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Fish pathology

HEMATO- AND HISTOPATHOLOGICAL CHANGES IN THE WHITEFISH (*COREGONUS ALBULA* (L.) INVADED BY METACERCARIAE OF *COTYLURUS ERRATICUS* (Syn. *ICHTHYOCOTYLURUS*) (RUDOLPHI, 1809)

ZMIANY HEMATO- I HISTOPATOLOGICZNE PRZY INWAZJI METACERKARII *COTYLURUS ERRATICUS* (Syn. *ICHTHYOCOTYLURUS*) (RUDOLPHI 1809) U SIELAWY (*COREGONUS ALBULA* (L.))

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A histopathological examination of parenchymal organs of the whitefish *Coregonus albula* (L.) infested with metacercariae of *Cotylurus erraticus* established the extent of inflammation and necrotic changes and justified a possiblity that the fish died as a result of the invasion. The examination revealed damaging inflammation of the cardiac muscle and retrogressive changes in the kidney with progressing damage of nephron elements, congestion, and hyperplasia of endoparenchyma of hemopoietic properties.

The high pathogenicity of *Cotylurus erraticus* metacercariae is evidenced by the scope of necrobiotic changes resulting from mechanical damage brought about by penetration, encystment, and intoxication.

INTRODUCTION

Invasions of metacercariae of numerous trematode species result in pathogenic changes occurring in different organs of the intermediate hosts affected. Pathogenic effects were documented for, i.a., metacercarias of *Diplostomum spathaceum, Posthodiplostomulum brevicaudatum*, and *Neodiplostomum scardinii*, parasitising in eyeballs and brain of numerous cyprinids. A strong invasion of metacercariae destroys a regular structure of the eye and produces disappearance of rods and causes blindness (Kozicka, 1958, 1959).

Similarly, *Bucephalus polymorphus* and *Rhipidocotyle illense* located in eyeballs, brain, spinal cord and nervous system coats were found to produce in, i.a., *Abramis brama* such effects as sight disturbances, cornea delamination, and retina damage leading to blindness (E. and J. Grabda, 1967; Baturo-Warszawska, 1980).

Under experimental conditions, cercariae of *Paralepoderma brumpti* enter underneath the skin, penetrate muscles, eyes, nervous system, heart, and kidney of hatchlings of the whitefish, lavaret, and other Cyprinids, and are lethal to the host (Kozicka and Niewiadomska, 1966).

Generally, except for invasions affecting larvae, metacercariae of *Posthodiplostomum cuticola, Apophallus muehlingi,* and *A. donicus* located in the skin, subcutaneous tissue, fins and gills of cyprinids and percids exert a less pronounced pathogenicity (Wierzbicka and Wierzbicki, 1973).

A mass invasion of the *Paracoenogonimus ovatus* metacercariae located in surficial parts of muscles (up to 95 larvae per 1 g of tissue), in fins, mouth cavity, and gills produces emaciation and muscle atrophy in *Abramis brama* and *Blicca bjoerkna* (Wierzbicka and Einszporn-Orecka, 1973).

According to Odening and Bockhardt (1971), invasion of the *Tetracotyle percae* fluviatilis metacercariae in *Acerina cernua* and *Perca fluviatilis* reaches 78% and 32 – 71%, respectively; the parasites were located in the swimming bladder wall, peritoneum, and in intestinal coils.

Szalkoczai and Molnar (1966) found pathological changes produced by *Tetracotyle percae fluviatilis* in *Hypophthalamichthys molitrix* and an invasion level of 500 parasites in a fish. Odening et al. (1970) determined invasion intensity of *Cotylurus c. cucullus* metacercariae in *Lucioperca lucioperca, Acerina cernua, Osmerus eper lanus, Gobio gobio,* and *Leuciscus idus* to reach 100% (150 – 500 cysts in a fish), 100%, 90%, and 40 – 50%, respectively, the cysts being found mainly on the heart ventricle and on the bulbus arteriosus. On the other hand, in *Abramis brama* and *Blicca bjoerkna* showing invasion incidence of 10 – 30% and intensity of up to 25 cysts, the parasites were located also on the intestinal peritoneum, liver, and gall bladder.

Metacercariae of *Cotylurus erraticus* Rudoplphi, 1809 are parasites specific for the genus *Coregonus,* particularly *C. albula* and *C. lavaretus.* The mature trematode *Cotylurus erraticus* and its metacercariae had been earlier known under the name of *Tetracotyle intermedia* Hughes, 1929 (synonym: *T. coregoni* Dogiel and Achmerov) (Niewiadomska and Kozicka, 1970). The connection between metacercariae of *T. intermedia* and the trematode *Cotylurus erraticus* was established by Razmaskin (1964, 1966) and Niewiadomska and Kozicka (1970). However, in his later monograph, Bauer (1987) gives another generic name, *Ichthyocotylurus erraticus* Rudolphi, 1809).

Within 1953–1956, Niewiadomska and Kozicka (1970) studying fish from 13 Masurian lakes found the presence and determined the invasion intensity of *Cotylurus erraticus* metacercariae in *Coregonus albula* (1–181 cysts) and *C. lavaretus* (1–40 cysts). J. Grabda (1971) recorded the maximum invasion of *C. lavaretus* to be 145 cysts found on a single heart.

The present paper is aimed at evaluating hemato- and histopathological changes in *Coregonus albula* invaded by *Cotylurus erraticus*.

MATERIALS AND METHODS

A mass invasion of *Cotylurus erraticus* metacercariae in the whitefish *Coregonus albula*, followed by die-out the fish affected, was recorded several times within 1960–1970 in some Masurian lakes.

The fish to be examined were caught in the Lake Lutry in spring and winter. The invasion incidence was 100%, while the intensity of invasion ranged from single to 50 metacercariae on a heart and 20 cysts in surficial kidney layers.

Materials for histopathologic examinations were collected from 25 metacercariae-infested and 10 control fish, the latter caught in the matacercariae-free Lake Łężany.

The materials were fixed in neutral formalin and in Zenker, Bouin, Susa, and Carnoy solutions.

The paraffin sections made were stained with the Delafield hamatoxylin with eosin and the Heidenhein hematoxylin with eosin. Some mounts were also stained using Passini, Mallory, and other techniques.

Smears were also made of the peripheral blood, collected from the heart and caudal vein and from the hematopoietic tissues (kidney and spleen); the smears were stained using the Pappenheim and Dominici technique.

RESULTS

Evaluation of pathologic changes

Heart. Cysts of the matacercariae are located mainly in the pericardial layer, usually all over the entire heart ventricle (more often in its tipped ending) or between the bulbus arteriosus, conus arteriosus, and the ventricle (up to 15 cysts along the longitudinal cross-section). Some cysts are suspended on the pericardial membrane processes, but seldom penetrate deeper into the muscle fibre layers close to the ventricle cavity (Fig. 1).

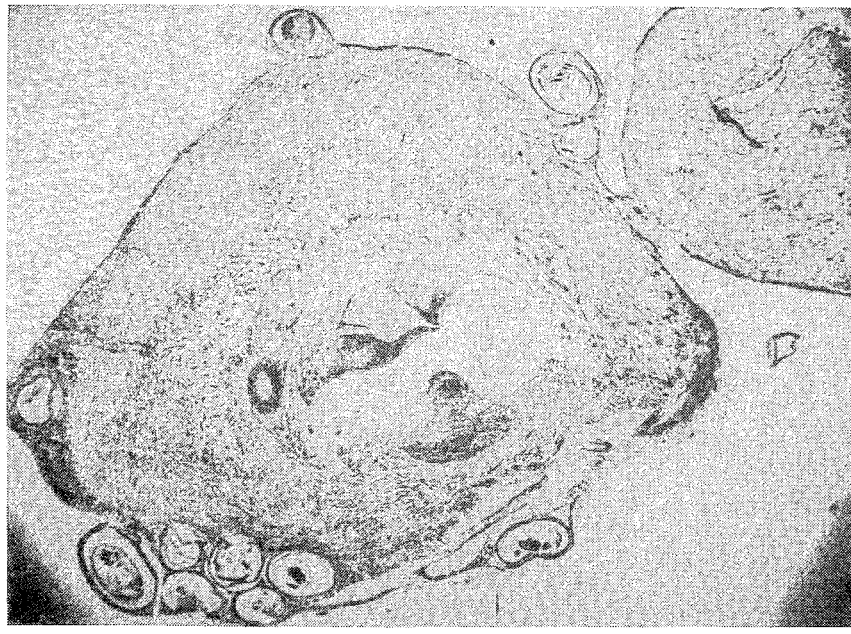


Fig. 1. Distribution of *Cotylurus erraticus* metacercariae cysts in the cardiac muscle: most parasites are located in the pericardium, fewer are found in the myocardium; location in the endocardium is sporadic

Fig. 2. Location of a metacercaria cyst in the peri- and myocardium with a weak infiltration, hyperplasia and degeneration of muscle fibres

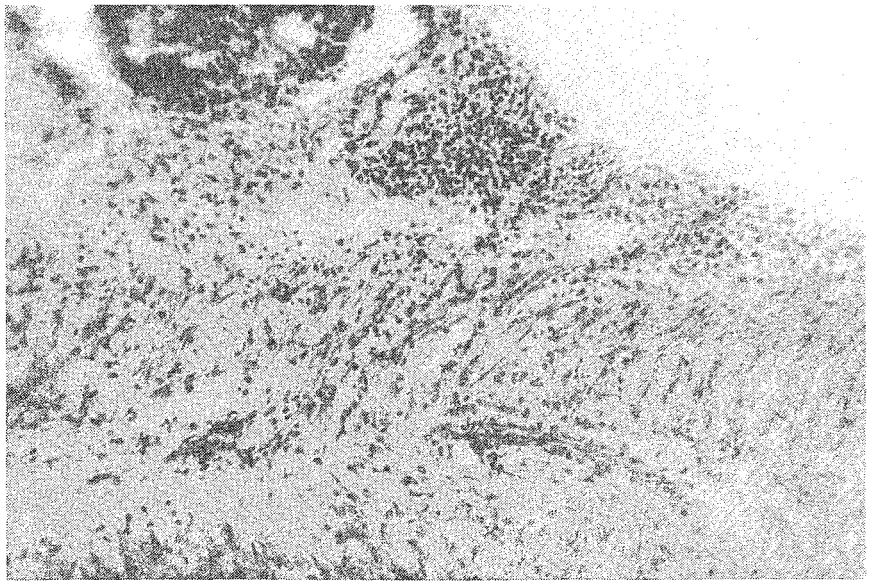


Fig. 3. Metacercaria in the pericardium with abundant inflammational infiltration and destroyed structure and fragmentation of muscle fibres; below clear losses of muscle tissue, changed during fibrination and scarring

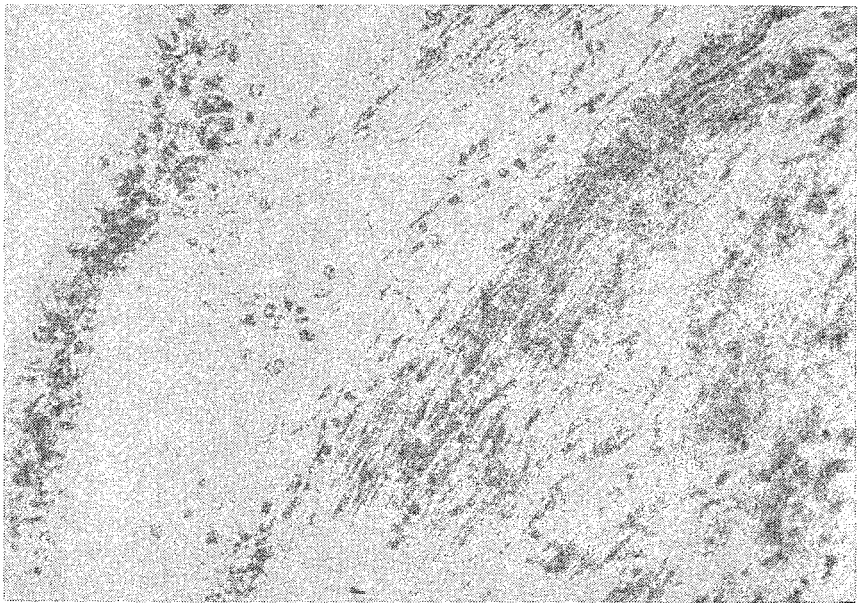


Fig. 4. Matacercariosis-associated necrotic lesion in the pericardium, with extravasation of erythrocytes, cytolysis of muscle fibres, and karyolysis or heteropyknosis of nuclei

Necrobiotic and hyperplastic changes are most pronounced around the cysts in the pericardial serosa, and less so in the myo- and endocardium (Fig. 2). The pericardial layer is variously hyperplastic within the entire komora, the hyperplasia being more intensive near the newly-encysted cercariae (Fig. 3). As a result of intensified proliferation, a granulation with predominating connective tissue fibres and morphotic blood elements forms around the cysts. The immediate surrounding of young cysts shows the presence of inflammational infiltration, more intensive than that around mature and formed cysts; the infiltration has a variable cellular composition with prevalence of non-differentiated, neutro-, and eosinophilous cells as well as phagocytes and pigmentophages. Losses of muscle tissue where the cercariae have penetrated are filled with connective tissue fibres producing scars.

Retrogressive changes concern also, and to a varying degree, deeper muscle layers of the heart ventricle within the myo- and endocardium. Muscle fibres in an inflammed tissue show an irregular arrangement with progressing fragmentation, disappearance of striation, and swelling and karyolysis of the nuclei. A necrobiotic process of a varying scope is observed, accompanied by vitrification and obliteration

Fig. 5. Cysts of *Cotylurus erraticus* metacercariae in necrotically changed kidney parenchyma, with vast lesions and haemorrhagic within-tissue extravasation

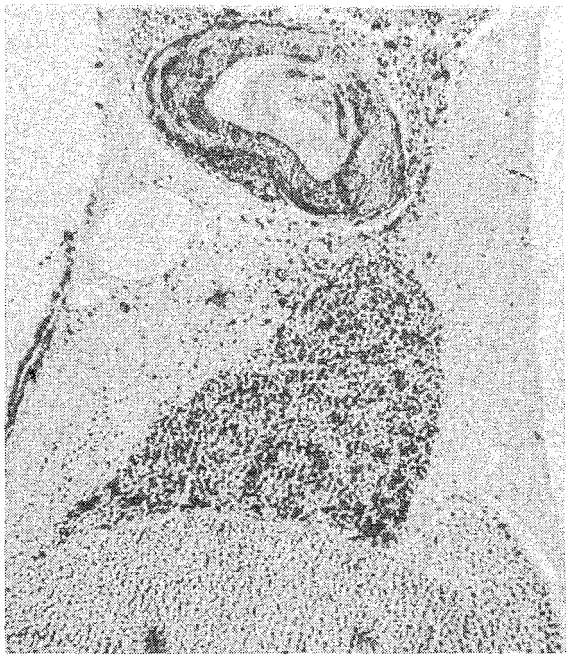


Fig. 6. Vast necrosis of the endoparenchymal tissue and haemorrhage form the main kidney vein near a metacercaria cyst

of fibre bundles and cellular elements of the inflammational infiltration (Fig. 4). Necrotic centres emerge also in the smooth muscles of the bulbus arteriosus and in the atrium wall.

The degenerative and inflammational processes are associated with disturbed circulation, visible as excessive filling of the coronary arterioles with irregular, pre-disintegrative blood cells (Fig. 4).

Kidney. All along the kidney, the cercariae are localised usually on the surface, under the external coat of connective tissue, and damage the adjacent tissue. Frequently, however, they produce much larger destruction of the endoparenchymal tissue and vast necrotic lesions (Figs 5, 6).

Depending on the timing of cercariae penetration, the range and intensity of retrogressive changes and hyperplasia in the kidney parenchymal tissue vary. A typical granulation of loosely connected fibres of the connective tissue, primordial reticularendothelial cells, and differentiating blood cells is formed zonally around the newly settled metacercariae (Fig. 7). As hyperplasia continues, a zone of connective fibres sets out and surrounds the parasites with a band of disintegrating tissue (Fig. 8). This layer gradually atrophies. The innermost, amorphous zone directly surrounds the parasite and, in older metacercariae, adheres rather tightly to the connective

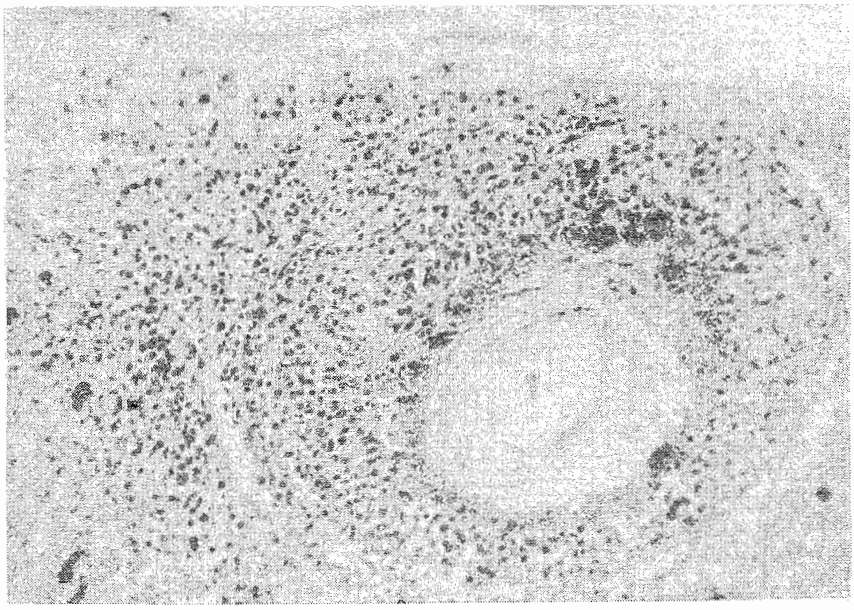


Fig. 7. An early cyst of *Cotylurus erraticus* in the kidney parenchyma with a complex granulation of conective tissue cells and blood morphotic elements

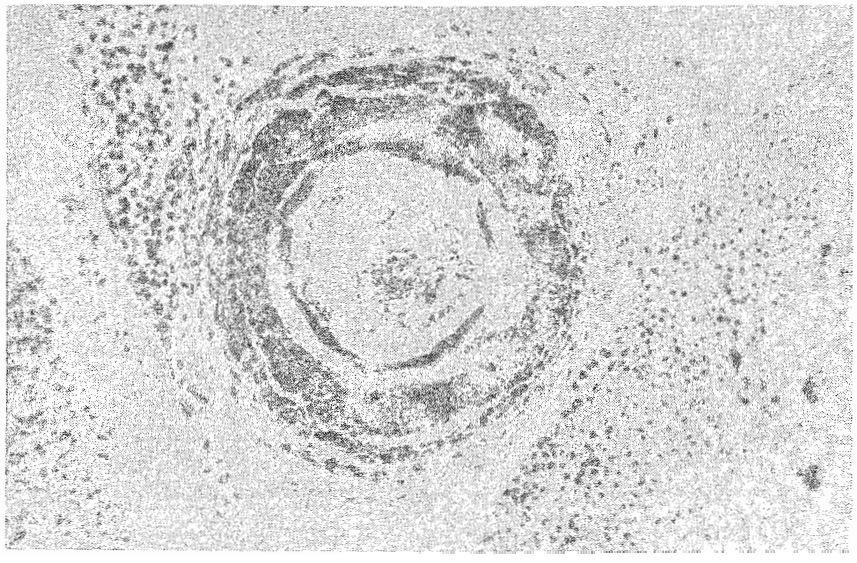


Fig. 8. A stratified arrangement of metacercaria cyst wall, with a predominance of conncetive tissue fibre layer and amorphous tissue of the host: the congested and necrotically changed kidney parenchyma around

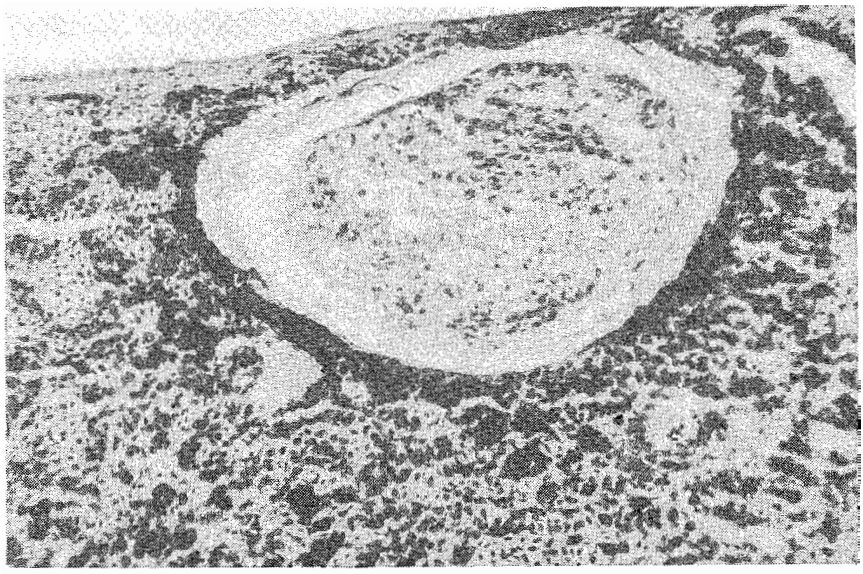


Fig. 9. A metacercaria cysts separated by a band of connective tissue fibres and dense hemosiderin deposits. Necrobiotically changed endoparenchymal tissue, congested with multiple centres, numerous deposits and cells of pigmentophages

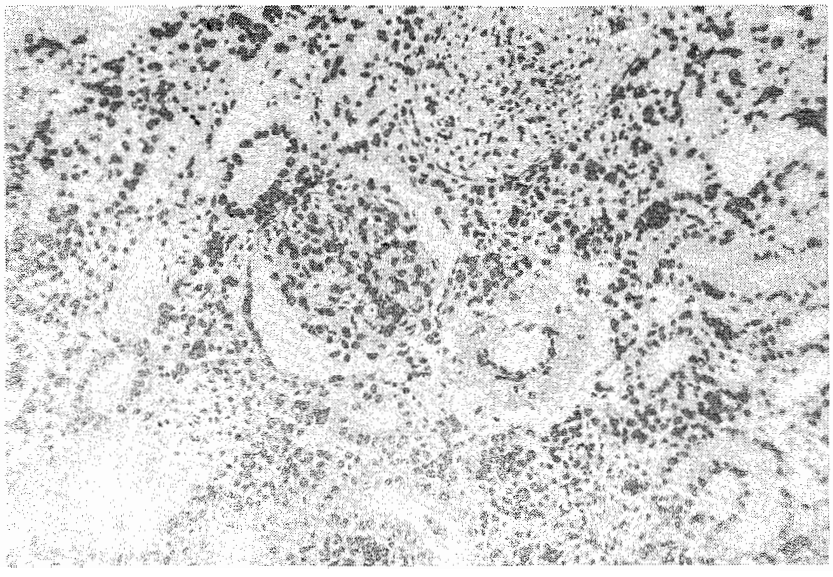


Fig. 10. Signs of degenerative necrosis of the kidney tissue with disintegration of nephron elements, blood vessels, and abundant parenchymal congestion

tissue coat produced by the host and enriched with pigment deposits and pigmentophages (Fig. 9).

Hyperplastic processes concern, to a varying extent, also deeper layers of the kidney tissue. The intercanalicular tissue of hemopoietic properties is subject to focal proliferation, the active mesenchyma of the reticular-endothelial system participating in the proliferation. The regenerative phenomena, however, are able to counteract in part only the retrogressive and necrotic processes concerning elements of both the nephron and the parenchymal tissues (Fig. 10).

Generally, the intercanalicular tissue is congested with blood vessels overly filled with pre-disintegrating blood cells. Where the veins are damaged, vast endoparenchymal haemorrhages are visible; other places feature smaller haemorrhagic centres, extravasations, and hematorrhages. The glomeruli, where retained, which occurs seldom, show signs of degeneration. The process proceeds in a variety of directions: some glomeruli are subject to proliferation of the endothelial cells, erythrocytes excessively filling the Henle loop. In other cases, the nephron structure becomes obliterated, vitrifies and succumbs to liquefaction. Most often, erythrocytes aggregate in a damaged Bowman sac, in the liquefied Henle loop and form separated clusters. The canaliculi become destroyed, too, as a result of epithelial cell membranes becoming liquefied and the nuclei, altered by pyknosis and karyolysis, dislocating.

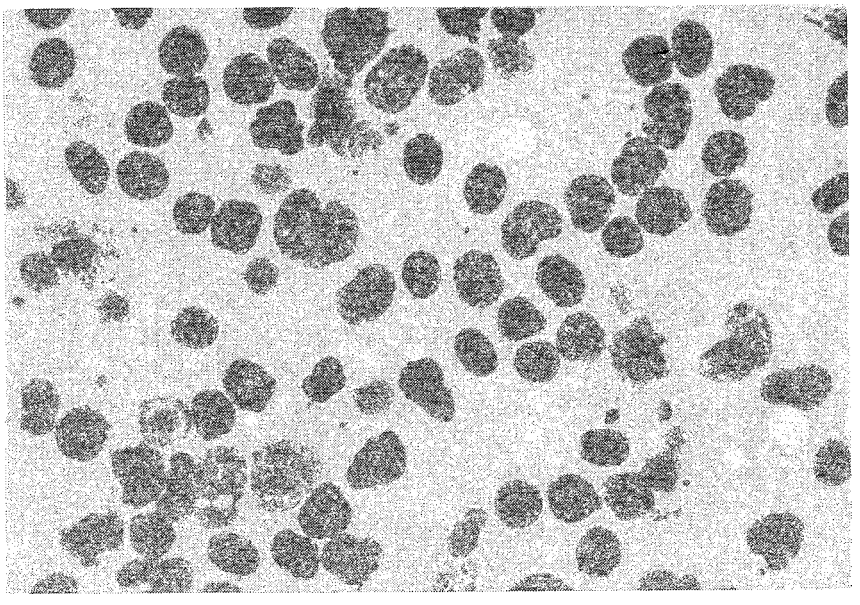


Fig. 11. Disintegration of morphotic blood elements in the kidney parenchyma

As a result of intensified necrobiotic processes, most morphotic blood elements in the hemopoietic tissue of the kidney degenerate. The erythrocyte population reveals the presence of non-typical forms showing signs of aniso-, poikilo- and schistocytosis at a varying stage of disintegration. Relatively seldom were immature blood cells at the polychromatic and basophilous erythroblast stage recorded. The primordial hemocytoblasts and non-differentiated and other cells of the leukocyte series show also features of degeneration and karyo- and cytolytic disintegration. Only few typical mature neutrophils and other cells are retained. On the other hand, naked nuclei, remains of disintegrated cytoplasm, and necrotic masses abundant (Fig. 11). The number of phagocytes (macrophages and hemosiderocytes) and pigment and pigment deposits increases.

Liver. No metacercaria cysts were found in the liver parenchyma. However, necrotic centres of denaturative character, varying in range, were observed. The whole organ shows a differing degree of destruction of the trabecular system, loose arrangement of hepatocytes which have obliterated contours and pyknotic and karyorrhexis--affected nuclei (Fig. 12).

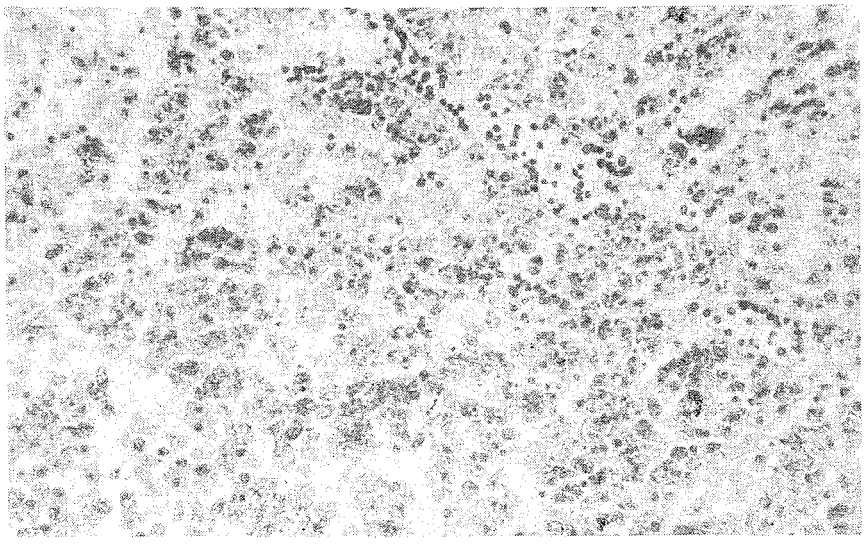


Fig. 12. Denaturative necrosis of the liver parenchyma associated with *Cotylurus erraticus* metacercariae infestation

The capillary and major veins are dilated and filled with oligochromatic erythrocytes showing signs of cyto- and karyolytic disintegration. Haemorrhages, extravasations, and hematorrhages are numerous. A weak inflammational infiltration

is observed. Granules and deposits of bilirubin and hemosiderin are scattered around in varying quantitites. The intercellular spaces contain amorphous masses of detritus remaining from disintegrated hepatocytes.

Spleen. No metacercariae settled in the spleen. However, necrobiotic and necrotic changes occurred with a varying intensity in the organ. The connective tissue interstice is hyperplastic and shows proliferation of the fibrous tissue with concurrent vitrification and homogenisation of the structure.

The spleen antra are abundantly filled with erythrocytes showing signs of plasmo- and karyolysis. Single polychromatophilous and basophilous erythroblasts occur. Degradation processes are pronounced also in the primordial cells of the leukocyte series and in the monoid non-differentiated cells. Damaged and necrotically changed cells prevail and are accompanied by numerous nuclei remaining from destroyed cellular structures. The cellular detritus is abundant.

Evaluation of blood morphotic elements

Erythrogram. The composition of morphotic elements and range of pathogenic changes in the metacercariae-infested whitefish differed depending on the blood collection site (caudal vein or heart).

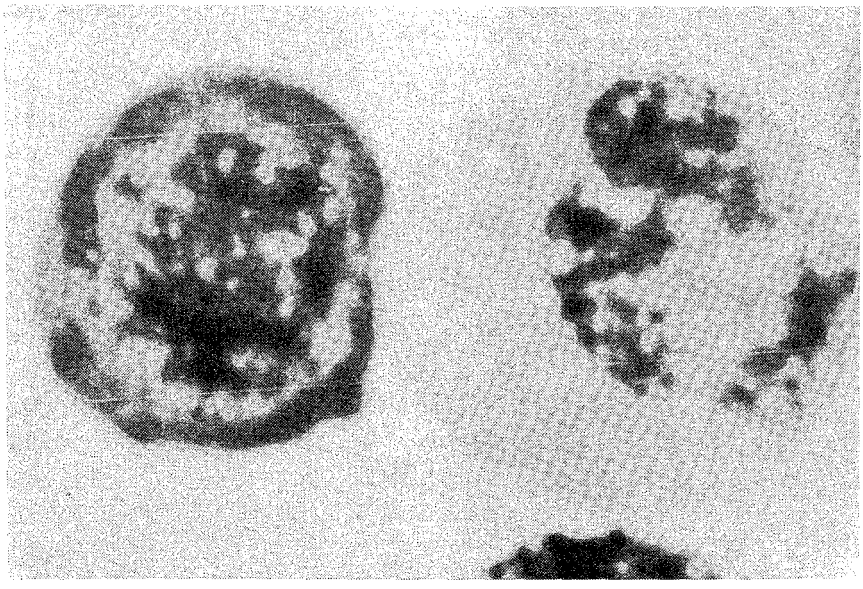
Most erythrocytes show deviations from the norm, more pronounced in the heart blood, with poikilo-, aniso-, and microcyte forms. Mature irregular erythrocytes are usually poly- or oligochromatic. The nuclei are differently dislocated and transfigured: they show chormatosea, and – more seldom – karyolysis and chromatolysis. A sizable number of erythrocytes were at a pre-disintegration stage, their structure being obliterated. Many cells were affected by plasmolysis and released misshaped homogenous nuclei. Additionally, amorphous masses of disintegrated cells were observed, particularly in the heart blood smears.

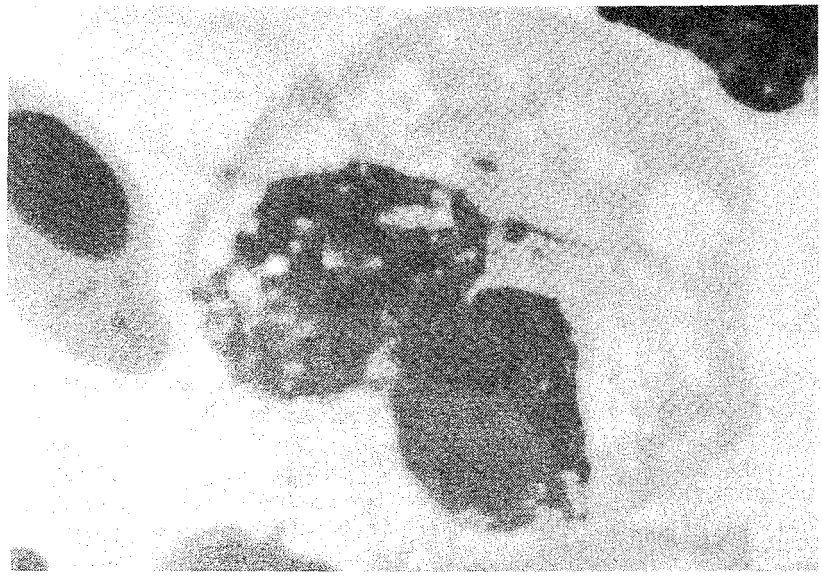
It was only very seldom that juvenile erythrocytes (polychromatic and basophilous erythroblasts) with signs of irregular differentiation and with a clear hyperchromasia of cytoplasm and nuclei were observed, mostly in the peripheral blood smears.

Leukogram. Differences in the leukocyte composition evidenced extremely different processes related to the inflammation of the heart, compared with the peripheral blood.

In the peripheral blood of both the metacercariae-affected whitefish and in the control ones, lymphocytes were prevailing, making up 81% and 89%, respectively. On the other hand, the cardiac blood of the infested fish showed lymphopenia, the contribution of lymphocytes dropping to 35.9% on the average. At the same time, the leukocytosis reaction with degenerative shift was identified, the number of cells reaching 64%, 38% of which being non-differentiated.

Generally, leukocytes show large variations in their staining properties, from hyperchromasia with simultaneous nuclear pyknosis to oligochromasia with progressing swelling, karyo-, and cytolysis. Most cells show features of vacuolar degeneration (Fig. 13). A large proportion (50%) of neutophiles and non-differentiated cells in the cardiac blood smears, is subject to degradation. Most liquefied cells are difficult to identify.





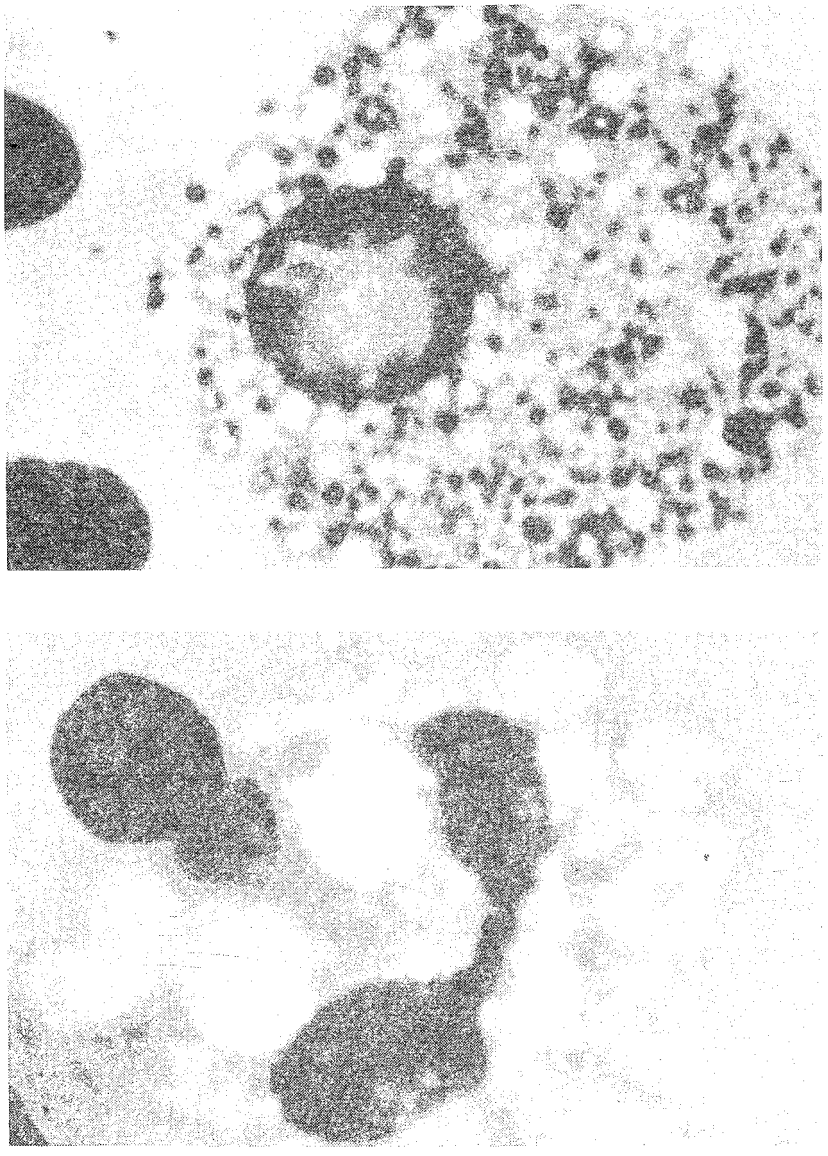


Fig. 13. Pathogenic leukocytes in the peripheral blood in *Cotylurus erraticus* metacercariae-invaded fish

DISCUSSION

Metacercariae of *Cotylurus erraticus* settled in *Coregonus albula* cause damaging and biotic changes in the organs affected. The histological evaluation reveals myo-

carditis with biotic changes and proliferation of endothelial elements towards fibrination and scarring. Intensity of the biotic processes depends on the timing and location of cercariae settlement in the heart muscle layers (pericardium; more seldom endo- and myocardium) or in the kidney parenchyma. The nature of a tissue and its reserves of the mesenchymal interstice determine the extent of hyperplasia and proliferation, particularly with respect to the inflammational infiltration, mostly from elements of the connective tissue (non-differentiated cells).

On the other hand, vast retrogressive changes in the kidney produce acute inflammation and disappearance of the nephron elements with progressing damage and liquefaction of glomeruli and canaliculi. The excessively congested and usually hyperplastic endoparenchymal interstices of hemopoietic properties forms a specific granulation, differently developed depending on the stage of host and parasite cyst formation. Proliferation of primordial hemocytoblasts and leukocyte series cells as well as phagocytes – pigmentophages (hemosiderocytes) proceeds with a varying intensity. A uniform pattern of necrobiotic changes in the liver, showing features of a vast denaturative necrosis justifies the suggestion of the parenchyma being intoxicated with endotoxins. Kidney disfunction, in terms of both excretion and hemopoiesis, is a manifestation of the generalsed toxemia. As a result of the intensified destruction of the blood morphpotic elements, hypersplenism of the spleen is pronounced, too.

As found by Baturo-Warszawska (1980), pathologic changes caused by cercariae of *Bucephalus polymorphus* Baer, 1827 and *Rhipidocotyle illense* (Ziegler, 1883) occurrd in all organs of the fish affected. Mechanical injuries and lytic changes in the vicinity of the parasites are produced by proteolytic glands.

Kozicka and Niewiadomska (1966) experimentally confirmed that cercariae are able to penetrate the hosts in masses, to produce mechanical and toxic damages during their migrations within the host's body, and to cause the fish larvae to die, deaths occurring also under natural conditions.

As shown by the present paper, the invasion of *Cotylurus erraticus* metacercariae can be considered responsible for the dieout of *Coregonus albula* in some Masurian lakes within 1960.

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Translated: Dr T. Radziejewska

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ZMIANY HEMATO- I HISTOPATOLOGICZNE PRZY INWAZJI METACERKARII *COTYLURUS ERRATICUS* (syn. *ICHTHYOCOTYLURUS*) (RUDOLPHI 1809) U SIELAWY (*COREGONUS ALBULA* (L.))

STRESZCZENIE

Wynik oceny klinicznej i histopatologicznej narządów wewnętrznych sielawy zarażonej metacerkariami *Cotylurus erraticus* uzasadniają śnięcie tych ryb wielokrotnie notowane, w latach 1960–70, w niektórych jeziorach Pojezierza Mazurskiego m.in. w jeziorze Lutry.

Cysty metacerkarii zlokalizowane były głównie w warstwie osierdziowej wzdłuż całej komory serca, prócz tego między opuszką tętniczą i przedsionkiem (maksymalnie do 50 cyst). Wywołują zapalenie uszkadzające mięśnia sercowego (*myocarditis*) z rozległymi ogniskami martwiczymi przy równoczesnym włóknieniu i bliznowaceniu tkanki. Zmiany nekrobiotyczne nasilone są w pobliżu cyst w błonie surowiczej worka osierdziowego (*pericaridum*), mniej w warstwach *myo-* i *endocardium*. Różnie zaznaczają się zmiany rozplemowe oraz proliferacja komórek nacieku zapalnego z elementów podścieliska łącznotkankowego.

W nerce, drugim narzędzie wybiórczo zasiedlonym, metacerkarie usytuowane są powierzchniowo pod łącznotkankową torebką (maksymalnie do 20 cyst). Powodują rozległe zmiany zanikowe elementów nefronu z postępującym uszkodzeniem i rozmyciem kłębków i kanalików nerkowych. Zapalenie zmienione, przekrwione i przeważnie przerośnięte utkanie śródmiąższowe tworzy swoistą ziarninę różnie wykształconą

w zależności od rozwoju cysty pasożyta. W zmiennym stopniu zachodzi różnicowanie komórek pierwotnych (hemocytoblastów) i komórek szeregu leukocytarnego oraz pobudzonych czynnościowo fagocytów typu makrofagów i hemosyderocytów. Leukogram krwi (pobranej z serca) ryb zarażonych cechuje limfopenia (36%) i granulocytoza (64%), przy czym większość komórek uległa zmianom zwyrodnieniowym i martwiczym. Natomiast we krwi obwodowej (pobranej z żyły ogonowej) zarówno przy matacerkariozie jak i u ryb kontrolnych, wyznaczono limfocytozę (81% i 91%) i granulopenię (19%).

Patogeniczność metacerkarii *Cotylurus erraticus* określa zasięg zmian zapalnych i martwicotwórczych w narządach bezpośrednio uszkodzonych przez pasożyty w wyniku penetracji, ocystowania i działania litycznego. Zmiany w wątrobie o cechach martwicy denaturacyjnej spowodowane są intoksykacją miąższu. Podobnie jak następstwem ogólnej toksemii jest niewydolność czynnościowa nerek w zakresie funkcji wydalniczej i hemopoetycznej.

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