"Sphaerosporosis", a New Kidney Disease of the Common Carp

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Parasitological surveys of cultured one-summer carp often revealed the presence of spores and developing stages of the myxosporidium *Sphaerospora angulata* Fujita, 1912 in renal tubules (Molnár, 1980). The rate of *S. angulata* invasion was especially high in fish stocks also affected by other parasitic or nonparasitic diseases (blood protozoans, gill sphaerosporosis, swim bladder disease). This prompted closer investigations into carp renal sphaerosporosis, a disease not previously known in Europe. The study covered the following aspects: (1) nature of renal lesions caused by *S. angulata;* (2) possible identity of the extracellular blood sporozoan of unknown systematic position described by Csaba (1976) with a developmental stage of *S. angulata;* (3) etiological responsibility of one or two different *Sphaerospora* spp. for the gill and kidney sphaerosporosis of carp; (4) possible causal involvement of *S. angulata* in the etiologically unknown swim bladder disease associated with swelling of the kidney.

Of the many known *Sphaerospora* spp. only the histozoic species *S. carassii, S. tincae,* and *S. reichenowi* have hitherto been regarded as pathogenic (Leger, 1930; Plehn, 1932; Hámory and Molnár, 1972; Kashkovskij et al., 1974; Molnár, 1979; Jacob, 1953). Information has been scarce on the pathogenicity of coelozoic parasites, of which little is known apart from their prevalence. In the USSR 15 such species have been identified (Shulman, 1966).

Of the *Sphaerospora* spp. parasitic in carp, only *S. carassii* has been shown to infect indigenous European hosts (Hámory and Molnár, 1972; Lom et al., 1976; Molnár, 1979), while *S. cyprini* and *S. angulata* were originally found only in Far Eastern habitats (Fujita, 1912; Shulman, 1966). Recently Razmashkin and Skriptsenko (1976) reported the occurrence of *S. cyprini* in Western Siberia, and Osmanov (1971) that of *S. angulata* in Central Asia.

Certain authors have taken into consideration the etiological involvement of protozoa in swim bladder disease. Since this condition is associated with renal hypertrophy, and protozoon-like bodies are often found in the wall of the affected air bladder (Szakolczai, 1967), the causal importance of sphaerospores cannot be excluded with certainty.

Observations on the occurrence, development, etiology, and pathogenesis of renal sphaerosporosis, and on its associations with infection by the blood sporozoan of Csaba (1976), gill sphaerosporosis, and swim bladder disease, are reported in this paper.

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Material and Methods

Carp populations from nine Hungarian pond farms were regularly screened for renal sphaerosporosis from 1976 to 1978. For the most one- and two-summer carp were examined, but occasionally older carp and other fish species from the canal system of the farms were also included in the study. During 1977–1978 fry and one-summer hosts, 20 of each from November 1977 to May 1978, were taken at biweekly intervals for parasitological examination from the two most heavily infected farms, in which fry rearing was also carried out. Older carp and other fish species were occasionally examined along with the regular sample.

Impression smears of the visceral organs, especially the kidneys, were examined microscopically and two infected kidneys from each sample were examined histologically.

In 1978 and 1979 complementary studies were performed on the relationship of renal sphaerosporosis with concomitant diseases. These studies covered the parasitological examination of 80 (one- to three-month-old, 156 three-to six-month-old carp fry and 32 overwintered one-summer and two-summer carp. Gross examination was followed by the study of impression smears from the blood, kidneys, gills, and swim bladder.

Six young carp reared in the laboratory under parasite-free conditions were infected experimentally by the intra-abdominal route with blood from a fish harboring Csaba's protozoa (C-hemoprotozoa). Gill biopsies were taken regularly and examined microscopically for sphaerospores, and two fish of each group were killed at appropriate intervals to examine the kidneys for renal sphaerosporosis.

For histological examination the organ specimes were fixed in 10% formalin or Bouin's solution, embedded in paraffin wax, and stained with hematoxylin and eosin, Farkas-Mallory technique or Gömöri's trichrome stain.

Results

S. angulata spores and developmental stages were detected in the kidney of several hosts in each farm under survey, but the percentage occurrence of the parasite differed between farms, ranging from 10%–15% to more than 50%.

The kidneys of hosts with sphaerosporosis showed no gross changes compared to noninfected controls.

The *S. angulata* spores and developmental stages equally localized in the renal tubules. Both could be easily identified in the impression smears. The mature spores had a characteristic angular shape, while the pansporoblasts and young spores had a granular appearance. The latter was due to the presence of 8–14 conspicuous nuclei in the 12–15-μm-wide pansporoblasts; 6 nuclei were still present in the developing spores.

Renal sphaerosporosis has usually been demonstrated at 1.5–3 months of age in the fry at the earliest, but quite recently we also detected the developing stages of *Sphaerospora* in 1.5-month-old hosts. The intensity of infection reached a peak at 4–6 months of age. Among the older fish infected individuals were found in all

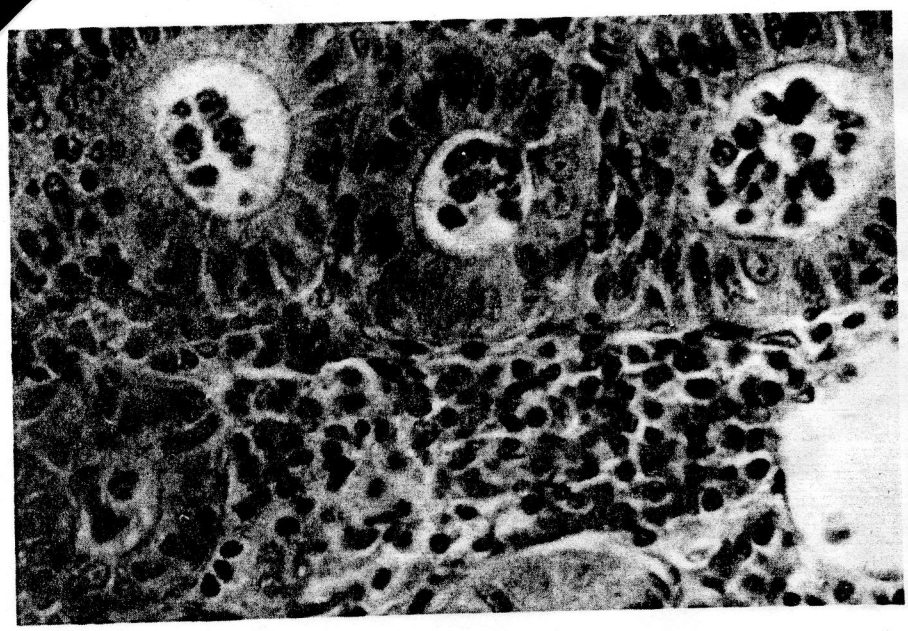

Fig. 1. Tubular lumen packed with sphaerospores. HE, x 300

seasons of the year, but the number of hosts harboring spores was low among the two-summer population.

Confirming the findings from unstained impression smears, histological examination also showed that the parasites localize exclusively in the tubular lumen, without invading the lining epithelium or parenchyma. Occasionally 80% of the tubules were filled with *S. angulata* stages (Fig. 1). In mild infections the parasites established themselves chiefly in the proximal segment of the tubules, whilst in massive infections they also appeared in the intermediate and distal segments.

The earliest stages of *S. angulata* found in renal tubules were multinucleated pansporoblasts; these contained developing pairs of spores at a more advanced stage of the infection (Fig. 2). Spore development was not synchronous. While some segments harbored mature spores, others were filled with developing forms. Furthermore, in some tubules the mature spores were surrounded by developmental stages. (Mature spores take on a yellow, developing spores a blue or red Farkas-Mallory stain.) In some preparations reticular residues of pansporoblast, with trapped spores, indicated an earlier infection. In the same preparations the narrow lumen of some distal tubular segments contained, in addition to spores, condensed tissue debris and starlet-shaped, needle-shaped, or cylindrical salt crystals. Presumably the condensed mass assumed a vivid red colour on staining with hematoxylin and eosin; it filled the lumen of the tubules as a compact cast (Fig. 3) and accounted through obliteration for a functional insufficiency of the nephrons.

Light-microscopic examination revealed no interaction between the tubular lining epithelium and the mass of parasites filling the lumen. However massive the infection, the tubular epithelium and its brush border appeared intact, although slightly reduced

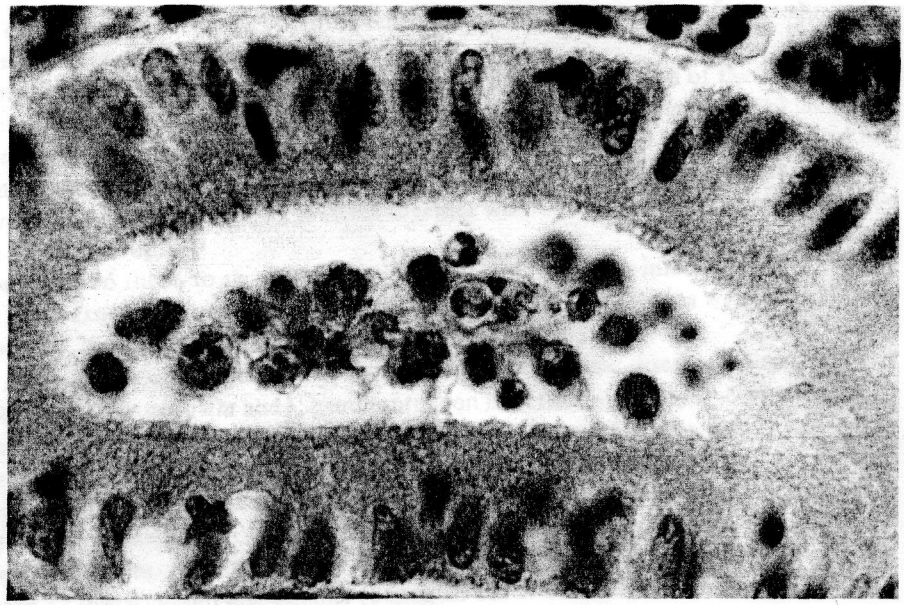

Fig. 2. Spores and developmental stages in a renal tubule. HE, x 500

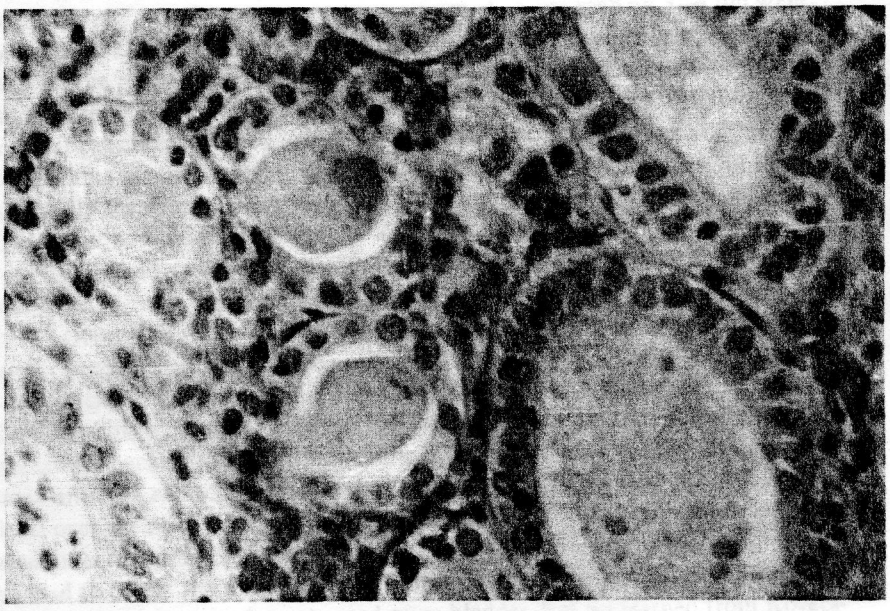

Fig. 3. Accumulation of tissue and other casts in renal tubules in consequence of *S. angulata* infection. HE, x 300

in height owing to dilation of the parasite-packed lumen. Epithelial atrophy followed only upon obstruction of the tubular lumen.

Among the 80 one- to three-month-old carp fry used for examination of the involvement of *S. angulata* in mixed infections only two harbored *S. angulata* in the kidney. Spores of the gill parasite *S. carassii* were found in 16 cases, and 9 hosts harbored the C-hemoprotozoon. No host had swim bladder disease. No simultaneous occurrence of the two *Sphaerospora* species, or of either together with C-hemoprotozoon was observed in this age group.

Among the 156 three- to six-month-old hosts 36 (23%) had been infected by *S. angulata. S. carassii* infection did not occur in this group. C-hemoprotozoa were found in 33 cases (21.2%), and 17 fish (10.9%) had swim bladder disease.

The analysis of mixed infections has shown that the air bladder disease occurred in association with *S. angulata* and C-hemoprotozoon infection in 4 cases (2.6%), with *S. angulata* infection alone in 12 cases (7.6%). A mixed C-hemoprotozoon and *S. angulata* infection was found in 17 hosts (10.9%). Among 17 hosts affected by air bladder disease 12 (70.5%) and among 33 hosts with C-hemoprotozoon infection 17 (51.1%) were simultaneously diseased with renal sphaerosporosis. Among the 139 hosts free from the swim bladder disease 24 (17.3%) had *S. angulata* infection.

Among 32 overwintered one-summer (6 months old or older) fish 5 harbored *S. angulata* and of these 3 also had swim bladder disease. The C-hemoprotozoon was found in 7 hosts, of which one also harbored *Sphaerospora.*

The spores isolated from the gills differed from those found in the kidneys in both shape and size. Spores from the gills were 9–12 μm in diameter, spherical in shape, with a smooth surface, and the two halves of the spore wall were connected by a thick, prominent suture. The polar capsules were pyriform, 4–5 μm long by 3–4 μm wide. The spores found in the renal tubules were not round but roundish, slightly tapering at one end (at the polar capsule), but somewhat angular at the other, with tiny ear-like projections on their surface. The renal spores were 6–7.5 μm long by 6–6.5 μm wide. The two polar capsules were nearly equal in size, 3.5–4 μm long by 2–2.5 μm wide. The suture uniting the two halves of the spore wall rose only slightly above the surface. Morphologically the spores found in the gills corresponded to *Sphaerospora carassii* Kudo, 1919, those found in the kidney to *S. angulata* Fujita, 1912.

As well as in blood smears the C-hemoprotozoon was detected in impression smears and histological sections from kidneys, gills, and swim bladder, but always within the circulation system (Fig. 4). C-hemoprotozoa were especially numerous in gill vessels with narrow lumina. The microscopic appearance of the 6–7-μm-long parasites, with their 6 or 8 nuclei, was reminiscent of the pansporoblast stages of *S. angulata* and *S. carassii.* Susceptible hosts inoculated intra-abdominally with blood containing C-hemoprotozoa always became infected by these parasites. The intensity of infection tended to increase until the end of the first month, then it gradually became stabilized at a lower level over the next two months. The hosts killed 1, 2, and 3 months after experimental infection did not harbour sphaerospores in the gills or in the kidney.

We failed to detect the parasite-like or fungus-like bodies described by Szakolczai (1967) in impression smears of the changed swim bladder, but we learned from Kovács-Gáyer by personal communication that similar formations have been frequently seen in histological sections from affected swim bladder.

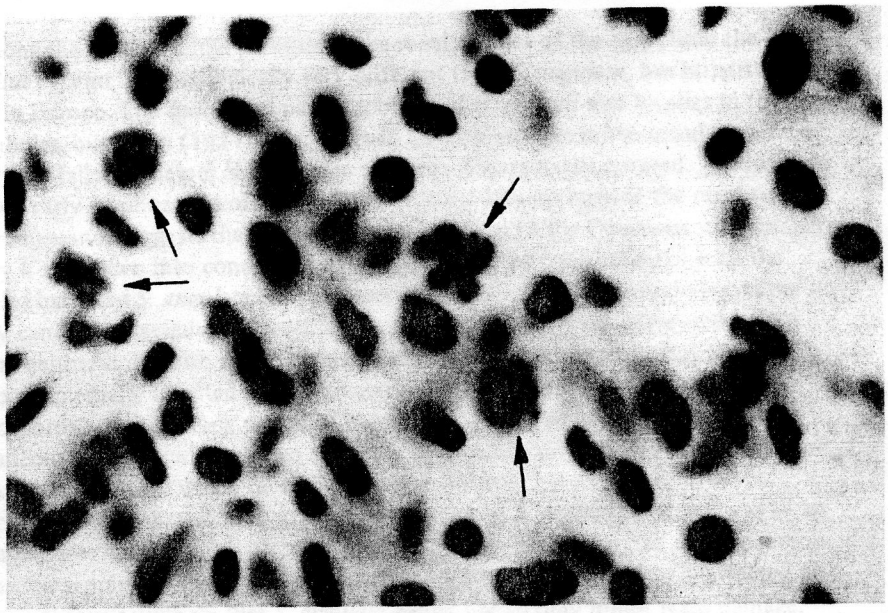

Fig. 4. Csaba's blood protozoon *(arrows)* in the gill vein. HE, x 800

Discussion

The present studies have shown that renal sphaerosporosis is a fairly frequent parasitosis in carp 3 months old and older in Hungary. Since gill sphaerosporosis was not infrequently also present in the affected host populations, it was postulated that the two conditions might possibly be due to stages of the same species established in different locations depending on the time of infection. The fact that not a single host was simultaneously diseased with gill and renal sphaerosporosis weighed in favor of this hypothesis, but the dissimilar morphology and size of the spores found in gill and kidney unequivocally indicated that they represented independent species, *S. carassii* and *S. angulata* respectively.

The spores and developing stages of *S. angulata* which filled the renal tubules in large masses are obviously coelozoic parasites and as such do not directly damage the tubular epithelium. But despite their ceolozoic nature they definitely affect the host organism by utilizing nutrients excreted but not yet reabsorbed by the kidney and, especially, by mechanical obstruction of the renal tubules. The salt crystals precipitating from retained urine, and debris resulting from spore development form in the obliterated distal tubules a homogeneous conglomeration which might cause nephrosis.

Leger (1930) and Plehn (1932) similarly established that the renal sphaerospora *S. tincae,* a parasite of the tench, accounted for a considerable enlargement of the kidney. With the renal *S. angulata* infection we saw no gross renal hypertrophy except in hosts having swim bladder disease as well. We have therefore regarded the enlargement of the kidney as a sequel to the latter condition.

Among the Myxosporidia parasitic in the renal tubules of the carp, only the species *Hoferellus cyprini,* morphologically very different from *S. angulata,* has hitherto been known in Europe. The spores and pansporoblasts of *H. cyprini* also localize in the renal tubules, but Plehn (1924) detected even its trophozoites in the tubular wall. In Hungary we failed to detect *H. cyprini* in the large host material covered. The hitherto unkonwn early trophozoites of *S. angulata* probably develop outside the renal tubules. This circumstance suggests the identity of *S. angulata* with the C-hemoprotozoon, particularly if it is taken into consideration that 51.5% of the hosts infected with the latter also harbored *S. angulata.* On the basis of light-microscopic morphology their identity cannot be excluded. A proof against identity is, however, presented by the fact that neither hosts with spontaneous C-hemoprotozoon infection, nor susceptible hosts experimentally infected with C-hemoprotozoon containing blood developed sphaerosporosis during a long period of observation in the laboratory. The frequent simultaneous occurrence of the two species is in all probability due to inadequate environmental conditions which favor the invasion of both.

A closer relationship seemed to exist between renal *S. angulata* invasion and swim bladder disease. Among fish with swim bladder disease 70.5% also had renal sphaerosporosis; this represents a four fold greater incidence than that found in unaffected populations. It seems unlikely that *S. angulata* giving rise to only minor renal damage, could be the direct cause of the swim bladder disease, but the parasite-like bodies observed by Kovács-Gáyer in the bladder wall need further detailed studies. It is quite likely that a decreased resistance may play the major role in the relationship of the two diseases, i.e., fish with decreased resistance are more susceptible to secondary infections or invasions. On the basis of the present findings it cannot be decided with certainty whether swim bladder disease or *S. angulata* invasion is primarily or secondarily involved.

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