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Histopathology

**NEUROPATHOLOGICAL PICTURE IN FISH AFFECTED BY CARP
SEPTICAEMIA (*Septicaemia haemorrhagica cyprinorum*)**
OBRAZ NEUROPATOLOGICZNY U RYB Z POSOCZNICĄ KARPI
(*Septicaemia haemorrhagica cyprinorum*)

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The paper deals with neuropathologic lesions in brains of septicaemia-affected carp and crutian carp. The structure and topography of primary viral encephalitis are determined.

INTRODUCTION

Amongst symptoms of carp septicaemia there are some indications of disturbances in the central nervous system such as sluggish paraetic swimming with equilibrium distempers or an adoption of a flounder-like position. Diseased individuals poorly respond to external stimuli, which makes them easy to catch by hand. These undoubtedly neurologic symptoms in septicaemia-affected fish have attracted the researchers' attention to the fish central nervous system (Nikolau and Dinu, 1952). The hitherto-accumulated data on fish central nervous system affected by septicaemia (Epstein et al., 1934; Pieszkow, 1954; Nikolau and Dinu, 1952; Gončarov, 1954; Faktorowicz, 1968, 1969; Kanajev, 1973) provide no exact description of a structure and topography of changes in the fish central nervous system.

MATERIAL AND METHODS

Fishes kept in ponds, 53 ill and 5 healthy carp individuals as well as 10 ill and 5 healthy crutian carp ones provided materials to be studied. A total number of 73 fish brains was examined. The studies were commenced in spring 1972. A living fish was intoxicated with alcohol penetrating from a cotton wool ball inserted under its opercular cover, and then killed by cutting its spinal cord abreast the fifth thoracic vertebra. Dissected brains together with adjacent sections of spinal cord were fixed in 8% formalin, after which they were mounted in paraffin block. The latter were sagittally microtome-cut along the brain long axis beginning from the lateral side. The 5 μ m-thick sections obtained, containing the entire sagittal plane of the brain were mounted on glass slides and stained with haematoxylin – eosin and cresil violet, glia and myelins being additionally stained with Kanzler Arendt-modified Holzer method and ferruginous haematoxylin after Heidenhain, respectively (Fig. 1).

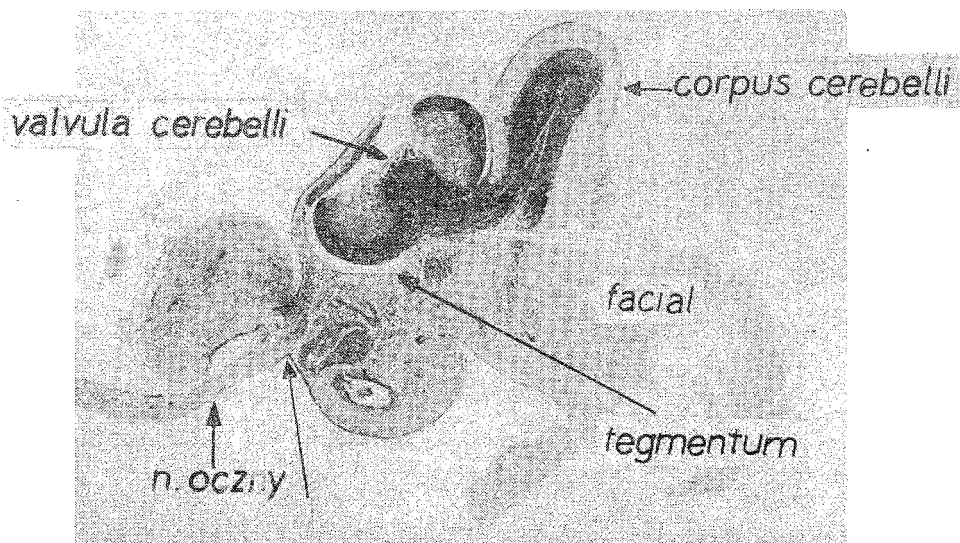


Fig. 1. Brain of carp (3-years old fish.). Cross-section in perpendicular plane

NEUROPATHOLOGICAL CHANGES ASSOCIATED WITH VARIOUS FORMS OF SEPTICAEMIA

Carp (*Cyprinus carpio* L.)

Acute (exudative) form of septicaemia (spring)

Glia-lymphoid infiltrations around blood vessels and changed neurons were revealed in the myelencephalon, moderate infiltrations being found in the diencephalon (Figs. 2 and 3). Within the *valvula cerebelli* a considerable proliferation of glia (Fig. 4, 5) with

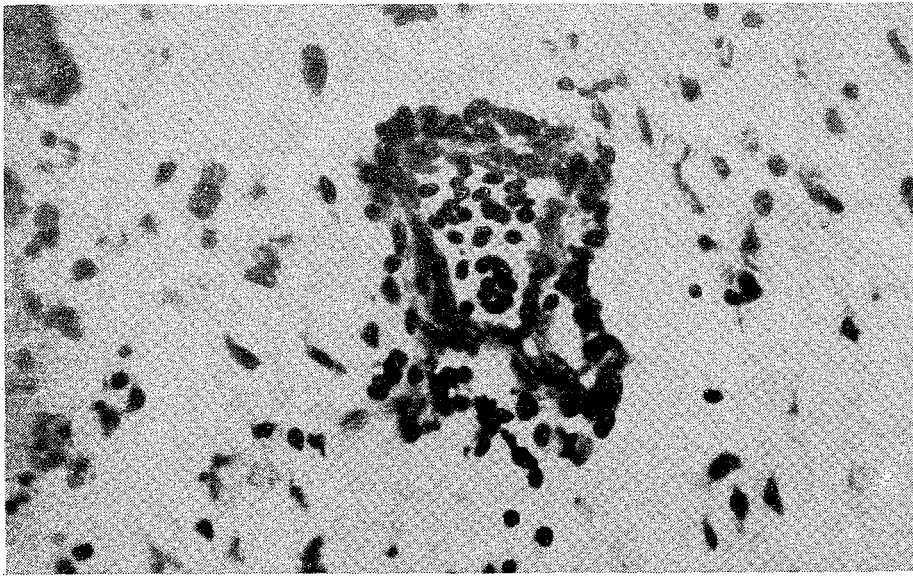
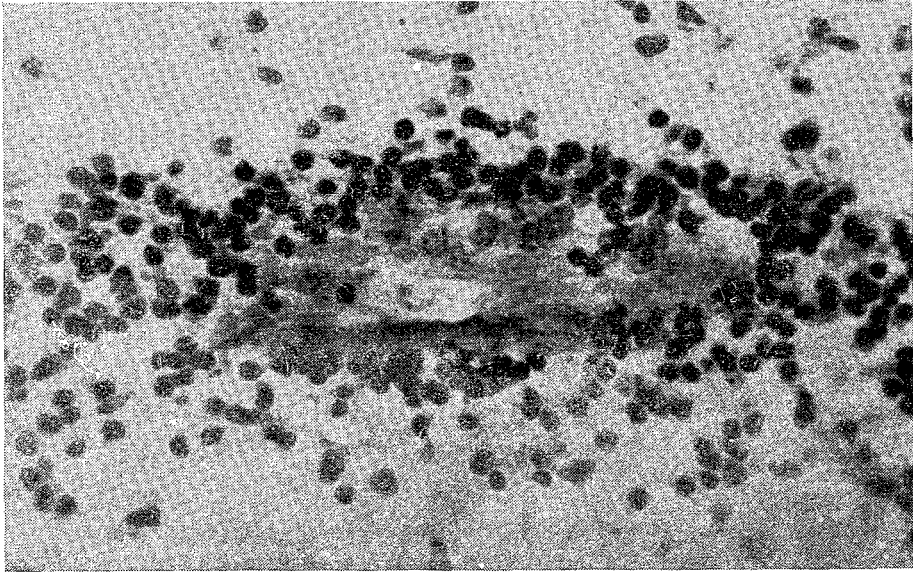


Fig. 2,3. Acute exudative carp septicaemia. Perivascular infiltration with rounded cells. CV. (cresil violet) staining. 400X

microglia (rhabdoid cells) (Figs. 6 and 7) and diluted chromatin-containing glia cells appeared, the latter also visible in the end-brain. Intracellular inclusion bodies were observed in neurons of the tegmentum and *truncus cerebri*.

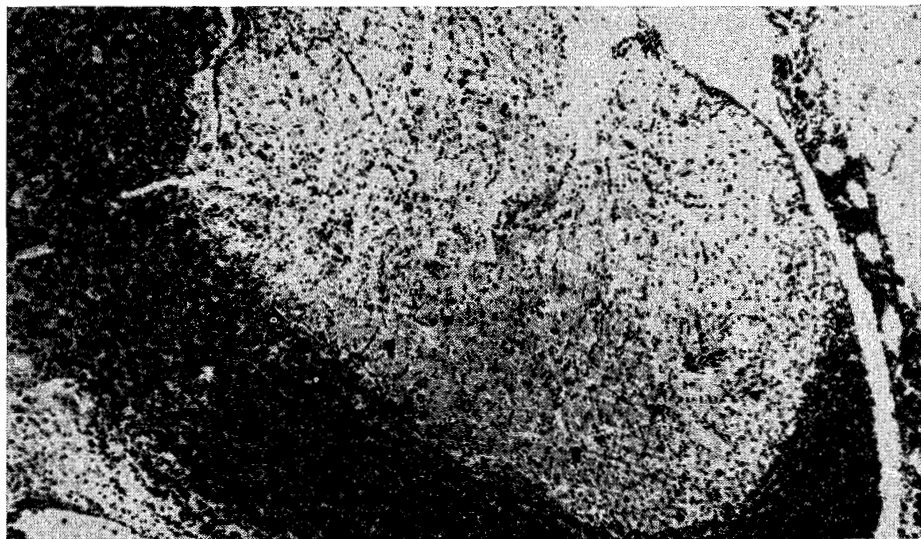


Fig. 4. Acute carp septicaemia (1-year old fish). Glia proliferation in mesencephalon (*valvula cerebelli*). CV. staining. 100 \times .

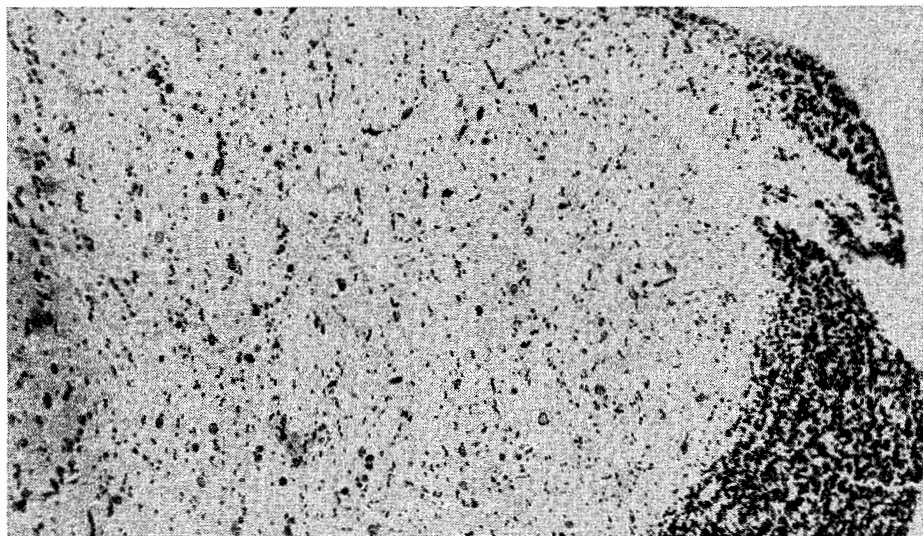


Fig. 5. Region of *valvula cerebelli* in mesencephalon of a control carp

Sub-acute (exudative-ulcerous) form of septicaemia (summer).

Most frequently observed were neurophagous papulae in the mesencephalon and cerebellum (Fig. 8). Neuronal lesions occurred as differing in their advancement acute or

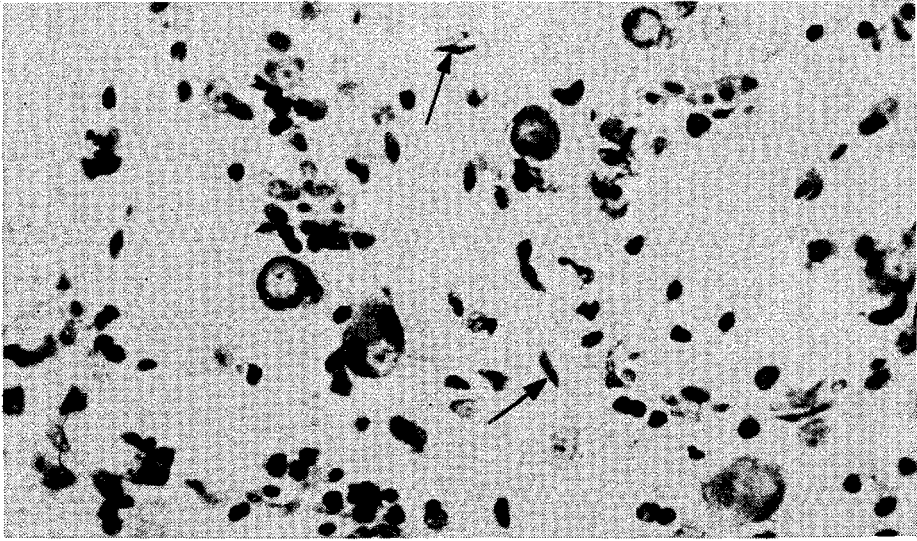


Fig. 6. Acute carp septicaemia. Glia proliferation in mesencephalon. Rhabdoid microglia cells visible (↑).
CV. staining. 400 ×

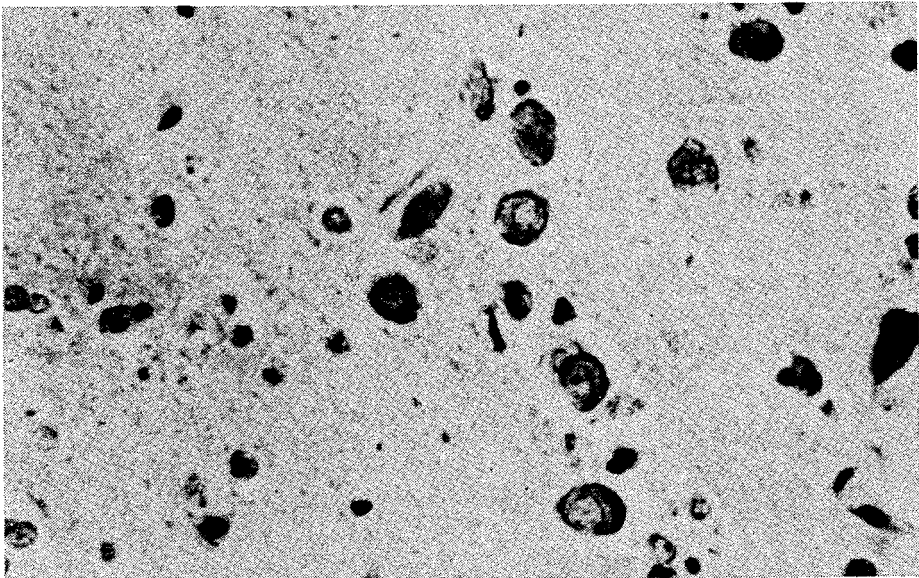


Fig. 7. Mesencephalon of a control carp.

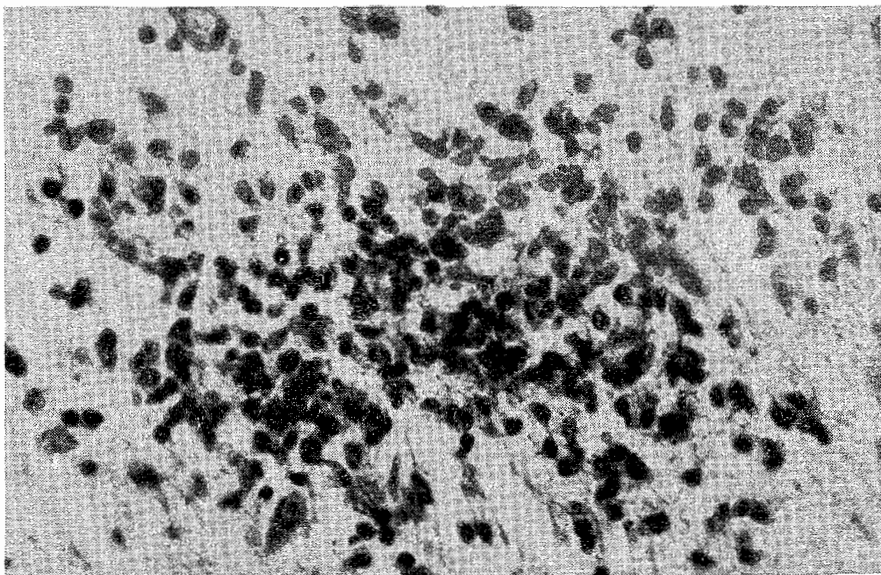


Fig. 8. Sub-acute carp septicaemia. Large glia papula in swollen tissue of brain.
CV. staining. 400 \times .

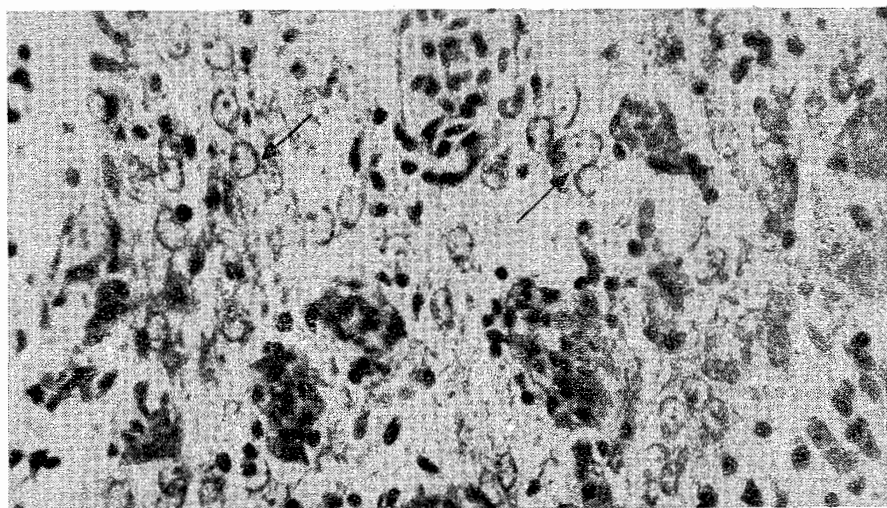


Fig. 9. Glia cells with transparent nucleus chromatin (the Alzheimer II cells (\uparrow)). Blood vessels filled with blood. CV. staining. 400 \times .

strong cellular diseases in neurons of the diencephalon, mesencephalon, and myelencephalon. Intranuclear inclusion bodies up to 8μ in diameter were encountered in neurons of the mesencephalon and myelencephalon. Both the infiltration and neuroglia showed the presence of cells with "empty nuclei" and invisible cytoplasm, morphologically resembling the Alzheimer II cells occurring in some human encephalopathies (Fig. 9).

Chronic ulcerous form of septicaemia (autumn)

In this form of the disease, neurons with enlarged nuclei and mulberry-like appearance were observed. A neuron thus changed sometimes showed an inclusion body seen as a homogenous formation pushing the micronucleus to the nucleus membrane (Fig. 10). The inclusion body was of a 14μ diameter, the nucleus diameter measuring $28\mu\text{m}$.

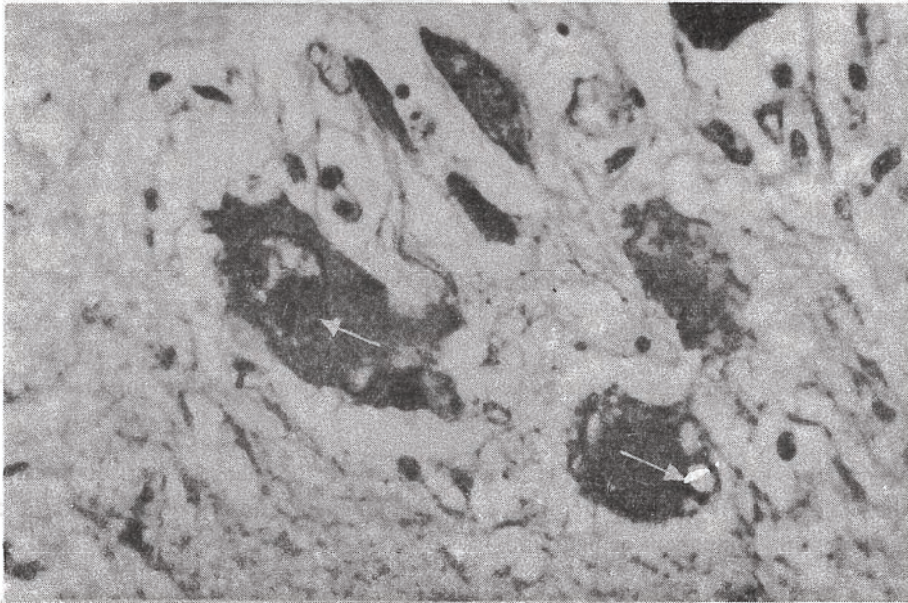


Fig. 10. Chronic carp septicaemia in autumn. Inclusion body fills the nucleus, micronucleus pushed aside to nucleus membrane (\uparrow). Heavily diseased neurons nearby. Right neuron with vacuolized cytoplasm margine. Myelencephalon H-E (Hematoxylin-Eosin) staining. 400X.

Chronic form of carp septicaemia (after ,methylene blue and detreomycin per os treatment)

Discrete perivascular infiltrations and those situated by the brain surface-limiting membrane were still in existence. Neuronal devastations were observed in the cortex of cerebellum while its granular layer showed gliosis. Large intracellular inclusion bodies were found in neurons of the mesencephalon and myelencephalon (Fig. 11).

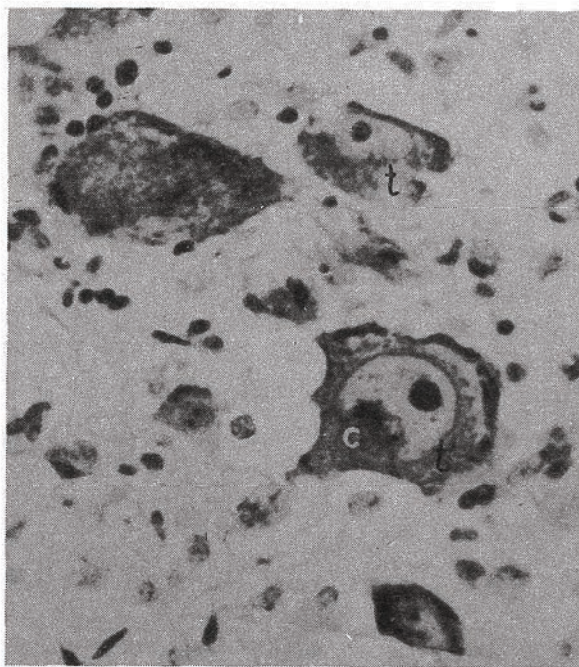


Fig. 11. Partly cured septicaemia. Inclusion body (C) in nucleus and central tigrolysis (t) in neurons. Myelencephalon. H-E staining. 400X.

CRUTIAN CARP (*Carasius carassius* L.) of the carp pond culture infected with septicaemia

The structure of neuropathologic lesions was similar to the changes described in carp, i.e., the same elements of the structure were observed, their intensity being weaker and no predominating component being found.

DISCUSSION

The histopathologic lesions described from brains of carp and crutian carp affected by septicaemia are similar in their structure and topography to those occurring in brains of other vertebrates suffering of viral inflammation of this organ (Osetowska, 1972, 1973). The following elements are common:

- a) glia-lymphoid infiltrations around vessels,
- b) glia proliferation around neurocytes with areas of glia proliferation, Alzheimer II, and microglia rhabdoid cells,
- c) intracellular inclusion bodies in neurocytes changed destructively.

Particularly interesting, during the inflammation described, are changes in the glia. They are characterised by its intense proliferation, particularly in the acute form of the disease at the valvula cerebelli level, as well by its qualitative changes. For example, cells

with "empty" nuclei and invisible cytoplasm are denoted the Alzheimer II cells since they appear to be pathologically transformed fibrous neuroglia cells. The occurrence of microglia, its rhabdoid form in particular, was somewhat astonishing. Healthy fishes, as stated by Bagiński (1955), have no microglia. Therefore, in my opinion, the antigen stimulation, while bringing the differentiation and hypertrophy of cerebral mesenchymal tissue about, reveals the presence of rhabdoid cells belonging to the microglia and originating within the mesenchyma, the latter – as it is widely known – showing great potential to undergo transformations (Osetowska, 1974).

Neuronal lesions occurring as different pictures of acute or strong cellular disease predominated particularly during the later phases of the illness (the chronic form in autumn). Here the neurons with neuropil margins jagged and transformed into a mulberry-like shape were observed. Most frequently the neurons altered in this way contained single Cowdry "A" – type intranuclear inclusion bodies of diameter equal to or exceeding that of the nucleus. Intracellular inclusion bodies are met with in human encephalitis (Chou and Cherry, 1967; Hideo et al., 1966; Martinez et al., 1974; Ostrowska, 1967) evidencing a contact between the cell and a virus (Osetowska, 1974). Nikolau and Dinu (1952) as well as Jankov (1972) are of opinion that carp septicaemia is initiated by a virus in the central nervous system. The virus of an acute exudative form of septicaemia (SVC) was named Rhabdovirus carpio by Fijan et al., (1971). It belongs – along with the rabies virus, Rabiesvirus canis – to the Rhabdovirus group (Larski, 1974). When the septicaemia-affected carp encephalitis is compared to the encephalitis in mammals suffering of rabies, common elements in the structure and topography of the process: glia proliferation areas with microglia-rhabdoid cells, neurophagous papulae, perivascular inflammation infiltrations, and degeneration of neurons become visible. The changes are observed primarily in the diencephalon, mesencephalon, tegmentum nuclei and in the spinal bulb. The intensity of neuropathologic pictures decreased toward the chronic form in which neuropathic lesion and restoration reactions of fibrous glia took place. Therefore it can be stated that the pictures of acute and chronic forms of septicaemia exhibit many common features and are the pictures of the same disease, primary viral encephalitis, in spite of an opposite view expressed by Fijan (1972).

CONCLUSIONS

The following conclusions could have been drawn from the studies presented:

- 1) Each form of carp septicaemia exhibit lesions in the central nervous system, the changes appearing as a primary encephalitis with features typical of a viral infection.
- 2) In various forms of carp septicaemia, the neuropathologic pictures of carp brains differ, with respect to the disease dynamics, in the intensity of inflammation occurring without any qualitative difference. The lesions concern various components of the nervous tissue; they cover:
 - a) glia-lymphoid infiltration around congested vessels;

- b) proliferation and active transformation of glia with the Alzheimer II cells, mesenchymal glia, and neurophagous papulae occurring in the areas of proliferation;
 - c) destructive changes in neurons, observed as acute, subacute, and chronic cellular disease;
 - d) the presence of intracellular inclusion bodies in neurocytes of the nucleus isthmi and truncus cerebri.
- 3) Similar lesions were observed in crutian carp in ponds populated by septicaemia-affected carp.

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OBRAZ NEUROPATHOLOGICZNY U RYB Z POSOCZNICĄ KARPI
(*Septicaemia haemorrhagica cyprinorum*)

Streszczenie

Na podstawie badań histopatologicznych 73 mózgów ryb (karp, karaś) opisano obraz neuropatologiczny w poszczególnych postaciach posocznicy karpi (*septicaemia haemorrhagica cyprinorum*). Stwierdzono, że w mózgach chorych ryb występują zmiany o charakterze pierwotnego zapalenia, charakterystyczne dla infekcji wirusowej. Zmiany te dotyczą różnych składników tkanki nerwowej i polegają na:

- a) nacieku gleju limfoidalnym wokół przekrwionych naczyń,
- b) rozplemie i przemianie aktywnej gleju z występowaniem w polach proliferacji gleju komórek Alzheimer II i gleju mezenchymalnego oraz grudek neuronofagicznych,
- c) zmianach destrukcyjnych neurocytów, występujących jako: ostre, ciężkie i przewlekłe schorzenie komórkowe,
- d) występowaniu ciałek wtrętowych wewnątrzjądrowych w neurocytach okolicy *nucleus isthmi* i *truncus cerebri*.

Э. Станкевич

НЕЙРОПАТОЛОГИЧЕСКАЯ КАРТИНА У РЫБ, БОЛЬНЫХ КРАСНУХОЙ
КАРПОВ (*SEPTICAEMIA HAEMORRHAGICA CYPRINORUM*)

Р е з ю м е

На основе гистопатологических исследований мозга 73 экз. рыб (каarp, карась) представлена нейрорпатологическая картина различных форм геморрагической септицемии карпов.

В результате исследований установлено, что в мозге больших рыб наблюдаются изменения прямого, специфического воспалительного характера, присущие вирусной инфекции. Эти изменения происходят в разных слоях нервной ткани и заключаются в следующем:

- a) в глеево - лимфоидном отеке (инфильтрат) вокруг переполненных кровью кровеносных сосудов;
- b) в пролиферации и активном обмене глея с появлением в области пролиферации глея клеток Alzheimer II и мезенхимального глея и нейронфагов фолликул;
- в) в деструктивных изменениях нейроцитов, протекающих как острые, так и хронические клеточные заболевания;
- г) в образовании телец включения в ядрах нервных клеток в области *nucleus isthmi* и *truncus cerebri*.

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