Parasit. hung. 19. 1986

Observations on the intracellular and coelozoic developmental stages of Hoferellus cyprini (Doflein, 1898) (Myxosporea, Myxozoa)

Dr. Kálmán MOLNÁR — Dr. Éva KOVÁCS-GAYER

Veterinary Medical Research Institute of the Hungarian Academy of Sciences, Budapest — Central Veterinary Institute, Budapest, Hungary

"Observations on the intracellular and coelozoic developmental stages of Hoferellus cyprini (Doflein, 1898) (Myxosporea, Myxozoa)" - Molnár, K. - Kovács-Gayer, É.- Parasit. hung., 19: 27-30. 1986.

ABSTRACT. The release of intracellular developmental stages of Hoferellus cyprini (Doflein, 1898) into the lumen of renal tubules was monitored histologically. In the autumn the trophozoites of H. cyprini transform epithelial cells into a syncytium in certain portions of the renal tubules. In January, after the syncytium becomes degenerated and disrupted, the trophozoites get into the lumen of the tubules, are transformed into coelozoic developmental stages, and colonize the ureter. After the epithelium of the renal tubule is destroyed, from the direction of the intact basement membrane fibrocytes and macrophages enter the lumen and surround the cellular debris and trophozoites stuck there with a connective-tissue capsule of several layers.

KEY WORDS. Hoferellus cyprini, development, intracellular and coelozoic stages, hostreaction, kidney, common carp.

In a previous paper (MOLNÁR, CSABA and KOVÁCS-GAYER, 1986) we reported our investigations confirming the statement made by PLEHN (1924), i.e. that Hoferellus cyprini (Doflein, 1898) is a parasite whose development follows a one-year cycle. Its early developmental stages are present in the epithelium of the renal tubules of the common carp (Cyprinus carpio) in the autumn: the trophozoites are located intracellularly in a circumscribed area. During the winter, the parasites gradually get into the lumen of the renal tubules where they develop further as coelozoic plasmodia up to April when they form spores and leave the host fish. In the opinion of MOLNÁR, CSABA and KOVÁCS-GAYER (1986) in spore morphology H. cyprini is identical with the species Mitraspora cyprini, and its development is consistent with that found by AHMED (1973) for M. cyprini. MOLNÁR, CSABA and KOVÁCS-GAYER (1986) demonstrated the different developmental stages by regular dissection of fish specimens; however, they could not furnish direct evidence of intracellular developmental stages getting into coelozoic position.

In the present paper, studies on disintegration of the so-called Hoferellus nodules and release of intracellular stages into the renal tubules are reported.

MATERIALS AND METHODS

The test material was obtained from a pond farm situated in Western Hungary. At the end of January 12 two-summer common carp were submitted to our laboratory alive and were dissected within a few days' time. The kidneys and the ureter were removed from the fish in their entirety, together with the urinary bladder. Certain portions of the organs were examin-

ed in fresh state; the remainder of the kidneys and ureter were divided into two parts, and fixed for histological examination in Bouin's solution and 10 % neutral formalin, respectively.

Squash preparations were made from pieces of the kidney under coverslips. Mucus squeezed out from the ureter was also studied in fresh state under microscope. Organs fixed for histological examination were embedded in paraffin, sectioned, and the 4 to 8 um thick sections were stained with haematoxylin and eosin.

RESULTS

In fresh squash preparations made from the ureter of all the 12 common carp studied, large numbers of Myxosporean plasmodia 15 to 40 um in size and situated freely in the lumen, stages identified by MOLNÁR, CSABA and KOVÁCS-GAYER (1986) with coelozoic forms of H. cyprini, were present. Mostly spherical foci 0.1 to 0.3 mm in diameter and free from parasites were demonstrable in the kidneys of all the fish examined. Renal tubular portions containing intracellular Hoferellus stages were found only for one fish exhibiting rather severe infection.

By histological examination only few areas infected by typical Hoferellus trophozoites were found. In these sections Hoferellus trophozoites were located intracellularly in a syncytium formed of epithelial cells, in formations 100-180 x 170-200 um in size. In most cases infection extended only to one side of the tubules, and on the other side intact epithelial cells were seen (Fig. 1). In the epithelial areas attacked by Hoferellus trophozoites a circumscribed tubulonephrosis, involving shorter or longer portions of the given tubule, developed. Epithelial cells transformed into cell syncytia were demarcated from the renal interstitium by a thin basement membrane, and towards the tubular lumen they were lined by an intact brush border. The cytoplasm of the syncytium was filled with masses of Hoferellus trophozoites which pushed the nuclei of injured epithelial cells to the basement membrane where they formed small clusters. In advanced stages of infection, the cytoplasm of the parasite-containing syncytium became degenerated, disintegrated, and the trophozoite-containing cytoplasm was expulsed, through the ruptured cell membrane, into the lumen of the tubules (Fig. 2). The remaining cytoplasm of the syncytium contained only the nuclei of infected epithelial cells

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Legend to the figures:

Fig. 1. Portion of a renal tubule infected by intracellular Hoferellus stages. Epithelial cells containing parasites have been transformed into syncytia. In certain parts of the tubule there still are intact epithelial cells. Haematoxylin and eosin, x 200

Fig. 2. Release of Hoferellus trophozoites into the lumen. Only the cell nuclei and a few trophozoites have remained in the degenerated syncytium. Most of the parasites are situated in the lumen. H. and E., x 200

Fig. 3. Release of Hoferellus trophozoites into the lumen. Note the mass of trophozoites and epithelial debris within the intact basement membrane. Remnants of the syncytium contain nuclei and trophozoites. In the distal efferent ducts there are Hoferellus plasmodia. H. and E., x 200

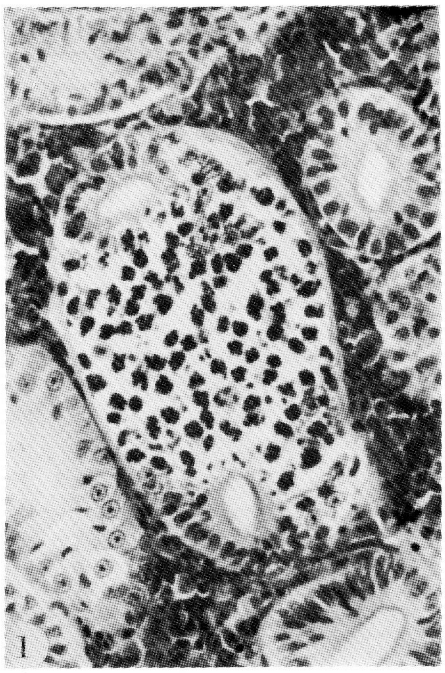
Fig. 4. Section of a distal part of a tubular portion infected by Hoferellus. Intracellular and coelozoic trophozoites occur together, side by side. The trophozoites situated in the lumen contain secondary formations. H. and E., x 200

Fig. 5. Cross-sections of tubular portions with passed-off Hoferellus infection. The lumen of the tubule is filled with tissue debris interwoven with fibroblasts. From the direction of the basement membrane the connective tissue becomes fibrillated. H. and E., x 200

Fig. 6. Advanced stage of regeneration. In the place of the lumen an amorphous debris, surrounded by fibrous connective tissue, can be seen. H. and E., x 200

Fig. 7. Focus formation in the place of the Hoferellus nodule. The fibrous focus is surrounded by epitheloid cells arranged in crescent shape. H. and E., x 200

Fig. 8. Hoferellus plasmodia in the lumen of the ureter. H. and E., x 100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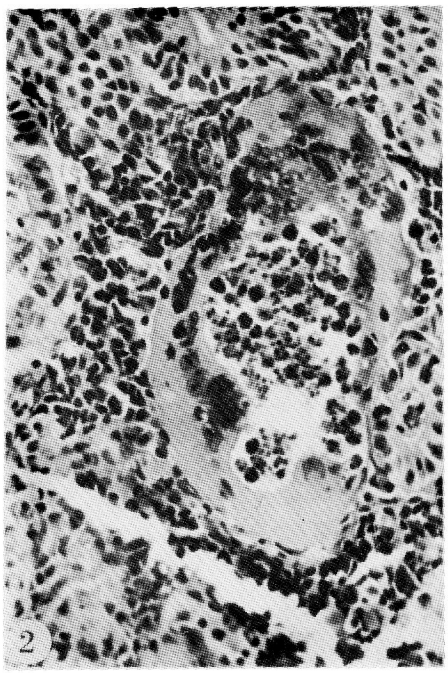
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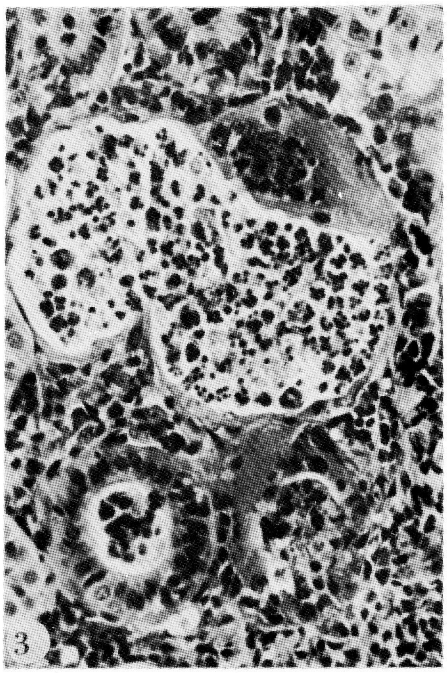
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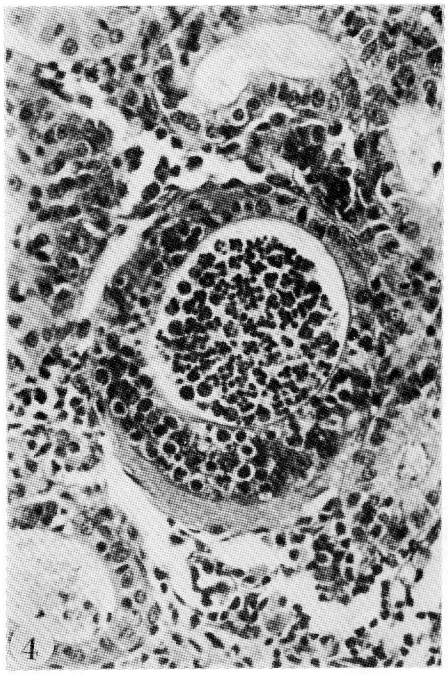
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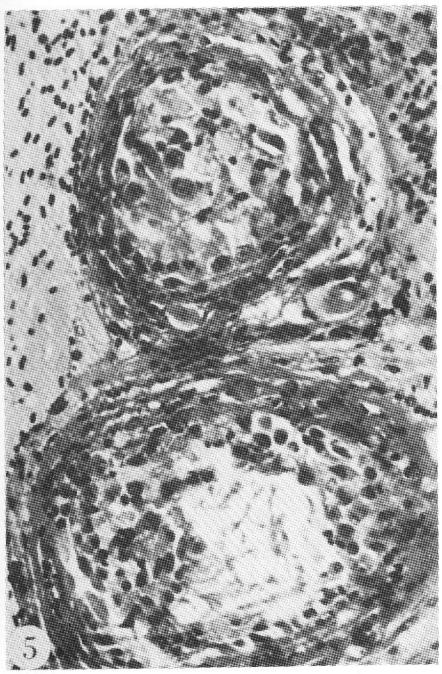
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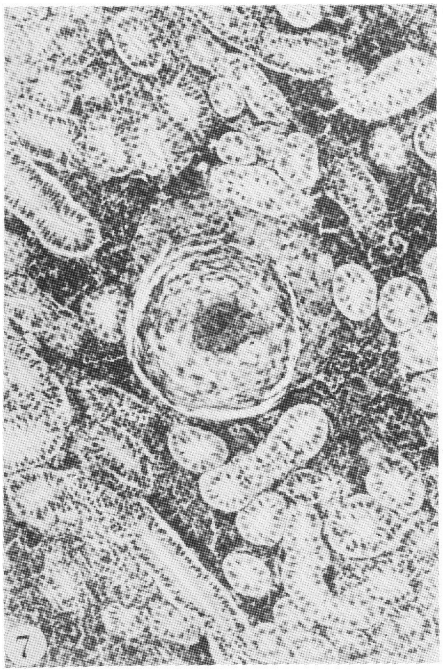
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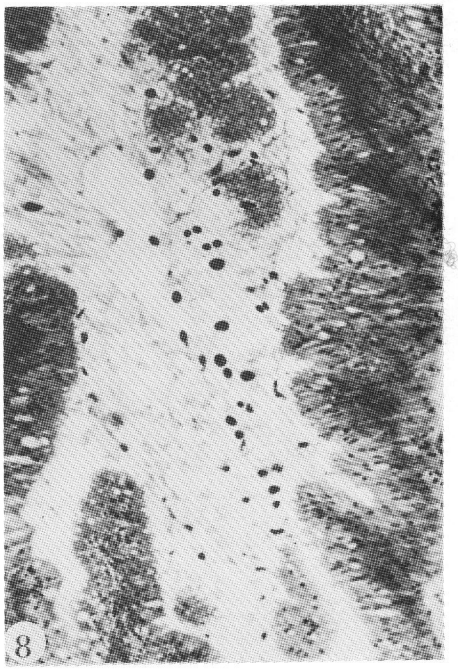
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and some trophozoites that had got stuck there. In some cases the epithelial cells of the tubules almost completely disappeared, and their presence was indicated only by a small islet containing cell nuclei and trophozites (Fig. 3). The lumen was filled with trophozoites, separated from the renal interstitium only by the basement membrane of the tubule In such cases the basement membrane was thickened and gradually became replaced with connective tissue. In the lumen of the tubules some trophozoites remained compact, but most of them had disintegrated to secondary units.

Disruption of the tubular epithelium and release of the trophozoites into the lumen must have started centrally in the Hoferellus-infected area. Therefore, in distal portions of the tubules there still were intracellular Hoferellus trophozoites, although the lumen was already filled with trophozoites released from proximal portions of the tubules (Fig. 4). However, signs indicative of degeneration were already visible on the cell membrane of the distal portions as well.

In the tubular portion containing remnants of syncytia and cell debris, left over after Hoferellus infection, proliferation of connective tissue had started from the direction of the peritubular connective tissue (Fig. 5), and fibroblasts accompanied by a few phagocytes penetrated the detritus containing epithelial cell nuclei, cytoplasmic debris, and one or two Hoferellus trophozoites (Fig. 6). The amorphous mass formed in this way had become surrounded by several layers of connective tissue. Parallel with connective-tissue proliferation, the centrally located debris became concentrated (Fig. 7) and islets formed of epitheloid cells appeared around it. In that stage, infection changed its location and spread to the lumen of the ureter where large numbers of plasmodia formed of trophozoites were seen (Fig. 8).

DISCUSSION

The present investigations have confirmed the results obtained by MOLNÁR, CSABA and KOVÁCS-GAYER (1986) on the development of H. cyprini.

In one of the 12 fish examined, infection was at the stage when intracellular Hoferellus stages get into the lumen of the renal tubules. In the remaining 11 fish this process must have taken place early in January or in the end of December, since in these fish there were only coelozoic developmental stages in the ureter, and infection of the convoluted tubules was indicated by debris-containing foci demarcated by connective tissue, formed in the place of intracellular developmental stages.

As it has been shown histologically, in a certain stage of development, usually in January, the intracellular trophozoites of H. cyprini get into the lumen of the renal tubules, into coelozoic position. This is not an active process, i.e. the trophozoites do not migrate into the lumen, but get there due to degeneration of the syncytium formed by the parasites. In histological preparations the areas containing intracellular parasite stages appear spherical or oval in shape. Actually, in the opinion of LOM and DYKOVÁ (1984), they are elongated formations situated along the convoluted tubules. An interesting feature of intracellular infection is that it usually involves only one side of the tubules, and on the other side there frequently are epithelial cells devoid of infection. If in certain portions of the infected area degeneration takes place earlier, in a tubule portions distal to that area intracellular and coelozoic forms will occur simultaneously (Fig. 4). In such a case H. cyprini stages situated in the lumen should not be mistaken for developmental stages of Sphaerospora renicola which differ from H. cyprini both in size and in seasonality. The excretion of trophozoites is soon followed by the destruction of nuclei pushed to the basement membrane of the syncytium-forming cells and of the intact tubular epithelial cells, since connective tissue proliferating from the direction of the basement membrane compresses the remnants of the epithelium and syncytium. Passed-off Hoferellus infection is characterized by foci encapsulated by several layers of connective tissue, similar to those developing in tuberculosis, Ichthyophonus infection, or infection by some other myxosporean. Obviously, DYKOVÁ, LOM and GRUPCHEVA (1983) and LOM and DYKOVÁ (1985) must have seen similar foci. The opinion of the above authors, i.e. that Hoferellus foci represent a blind alley of development, must be due to the fact that

among the degenerating cells there always are Hoferellus trophozoites that had stuck there. Actually, the formation of the connective-tissue capsule is part of the host's regeneration after a passed-off infection, when parasite development continues in a coelozoic manner in the ureters where H. cyprini spores are formed in the spring.

AHMED (1973) and HOFFMANN (1984) studied the development of Mitraspora cyprini, a species considered by us a synonym of H. cyprini, thoroughly. However, they did not report the formation of foci surrounded by connective tissue. At the same time, LOM and DYKOVÁ (1984) are of the opinion that at the end of Hoferellus infection a granulomatous inflammation develops, and the necrotic foci become surrounded by fibrocytes. Our opinion differs from that of the above authors in two different respects. LOM and DYKOVÁ (1984) assume that the trophozoites are released into the interstitium after the disruption of the basement membrane, while our results prove that they get into the lumen of the tubules. As opposed to LOM and DYKOVÁ (1984), we failed to find an inflammatory response after the disruption of the Hoferellus nodule. At the site of infection degenerative processes, followed by regeneration by connective tissue, took place. Lymphocytes and granulocytes were not typical, whereas the appearance of macrophages and fibroblasts was considered by us a sign of restitution.

MOLNÁR, K. — KOVÁCS-GAYER, É.: Megfigyelések a Hoferellus cyprini (Doflein, 1898) (Myxosporea, Myxozoa) intracelluláris és coelozoicus fejlődési stádiumaira vonatkozóan

A szerzők a Hoferellus cyprini (Doflein, 1898) intracelluláris fejlődési stádiumainak a vesecsatornák lumenébe való kiürülését követték nyomon szövettani módszerrel. A Hoferellus cyprini trophozoitái, melyek az őszi hónapok során a vesecsatornák egy adott szakaszán a vesehámsejteket sejtsyncytiummá alakítják, januárban a syncytium degenerálódása és szétesése után a tubulusok üregébe kerülnek, coelozoicus fejlődési stádiumokká alakulnak és az ureterben telepednek meg. A csatorna hámjának szétesése után az épen maradt alaphártya felől a lumenbe fibrocyták és macrophagok hatolnak be és a sejttörmeléket, valamint az ott rekedt trophozoitákat többrétegű kötőszövetes tokkal veszik körül.

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Received: 18 April, 1986

Dr. MOLNÁR, K.
Veterinary Medical Research Institute,
Hungarian Academy of Sciences,
H-1581 Budapest, P.O. Box 18,
HUNGARY

Dr. KOVÁCS-GAYER, Éva
Central Veterinary Institute,
H-1581 Budapest, 146,
P.O. Box 2,
HUNGARY