхода заболевания.

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