INCLUSIONS IN THE LIVER CELLS OF SILVER CARP (HYPOPHTHALMICHTHYS MOLITRIX VAL.) FROM THE KISKÖRE STORAGE-LAKE

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Abstract

Annual deaths of silver carps *(Hypophthalmichthys molitrix* VAL.*)* with a body mass of 6—8 kg occur in the spring in the Kisköre storage-lake. In the course of investigations of a weak silver carp specimen no parasites were found which would cause the death of the fish. Sections were pres pared from the interior organs of the fish. In the liver sections a considerable number of inclusion was found in the hepatocytes. The stainability of one bigger or several smaller inclusions according to Masson, with Giemsa or hematoxilin-eosin differed from that of the cytoplasm. The inclusions having mainly spherical shapes were located in the cavities of the cytoplasm. In the liver tissue microscopic necrotic foci were observed as well. It is unlikely that the appearance of inclusions is caused by endogenous degeneration of the cells. According to ANDERSON et *al.* (1965) and MOLNÁR et BOROS (1981) viruses or mycoplasma, as well as clamidia- or rickettsia-like organisms can cause inclusion formation. Striking similarity was found with the observations reported by LANGDON (1988), according to which hepatocyte inclusions have been found in rainbow trout infected by iridovirus.

Introduction

At present silver carp, bighead carp and their hybrids occur already in all natural waters of Hungary as described by BAKOS et al (1979) and MARIÁN et *al.* (1986). Silver carp grows intensively in the Tisza at Kisköre. Its growth is limited by an interesting phenomenon: every year with the increasing of water temperature deaths of a considerable number of silver carps with a body mass of 6—8 kg occur.

The investigation of the causes of the observed event is not easy because of difficulties encountered in catching suitable specimens in natural waters.

Materials and Methods

In May, 1988, we succeeded in catching a specimen from the Kisköre storage-lake. The signs of approaching death were clearly visible: the movement of the fish was slow, the mucous layer of the epithelium was damaged, greyish in colour, at places in tetters.

The specimen was subjected to macroscopic, and subsequently to microscopic investigation. The scrapes collected from the body surface of the fish were studied in native state and impressions were prepared from the internal organs. Liver was fixed in Bouin and formaline, embedded in paraffin and sections were prepared. They were stained with hematoxilin-eosin, Giemsa and according to Masson.

Results

In the course of macroscopic investigations no parasites were osberved which could cause the death of a fish of that size.

In microscopic investigations a considerable amount of bacteria was found in the epithelium, which is natural for the extremely poor condition of the fish. The identification of bacteria was not performed.

Among seemingly healthy viscera our attention was drawn by the liver, which was colourless, on the cut surfaces small cavities were visible. The cavities contained a gaseous substance and liquid, the colour of which corresponded to the colour of the liver. The liver tissue was disintegrating and friable.

The cytoplasm of the hepatocytes in the sections was in general faintly stainable, containing vacuoles. Occasionally, the disorganized cells shrunk, the sinusoides between them becoming broader. On the extended liver tissue surfaces inclusions with a stainability different from that of the cytoplasm could be seen. One bigger or several smaller inclusions, having generally spherical shape were stained in orange-reddish colour by hemotoxilineosin; in case of Masson staining they were usually grey and with Giemsa they were stained in dark blue, sometimes tending to purple, similarly to the nucleolus of the liver cells. Thus, in all cases the inclusions could be easily distinguished in the clearly visible cavities of the cytoplasm. Occasionally, a shiny buble-like formation attached to the spherically shaped inclusion could be observed. The diameter of the shiny buble reached maximally one third of the diameter of the inclusion.

In the liver tissue microscopic necrotic foci were visible as well. In the smaller ones even the contoures of the hepatocytes were recognizable, and in these cell-size cavities cell nucleus clots (?) were found, which in general have similar sizes but differ from each other in shape. The necrotic foci were surrounded by homogenously stainable necrotic cells. At places in the center of the necrotic focus a small gaseous buble was found. In these cases cells and cell nucli floating in liquid could be observed.

Discussion

Data on histological changes as those shown in the figures, description of characteristic hepatocyte inclusions and causes for their appearance are scarce in the literature. It is unlikely that they appear in the cytoplasm as a consequence of endogenous degeneration of the cells. Clamidia-rickettsia-like organisms, viruses, as well as mycoplasma cause formation of inclusions in tissue cultures (ANDERSON et *al.* 1965). According to MOLNÁR and BOROS (1981) the appearance of big inclusions in the gills of silver carp observed in their studies of mucophilosis, can be caused by clamidia-rickettsia-like organisms. PAPERNA et *al.* (1978) reported that clamidia-rickettsia-like organisms caused the appearance of inclusions in the gill tissue of two fish species. Hepatocyte inclusions are discussed by LANGDON (1988). In a paper published in 1986 the author reports on a typical cythophage effect of iridovirus on rainbow trout gonad tissue culture (RTG—2), as well as on the appearance of a roughly spherically shaped, solid nucleoid located in a polyhedral capsule in the infected culture. On the other hand, fish specimens were infected with the virus. Subsequently virus isolation was performed from infected rainbow trout specimens. In the hepatocytes of the sections obtained from infected specimens inclusions were observed (LANGDON 1988), similar to those found in the liver of the silver carp. Since

two different fish species are concerned, we emphasize only the similarity of the histological changes.

Further investigations are necessary to elucidate the causes of the regular spring leaths of silver carps, and to reveal connections between the described changes in, liver and the spring deaths of this species. It is important to clarify the causes for the appearance of inclusions in hepatocytes and the frequency of this phenomenon.

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Zárványok a Kiskörei tározóból származó fehér busa (Hypophthalmichthys molitrix Val.) májsejtjeiben

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Kivonat

A Kiskörei Tározóban évenként előfordul, hogy tavasszal 6—8 kg súlyú fehér busák *(Hypophthalmichthys molitrix* Val.*)* pusztulnak el. Egy gyenge fehér busa vizsgálata során nem találtam olyan parazitát, ami a hal elhullását okozhatná. A belső szervekből metszeteket készítettem. A máj metszeteiben, a hepatocitákban nagy mennyiségű zárványt találtam. Az egy nagyobb v. több kisebb zárvány Masson festéssel, Giemsa-val festve és hematoxilin-eosin festéssel is a citoplazmától eltérő festődésű. A többnyire gömb alakú zárványok a citoplazma üregeiben helyezkednek el. A májszövetben mikroszkopikus méretű nekrotikus gócok is megfigyelhetők. A zárványok nem valószínű, hogy a sejtek endogén degenerációja következtében jöttek létre. ANDERSON és mtsai (1965), MOLNÁR és BOROS (1981) szerint vírusok vagy mikoplazma, ill. clamidia-, rickettsia-szerű élőlények zárványok létrejöttét okozhatják. Feltűnő a hasonlóság LANGDON (1988) által közölt irodalommal, amiben iridovirus fertőzés eredményeképpen találtak pisztrángban hepatocita zárványokat.

Включения, наблюдаемые в клетках печени белого толстолобика (Hypophthalmichthys molitrix val.) из водохранилища в Кишкёре

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Резюме

Весной в водохранилище Кишкёре наблюдается гибель белого толстолобика *(Hypopthalmichthys molitrix* Val.*)* весом в 6—8 кг. При обследовании ослабевшей особи белого толстолобика не было обнаружено паразитов, которые могли бы вызвать гибель рыб. Были приготовлены срезы с внутренних органов особи. В гепатоцитах из срезов печени обнаружено большое количество включений. Наблюдаемые одно большое или несколько меньших включений окрашивали по методу Массона, красителями Гиемса и хематоксилин-эозин, при этом их окраска отличалась от окраски цитоплазмы. Включения, которые в большинстве случаев имели сферическую форму, находились в полостях цитоплазмы. В ткани печени наблюдали также микроскопические центры некроза. Маловероятно, что образование включений вызвано эндогенной дегенерацией клеток. По мнению Андерсона и соавт. (1965) и Молнара и Бороша (1981) вирусы или микоплазма, а также организмы, подобные кламидии или рикеттсии, могут вызвать образование включений. Очевидно большое сходство результатов, полученных в настоящей работе, и данных Лангдона (1988), указывающих на наличие включений в гепатоцитах форели, зараженной иридовирусом.

Kristali u stanicama jetre Hypophthalmichthys molitrix Val. iz rezervoara za vodu Kisköre

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Abstrakt

Svakog proleća se desi da *Hypophthalmichthys molitrix* Val. jedinke od šest-osam kilograma poginu.

Autorica je ispitala jednu slabu jedinku i nije našla parazitu od čega bi riba crknula.

Zato, pravila je preseke od nutarnjih organa.

U stanicama jetre (u hepaocitama) našla je kristale u većim količinama.

Kristali — većeg ili manjeg oblika — se bojadisaju sa Masson-, Giemsa- i sa hematoksilin-eosin bojama. Općenito, kristali su sfernog oblika i nalaze se u šupljinama citoplazme.

Nađeni su nekrolična zarišta u jetri. Čini se da kristali nisu od endogen-degeneracijskog porekla.

Prema ranijim radima Andersona i dr. (1965), Molnara i Boroša (1981) razlozi su sledeći: virus ili mikoplazme, odnosno uzročnici clamidia i rikettsia su sposobni da prouzrokuju nastajanje kristala.

Našla je sličnost sa radom Langdona (1989), koja je našao kristale u hepatocitama pastrmke uzročen sa iridovirusima.