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# Blood stages of *Sphaerospora* spp. (Myxosporea) in cyprinid fishes

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ABSTRACT: A total of 398 specimens of 14 cyprinid species were examined for the presence of renal sphaerospores and *Sphaerospora* blood stages (C blood protozoa). *Sphaerospora* infection of the kidney was demonstrated in 122 fish of 12 species, and blood stages were found in the blood of 30 fish of 6 species. Blood stages only occurred in fish infected by renal sphaerospores and were primarily demonstrable in fingerlings. Blood forms found in asp *Aspius aspius* and white bream *Blicca bjoerkna* differed from those described earlier and contained 16 to 32 secondary cells resembling *Sphaerospora* swimbladder stages (K protozoa) of common carp. In the blood stages observed in bleak *Alburnus alburnus*, secondary cells were situated together in an inclusion-like mass within the primary cell, with 1 to 4 nuclei. In bleak and white bream, forms consisting of 1 secondary and 2 tertiary units were also demonstrable. These can be identified as the earliest renal stages of *Sphaerospora* species. Organisms circulating in the blood so far referred to as 'C blood protozoa' are probably the early developmental stages of renal sphaerospores.

## INTRODUCTION

In the course of studies on the blood of cyprinids infected by renal sphaerospores, Lom et al. (1985) frequently observed parasites resembling the blood protozoa described from the common carp by Csaba (1976). They arrived at the conclusion that these parasites corresponded to early developmental stages of renal *Sphaerospora* spp. specific for the different fish species. Blood protozoa commonly occurring in the common carp were designated by Molnár (1980) as Csaba-parasites (C protozoa) and by Lom et al. (1983) as unidentified blood organisms (UBO). The above authors and others (Csaba et al. 1984, Ter Höfte et al. 1984, Grupcheva et al. 1985) have attempted to identify these organisms as developmental stages of *Sphaerospora renicola*. This theory, however, has only recently been confirmed experimentally by Molnár (1988). Kovács-Gayer et al. (1982) and Körting (1982) reported not only on blood stages, but also on the occurrence of myxosporean developmental stages in the swimbladder of *Sphaerospora*-infected common carp. These stages, which seemed to represent intermediate forms between blood stages and renal stages, were called K protozoa by many authors – referring to the initials of Kovács-Gayer and Körting. Inoculation of K stages into infection-free common carp resulted in renal sphaerosporosis (Molnár 1984, Molnár & Kovács-Gayer 1986). No such K protozoa have been reported from other cyprinids.

The present paper reports observations proving that in certain cyprinids the K stages, and within them the so-called triple formations (units composed of a secondary cell and 2 tertiary cells) identifiable with precursors of renal sphaerospore sporogonic stages, are already formed in the blood.

## MATERIAL AND METHODS

Fishes were seined from natural waters of Hungary (Lake Balaton, River Tisza, River Danube, dead channels of the River Körös) and transferred to the laboratory alive. In the majority of fish (total $n$ = 398) only the kidney and swimbladder were examined for *Sphaerospora* infection; from 150 fish, however, blood smears were also prepared.

Data on the species composition of the fish, rate of *Sphaerospora* infection, and prevalence of C blood protozoa are shown in Table 1.

From April to July blood samples were taken randomly via a capillary tube from the caudal vein of the fish. Subsequently the fishes were killed, dissected, and examined for presence of *Sphaerospora* infection in the



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Table 1. Occurrence of different renal and blood stages of *Sphaerospora* spp. in cyprinids

| Species | Fishes tested: Size (cm) | *n* | *Sphaerospora* infection in kidney | Blood stages[a] |
|---|---|---|---|---|
| *Alburnus alburnus* (L.) | 3.0–11.0 | 90 | 44 | + (11) |
| Fingerling | 2.0– 2.5 | 5 | 3 | + ( 1) |
| *Aspius aspius* (L.) | 28.0–36.0 | 2 | 1 | – |
| Fingerling | 2.5– 4.0 | 12 | 11 | + ( 4) |
| *Blicca bjoerkna* (L.) | 3.0–15.0 | 56 | 12 | + ( 8) |
| Fingerling | 3.0 | 1 | 1 | + ( 1) |
| *Abramis brama* (L.) | 7.0–21.0 | 12 | 5 | – |
| Fingerling | 3.5 | 1 | – | – |
| *Abramis ballerus* (L.) | 14.0 | 1 | 1 | + ( 1) |
| *Rutilus rutilus* (L.) | 3.0–17.0 | 27 | 5 | – |
| Fingerling | 2.5– 3.0 | 52 | 16 | + ( 3) |
| *Scardinius erythrophthalmus* (L.) | 4.0–16.0 | 13 | 1 | + ( 1) |
| *Pelecus cultratus* (L.) | 20.0–34.0 | 3 | 2 | Not examined |
| *Rhodeus sericeus* (Pallas) | 3.0– 5.0 | 20 | 11 | Not examined |
| *Phoxinus phoxinus* (L.) | 3.0– 5.0 | 20 | – | Not examined |
| *Gobio gobio* (L.) | 3.0– 8.0 | 13 | 4 | Not examined |
| *Carassius auratus gibelio* (Bloch) | 4.0–16.0 | 6 | – | Not examined |
| *Ctenopharyngodon idella* (Valenciennes) | 5.0–10.0 | 32 | 2 | Not examined |
| *Hypophthalmichthys molitrix* (Valenc.) | 4.0–16.0 | 32 | 3 | Not examined |

[a]+: Species infected by *Sphaerospora* blood stages; in parentheses, no. of fish that proved to be infected by *Sphaerospora* blood stages

kidney. Blood samples were examined first in fresh state under a coverslip, then as Giemsa-stained smears. Infection of the kidney and swimbladder was checked by studying pieces of these organs in squash preparations under a coverslip. In doubtful cases histological sections were also prepared from the organs.

On the blood smears the place of origin, date of collection, species and size of the fish were indicated, and another sign marked whether the fish was infected by renal sphaerospores.

For histological examination the organs were fixed in 10% buffered formalin or in Bouin's solution, embedded in paraffin, sectioned, and stained with haematoxylin and eosin.

## RESULTS

*Sphaerospora* spp. parasitizing the kidney were demonstrable from the majority of the fish species tested, i.e. from 12 out of 14 cyprinids. In all cases both spores and developmental stages were found in the renal tubules. The identification of spores extended only to genus level. Neither histological lesions nor K stages were found in the swimbladder of the dissected fishes.

C blood protozoa were only found in fish infected by renal sphaerospores and were primarily demonstrable in fingerlings a few centimetres in size. In asp *Aspius aspius* they were found in the blood of almost all sphaerospore-infected fingerlings. In bleak *Alburnus alburnus* C blood protozoa were often also found in the blood of older fish. In Table 1 the prevalence of blood stages is marked only with a cross (+). We did not use a more exact marking since blood stages are very difficult to detect if the intensity of infection is low. There were cases when only 1 out of 10 blood smears of the same fish specimen was found to contain C blood stages.

C blood stages demonstrated in different fish species exhibited characteristic morphological differences.

### Roach *Rutilus rutilus*, rudd *Scardinius erythrophthalmus*, blue bream *Abramis ballerus* and gudgeon *Gobio gobio*

In these fishes blood stages 7 to 15 µm in size and resembling in shape those found in common carp *Cyprinus carpio* occurred (Fig. 1A to F). Among them there were forms similar to those described by Lom et al. (1985) from roach. These included stages containing a single primary and a single secondary cell and those having 6 to 8 secondary cells in a single primary cell. The more advanced secondary cells contained a tertiary cell each. In some cases 'simple' secondary cells and 'double' ones containing a tertiary cell occurred together in the primary cells (Fig. 1D to F).

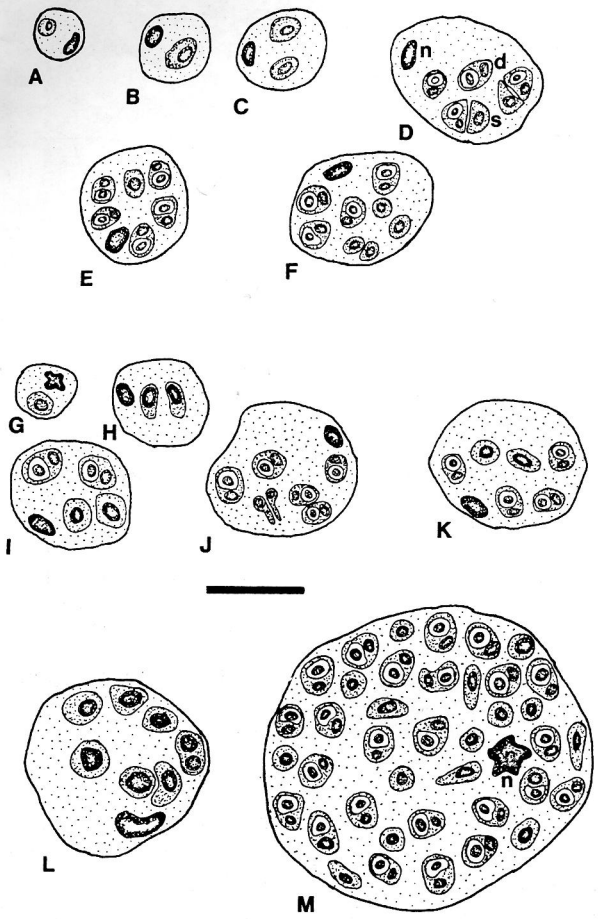


Fig. 1. *Sphaerospora* developmental stages from the blood of blue bream *Abramis ballerus* and white bream *Blicca bjoerkna*. (A to F) Stages resembling blood stages of common carp in the blood of blue bream. Besides the nucleus (n) there are at most 8 secondary cells within a primary cell. The secondary cells are either simple (s), i.e. contain a single nucleus, or double (d), i.e. also contain a tertiary cell. (G to L) Stages resembling blood stages of common carp in the blood of white bream. (M) Stage resembling K stage of common carp in the blood of white bream. The primary cell contains almost 40 secondary cells. n: nucleus of the primary cell. Bar = 10 µm

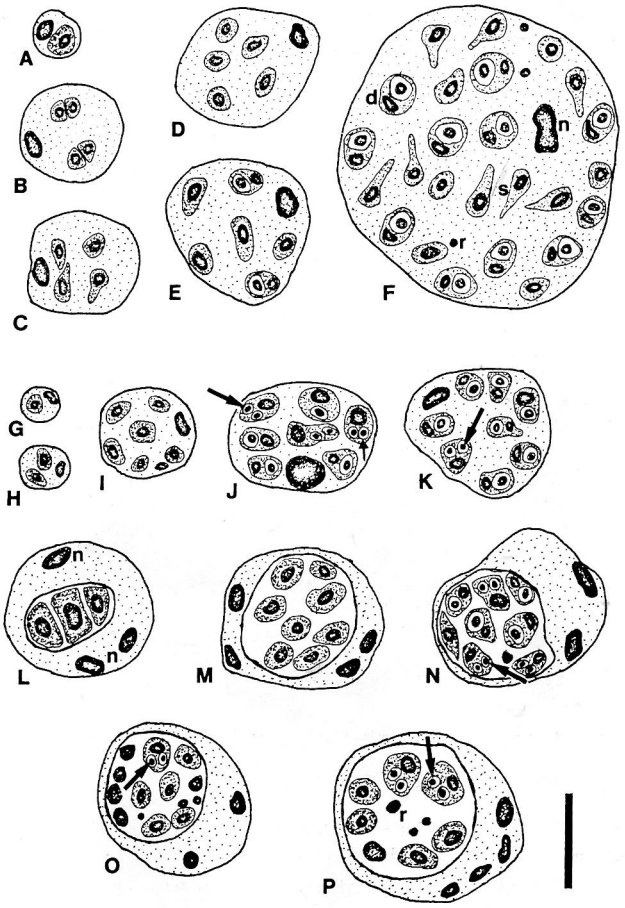


Fig. 2. *Sphaerospora* developmental stages from the blood of asp *Aspius aspius* and bleak *Alburnus alburnus*. (A to E) Stages resembling blood stages of common carp in the blood of asp. (F) Stages resembling K stages of common carp in the blood of asp. Besides the nucleus (n), the primary cell contains 11 double (d), 14 simple (s) secondary cells, and 3 so-called residual bodies (r). (G to K) *Sphaerospora* blood stages of conventional structure from bleak. The well-developed secondary cells contain 2 tertiary cells (arrow). (L to P) Special *Sphaerospora* blood stages from bleak. Secondary cells are seen as a distinct vacuole in the cytoplasm of the primary cell. Primary cells may have 2 to 4 nuclei (n). Some secondary cells contain 2 tertiary cells (arrow). Secondary cells resembling the residual body and having little cytoplasm are common (r). Bar = 10 µm

### White bream *Blicca bjoerkna* and asp *Aspius aspius*

In these fishes 2 types of C blood protozoa were commonly discernible. Besides stages comprising a single primary cell and 6 to 8 secondary cells (Figs. 1G to L, 2A to E, and 3A, B) there frequently occurred forms 20 to 60 µm in size and resembling the K stages known from the swimbladder of common carp. In the cytoplasm of the enormous primary cell, besides the nucleus of the primary cell there were 16 to 40 secondary cells and, occasionally, a few dark-red staining bodies (compact cell nuclei surrounded by a narrow cytoplasmic margin). Most of the secondary cells contained a tertiary cell each (Figs. 1M, 2F, and 3C to E).

### Bleak *Alburnus alburnus*

In this species, in addition to stages resembling those seen in the common carp (Figs. 2G to I and 3F to H), there were forms that differed from the former in 2 important features. First, the secondary cells occurred as a compact, inclusion-like unit within the primary cells, which were 15 to 30 µm in size and had a pale cytoplasm in Giemsa-stained preparations. Second, the primary cells often had 2 to 4 nuclei (Figs. 2L to P and 3I

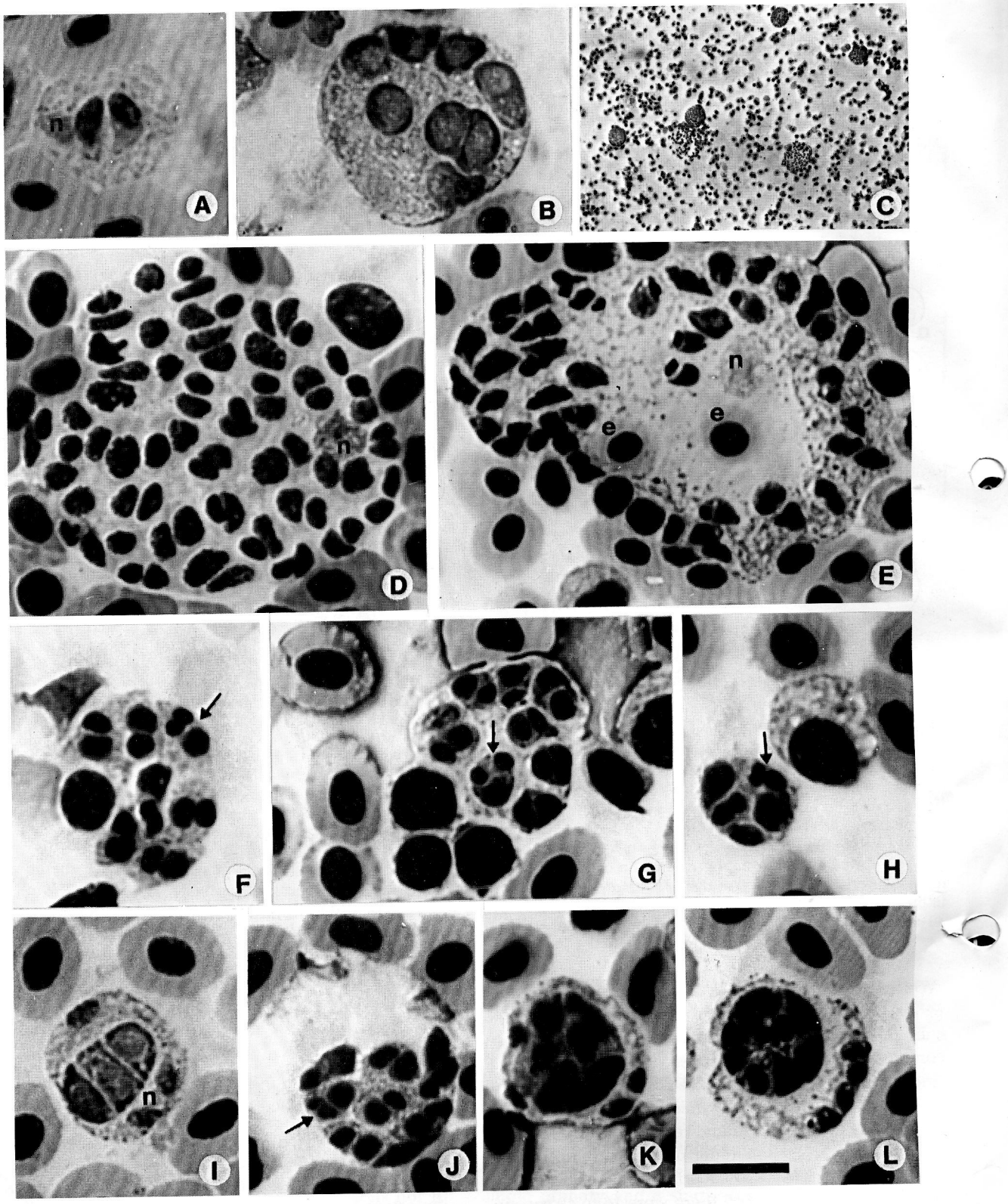


Fig. 3. *Sphaerospora* blood stages in Giemsa-stained blood preparations of white bream *Blicca bjoerkna*, asp *Aspius aspius*, and bleak *Alburnus alburnus*. (A and B) Young stages containing 2 and 8 secondary cells from white bream. (C and D) Blood stages resembling K stages of common carp and containing numerous secondary cells, from white bream. n: nucleus of the primary cell. (E) Blood stage containing numerous secondary cells from an asp. n: nucleus of the primary cell; e = erythrocyte. (F to H) Blood stage containing a primary cell nucleus and 6 to 8 secondary cells from bleak. Some secondary cells contain 2 tertiary cells which, together with the secondary cell nucleus, constitute the so-called triple formations (arrow). (I to L) Secondary cells situated like a distinct vacuole in the cytoplasm of a primary cell with 2 to 4 nuclei (n), from the blood of bleak. Arrow: triple formation. Bar in (L) = 10 μm, applies to all except (C) where bar = 200 μm

to L). In the majority of cases the secondary cells contained a single tertiary cell. In some cases, however, there were 2 tertiary cells within both the inclusion-like secondary cells and the normally located ones (Fig. 2J, K, N to P and 3F, G, H, J). These forms, consisting of a single secondary and 2 tertiary cells, resembled the triple formations developing in the K stages known from the swimbladder of common carp, and identified by Csaba et al. (1984) as pansporoblasts of *Sphaerospora renicola*. On one occasion such triple formations were also demonstrable in a blood smear from an asp.

## DISCUSSION

Results indicate that C blood protozoa are rather common in cyprinids. The coincidence of C blood protozoan infection and *Sphaerospora* infestation of the kidney is striking. In common carp numerous investigators (Molnár 1980, Kovács-Gayer et al. 1982, Lom et al. 1983, Csaba et al. 1984, Ter-Höfte et al. 1984) suggested that these blood parasites, together with K protozoa found in the swimbladder, were developmental stages of myxosporeans, more precisely of sphaerospores. This hypothesis was supported by investigations by Lom et al. (1985) who also demonstrated the presence of blood stages in *Sphaerospora*-infected gudgeon, roach and tench. The present studies have furnished new evidence that the appearance of blood stages in sphaerospore-infected cyprinids can be considered normal. Together with the experimental data of Molnár (1984, 1988), results indicate that C and K stages, whose taxonomic position has long been a subject of controversy, are developmental stages of *Sphaerospora* spp.

The development of sphaerospores (and possibly also that of closely related species, e.g. the PKX organism, the causative agent of proliferative kidney disease in salmonids: Kent & Hedrick 1986) obviously has several phases and takes place in more than one organ. This development always includes a blood stage. It is recommended, therefore, that parasites so far referred to as 'C blood protozoa' (or 'UBO') and 'K protozoa' should in future be called *Sphaerospora* blood stages (C stage) and *Sphaerospora* swimbladder stage (K stage).

Results also show that blood stages occurring in different fish species may vary considerably in morphology and are characteristic of the given fish species. This morphological diversity arises from 4 properties.

(1) *Sphaerospora* blood stages characteristic of common carp contain 8 secondary and 8 tertiary cells in a primary cell, while in asp and white bream stages containing 16 to 40 secondary and tertiary cells are also common.

(2) In the cytoplasm of the primary cell the secondary cells are sometimes segregated like inclusions.

(3) Primary cells may have as many as 2 to 4 nuclei.

(4) Some secondary cells contain 2 tertiary cells.

The morphological diversity of *Sphaerospora* stages in the blood of common carp has been documented abundantly by Csaba (1976), Lom et al. (1983) and Ter-Höfte et al. (1984). However, none of these research groups could find in the blood of common carp primary cells containing more than 8 secondary cells. Lom et al. (1985) were the first to suggest that primary cells might contain more than 8 secondary cells: they found 6 double and 7 single secondary cells in the blood stage found in *Gobio gobio*. On one occasion the same authors observed in common carp a blood stage containing 15 secondary cells. They state, however, that stages containing numerous secondary cells typically occur in organs other the blood (e.g. in the swimbladder and eye). In the present studies, in white bream and asp *Sphaerospora* blood stages containing numerous secondary cells were very common, and these developmental stages were morphologically identical to K stages known from the swimbladder of common carp. In these species development in the blood seems to be continuous and is not interrupted at a certain stage; rather, the swimbladder stage is omitted and the cycle is completed. The multinucleated forms characteristic of K stages develop in the blood itself and near the end of development triple formations appear. These latter can be regarded as precursors of the earliest sporogonic *Sphaerospora* stages observed in the renal tubules, which are pansporoblasts with 2 sporoblasts.

Another important feature of *Sphaerospora* blood stages described from cyprinids is that the secondary cells constitute a single closed unit surrounded by the cytoplasm of the primary cell. This peculiarity in location has been reported by Csaba et al. (1984) and Ter-Höfte et al. (1984). Csaba et al. (1984) demonstrated such forms in K stages of common carp while Ter-Höfte et al. (1984) found them in blood stages. In these blood stages secondary cells were located in the cytoplasm of the primary cell as if in a vacuole. These secondary cells, seen as distinctly segregated units in the pale cytoplasm of the primary cell, were particularly common in the blood of bleak.

Another striking feature of the stages found in bleak was that the primary cells occasionally had 2, 3 or even 4 nuclei. Myxosporeans are known to have, in addition to the generative cells, numerous vegetative nuclei of trophic function within the plasmodium. In the present case the supernumerary nuclei of the primary cell can be considered such vegetative nuclei. These nuclei were only found in bleak. Lom et al. (1985) published a photograph of a blood stage which they regarded as a primary cell with 2 secondary cells and phagocytised

blood cell. However, we cannot exclude the possibility that this blood stage represented a multinucleated primary cell with inclusion-like secondary cells.

The *Sphaerospora* nature of the blood stages is, however, best proved by the triple formations that were detected in bleak and asp on several occasions. These triple formations completely corresponded to those found in K stages of common carp and these latter, in turn, are identical to the earliest *Sphaerospora* developmental stages present in the renal tubules, as has been proved experimentally by Molnár & Kovács-Gayer (1986).

In the cyprinids examined by us, especially in bleak, asp and white bream, the *Sphaerospora* blood stages had morphological characteristics mostly typical for the given fish species. These morphological features can be considered species characters and seem to support the suggestion by Lom et al. (1985) that sphaerospores inhabiting the renal tubules of taxonomically closely related cyprinids, and hardly distinguishable by spore morphology, actually represent separate species.

## LITERATURE CITED


Csaba, G. (1976). An unidentifiable extracellular sporozoan parasite from the blood of the carp. Parasit. Hung. 9: 21–24

Csaba, G., Kovács-Gayer, É., Békési, L, Bucsek, M., Szakolczai, J., Molnár, K. (1984). Studies into the possible protozoan aetiology of swimbladder inflammation in carp fry. J. Fish Dis. 7: 39–56

Grupcheva, G., Dykova, I., Lom, J. (1985). Seasonal fluctuation in the prevalence of *Sphaerosopra renicola* and myxosporean blood-stream stages in carp fingerlings in Bulgaria. Fol. Paras. (Praha) 32: 193–203

Kent, M. L., Hedrick, R. P. (1986). Development of the PKX myxosporean in rainbow trout *Salmo gairdneri*. Dis. Aquat. Org. 1: 169–182

Kovács-Gayer, É., Csaba, G., Békési, L., Bucsek, M., Szakolczai, J., Molnár, K. (1982). Studies on the protozoan etiology of Swimbladder Inflammation in common carp fry. Bull. Eur. Ass. Fish Pathol. 2: 22–24

Körting, W. (1982). Protozoan parasites associated with swimbladder inflammation (SBI) in young carp. Bull. Eur. Ass. Fish Pathol. 2: 25–28

Lom, J., Dykova, I., Pavlásková, M. (1983). 'Unidentified' mobile protozoans from the blood of carp and some unsolved problems of myxosporean life cycles. J. Protozool. 30: 497–508

Lom, J., Pavlásková, M., Dykova, I. (1985). Notes on kidney-infecting species of the genus *Sphaerospora* Thélohan (Myxosporea), including a new species *S. gobionis* sp. nov., and on myxosporean life cycle stages in the blood of some freshwater fish. J. Fish Dis. 8: 221–232

Molnár, K. (1980). 'Sphaerosporosis', a new kidney disease of the common carp. In: Ahne, W. (ed.) Fish diseases. Springer-Verlag, Berlin, p. 157–164

Molnár, K. (1984). Experimental evidence that protozoa causing swimbladder inflammation in common carp *(Cyprinus carpio)* are stages of *Sphaerospora renicola*. Bull Eur. Ass. Fish Pathol. 4: 14–15

Molnár, K. (1988). Evidence that C-blood protozoa of the common carp are stages of *Sphaerospora renicola* Dyková et Lom, 1982. Bull. Eur. Ass. Fish Pathol. 8: 3–4

Molnár, K., Kovács-Gayer, É. (1986). Experimental induction of *Sphaerospora renicola* (Myxosporea) infection in common carp *(Cyprinus carpio)* by transmission of SB-protozoans. J. appl. Ichthyol. 2: 86–94

Ter-Höfte, B. B., Körting, W., Lehmann, J. (1984). 'C und K-Protozoen', Endoparasiten unsicherer systematischer Zuordnung bei jungen Karpfen (*Cyprinus carpio L.*). Derzeitiger Wissensstand und bildliche Dokumentation. Fisch und Umwelt 13: 89–99. G. Fischer Verlag, Stuttgart, New York


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# Presporogonic development of *Sphaerospora renicola* Dyková & Lom, 1982, in the swimbladder of the common carp, *Cyprinus carpio* L.

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**Abstract.** The presporogonic developmental stages of *Sphaerospora renicola*, the causative agent of swimbladder inflammation (SBI), develop in the swimbladder of common carp by multiple internal cleavage. The earliest development stage is the secondary cell enclosed by the primary cell. The secondary cells undergo multiple amitotic division within the mother cell and the dividing forms are connected by narrow cytoplasmic bridges. As a result of further internal cleavage, two tertiary cells appear in each secondary cell. The triple formation enclosing a secondary and two tertiary cells is identical with the pansporoblast containing two sporoblasts which occurs in the renal tubules.

## Introduction

According to Kovács-Gayer, Csaba, Békési, Bucsek, Szakolczai & Molnár (1982), Körting (1982) and Csaba, Kovács-Gayer, Békési, Bucsek, Szakolczai & Molnár (1984), swimbladder inflammation (SBI), one of the most important diseases of the common carp, *Cyprinus carpio* L., is caused by the presporogonic developmental stages of a *Sphaerospora* sp. parasitizing the renal tubules. Accepting the views of Molnár (1980a), these authors identified this parasite as *S. angulata* Fujita, 1912, a species reported from Japan. Kovács-Gayer *et al.* (1982) and Körting (1982) were the first to describe developmental stages in the swimbladder. In their opinion, the final stages of the parasites are formations consisting of a mother cell and two daughter cells. Csaba *et al.* (1984) considered these formations to be identical with the youngest *S. angulata* developmental stages occurring in the lumen of the renal tubules, i.e. with the two sporoblasts enclosed by a pansporoblast. Subsequently, the observations of Csaba *et al.* (1984) were experimentally proven by Molnár (1984) and Molnár & Kovács-Gayer (1986). While studying sphaerosporosis of common carp, Dyková & Lom (1982) arrived at the conclusion that the species occurring in the kidney of the common carp in Europe was not identical with *S. angulata*, and described it as a new species named *S. renicola*. Lom, Dyková & Lhotáková (1983) as well as Desser, Molnár & Horváth (1983a) performed electron-microscopic examinations to reveal the development and fine structure of the parasite stages in the kidney.

Electron-microscopic investigations into the presporogonic development of the swimbladder stages of *S. renicola* are reported in this paper. The species parasitizing the kidney of common carp in Europe is referred to as *S. renicola* throughout the text, although an absolutely reliable differentiation of the two species would require a re-description of *S. angulata*.

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## Materials and methods

Samples were taken from 2 to 2·5-month-old common carp fry affected by SBI. Swimbladders with incipient lesions (dilated capillaries) as well as those with thickened walls and advanced lesions were sampled.

Tissues pieces 1 $mm^3$ in size were excised from areas showing signs of inflammation, fixed in 5% glutaraldehyde, post-fixed in osmium tetroxide, washed in 0·13 M sodium cacodylate buffer (pH 7·4), dehydrated in an ascending series of ethanol, and embedded in Durcupan ACM resin. The ultrathin sections prepared with a glass knife were counterstained with uranyl acetate and lead hydroxide, and examined in a JEOL 100S electron microscope.

## Results

Electron microscopy of *Sphaerospora renicola* revealed that the early parasite stages were round or shortly elongated primary cells containing a nucleus and a secondary cell in the cytoplasm. The youngest stage found was spherical and 3·1 $\mu$m in size. This stage had an electron-opaque, frothy cytoplasm in which a nucleus enclosing a large nucleolus and a

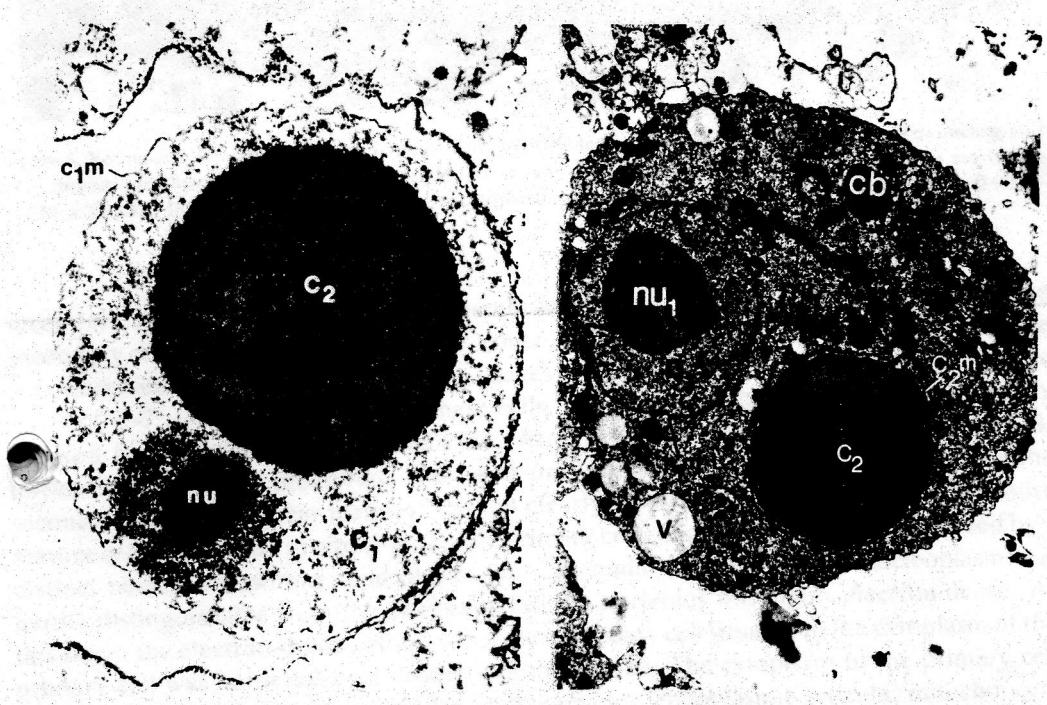


**Figure 1.** Young developmental stage of *S. renicola* in the swimbladder wall. The primary cell ($c_1$) encloses a single secondary cell ($c_2$): $n_1$= nucleus of the primary cell; nu=nucleolus; $c_1m$=cell membrane of primary cell (TEM. ×19 000).

**Figure 2.** Young developmental stage of *S. renicola*. The cytoplasm of the primary cell contains the nu nucleolus ($n_1$) and nuclear membrane ($n_1m$) of the primary cell, mitochondria (mi), vacuoles (v), the second (cb), and the cell membrane, formed of the endoplasmic reticulum, around the secondary cell ($c_2m$) ( ×67 000).

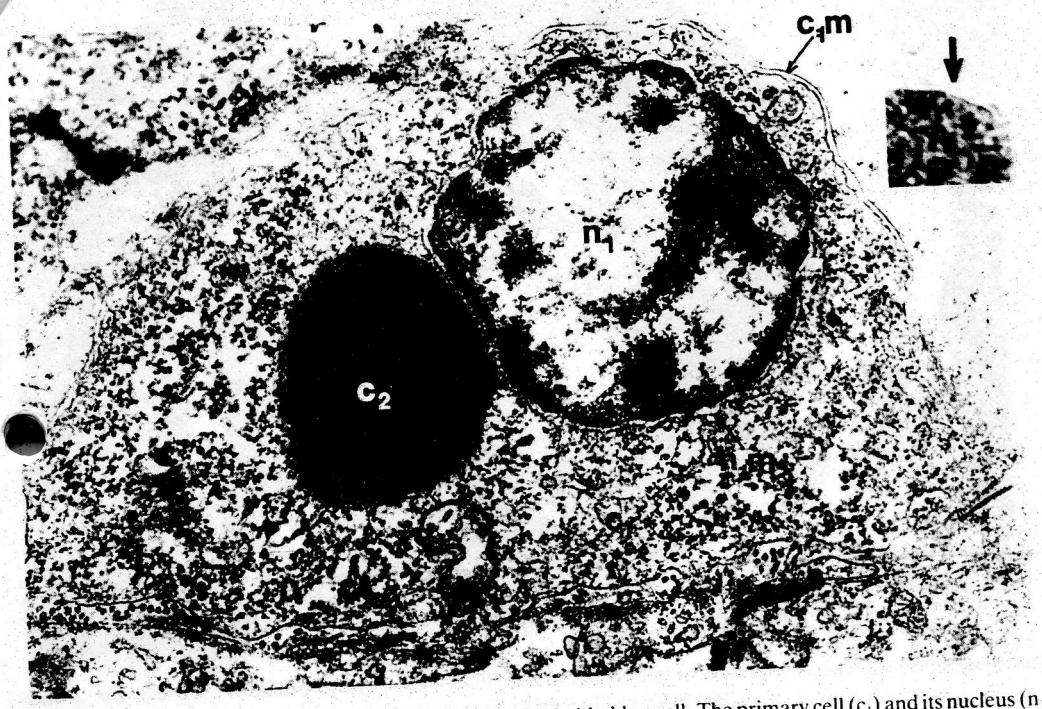


**Figure 3.** Young developmental stage of *S. renicola* in the swimbladder wall. The primary cell ($c_1$) and its nucleus ($n_1$) have double membranes ($c_1m$, $n_1m$). The secondary cell ($c_2$) is electron-dense and its nucleus is not yet visible (TEM, ×20 000). Inset: double membrane of the cytoplasm of the secondary cell, broad arrow (TEM, ×60 000).

secondary cell, but devoid of nuclear membrane, was seen (Fig. 1). The cytoplasm of the secondary cell was highly electron dense, contained no nuclear matrix and no cell membrane was observed around it. Other young stages containing only one secondary cell were 4–4·5×6·2–7 μm in size (Fig. 3). In these cells, the nucleus of the primary cell was already covered by a distinct double membrane. Around the cytoplasm of the secondary cell, however, the double membrane was seen only in short lengths (Fig. 3, inset). In the secondary cell, the nuclear material was not clearly separated from the cytoplasm. In more mature cells, 12–16 μm in size (Fig. 2), the primary cell contained a nucleus surrounded by a distinct nuclear membrane, and one or more secondary cells. The pale nucleoplasm was hardly distinguishable from the cytoplasm but the nucleolus was highly electron dense. At this stage, the electron-dense cytoplasm of the secondary cell situated in the cytoplasm of the primary cell was bordered by a distinct cell membrane. The cytoplasm of the primary cell contained, in addition to the secondary cell, vacuoles, endoplasmic reticula, mitochondria and small electron-dense areas resembling the cytoplasm of the secondary cells. Within the cytoplasm of the primary cells the secondary cells underwent amitotic division and were interconnected by narrow cytoplasmic bridges (Figs 4 & 5). Electron microscopy revealed eight to 10 secondary cells within the primary cell (Figs 5–7), whereas, in impression smears, as many as 40 secondary cells were seen (Csaba *et al.* 1984). In parallel with maturation of the secondary cells, electron-opaque nuclear areas containing the electron-dense nucleoli

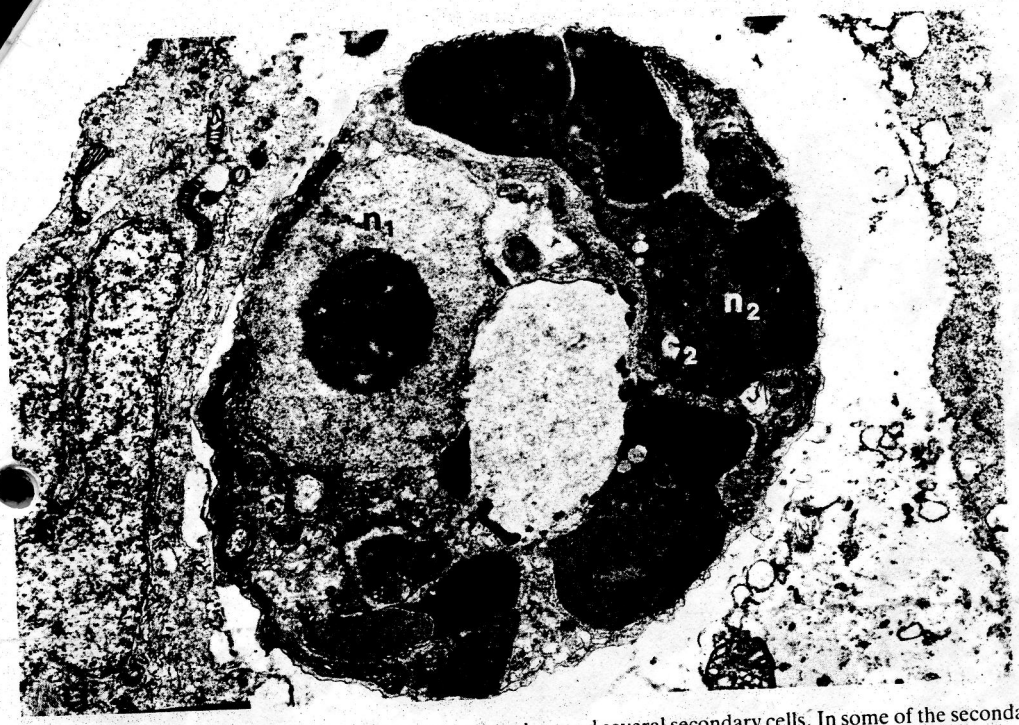


**Figure 6.** A primary cell enclosing a large primary cell nucleus and several secondary cells. In some of the secondary cells, the nucleus and the nucleolus are clearly distinguishable (TEM, ×10 000).

appeared in the electron-dense cytoplasm of the secondary cells (Figs 4 & 6). In the multinucleate cells, the nucleus of the primary cell differed from that of the secondary cells in its considerably larger size (Figs 5 & 6). The cell membrane of the secondary cells apparently developed from the endoplasmic reticulum of the primary cell, which clustered around the secondary cell (Fig. 7). In the younger developmental stages, the secondary cells were scattered in the cytoplasm of the primary cell (Figs 5 & 7), but subsequently they occupied much more of the cell (Fig. 6). In a later stage of secondary cell development, two tertiary cells appeared, by internal cleavage, in the cytoplasm of the secondary cell (Fig. 8). The two tertiary cells and the nucleus of the secondary cell made up the characteristic triple formation described by Kovács-Gayer *et al.* (1982) and Csaba *et al.* (1984). By that stage, the primary cell was already disrupted and the triple formations were seen in the cell debris.

## Discussion

The electron-microscopic observations of the presporogonic development of *S. renicola* confirmed the light-microscopic observations of Csaba *et al.* (1984) in practically every respect. During this development, a secondary cell develops within the primary one by internal cleavage and subsequently divides into further secondary cells by amitosis. Amitosis is primarily indicated by the presence of cytoplasmic bridges between the secondary cells. As a result of further internal cleavage, two tertiary cells appear in each secondary cell. The

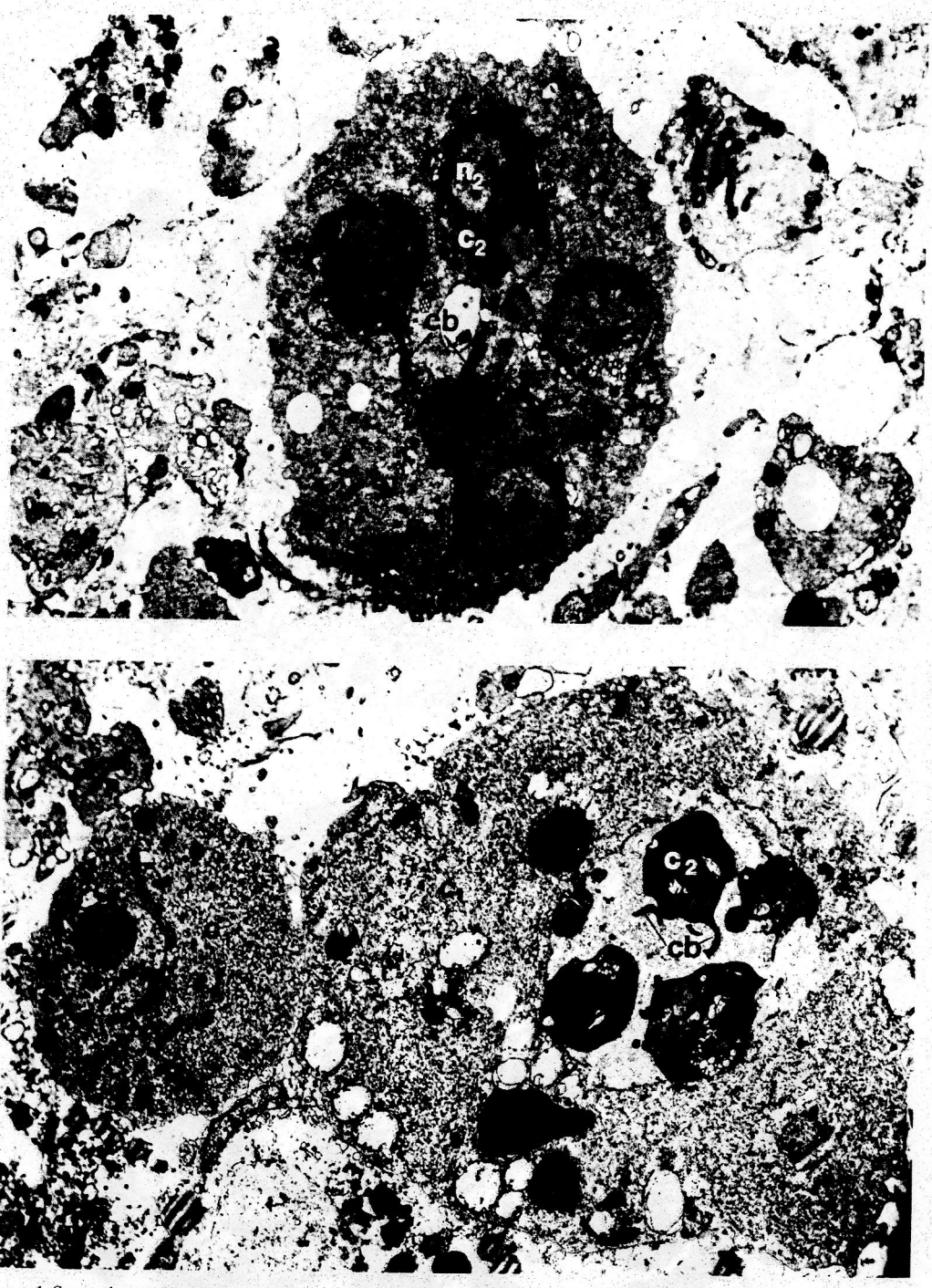


**Figure 4.** Secondary cells dividing by amitosis in the cytoplasm of the primary cell. The secondary cells are connected by cytoplasmic bridges, and contain a distinct electron-opaque nucleus ($n_2$) and an electron-dense nucleolus (TEM, ×6000).

**Figure 5.** Presporogonic stages of *S. renicola* in the swimbladder wall. Left: nucleus and a mitochondrium-rich portion of a primary cell. Right: portion of another primary cell, containing secondary cells and cytoplasmic bridges (TEM, ×42 000).

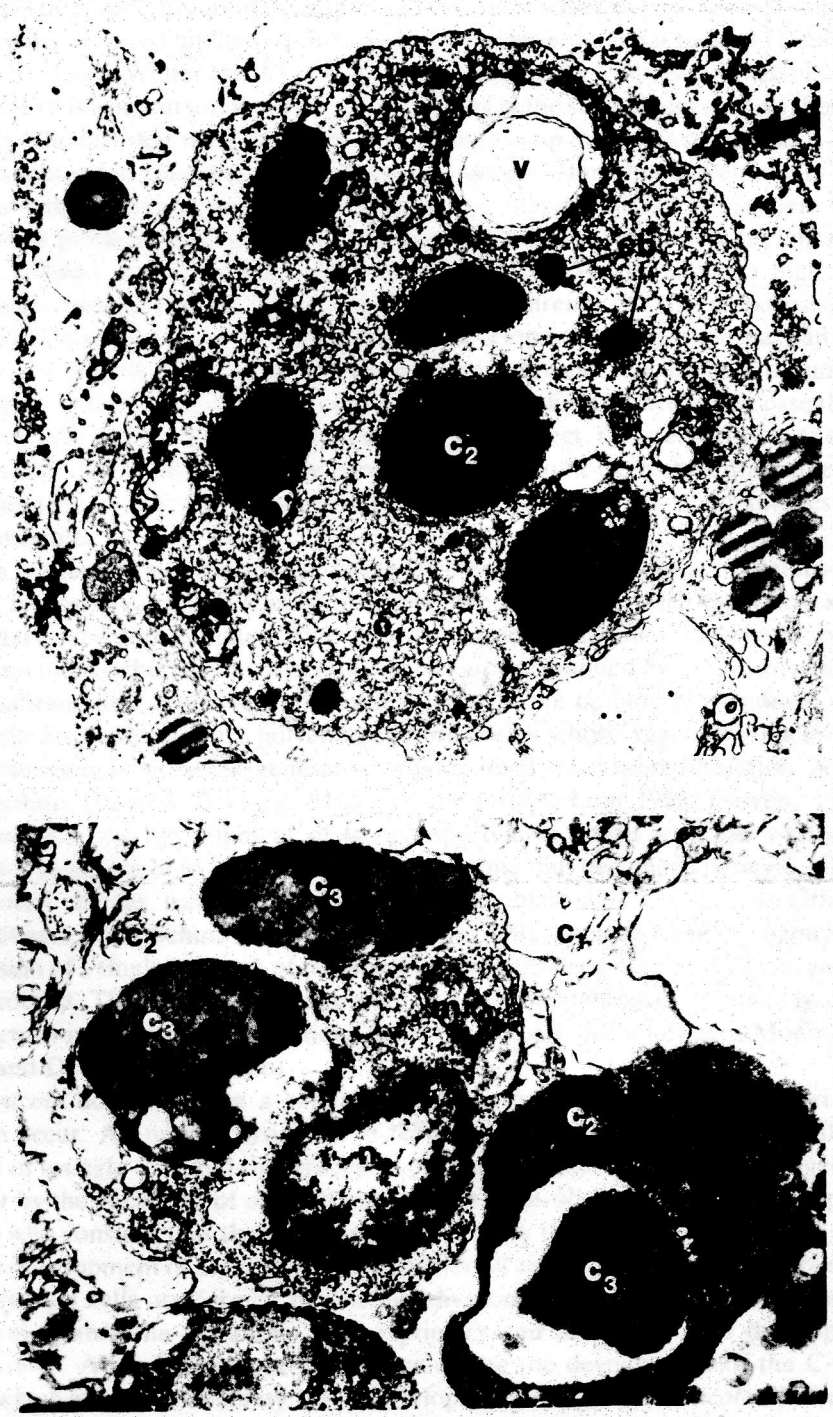


**Figure 7.** Cross-sections of secondary cells and cytoplasmic bridges in the cytoplasm of the primary cell. Around the secondary cells and vacuoles there is clearly visible endoplasmic reticulum (TEM, × 6000).

**Figure. 8.** A secondary cell enclosing two tertiary cells ($c_3$) in the cytoplasm of the primary cell. Within the secondary cell there are mitochondria, vacuoles and endoplasmic reticulum around the tertiary cells (TEM, × 20 000).

racteristic triple formations are identical with the earliest developmental stages, consisting of a pansporoblast and two sporoblasts, appearing in the renal tubules (Lom *et al.* 1983; Desser, Molnár & Weller 1983b). The question whether the C-blood-protozoan described by Csaba (1976) is a precursor of the stages developing in the swimbladder seems therefore to be resolved. The fact that the C-blood-protozoan breaks up into units containing a mother cell and a daughter cell, and that the swimbladder parasites start their development with stages identical with the former, suggested that in the development of *S. renicola* there was a blood stage which preceded that in the swimbladder. This was supported by the observations of Molnár (1980b) and Grupcheva, Dyková & Lom (1985) who found a high correlation between the presence of the blood forms and the occurence of sphaerosporosis.

Much less is known about the presporogonic development of myxosporea than about their subsequent, i.e. sporogonic, stage of development. It is especially difficult to study these two stages in species which develop in a cyst (a giant plasmodium), since in that case the different developmental stages occur simultaneously. In this respect, the presporogonic development of *S. renicola* is an ideal model since here the presporogonic developmental stages formed in the swimbladder and the sporogonic forms developing in the renal tubules are easily distinguishable.

There is a general agreement that during the presporogonic, so-called vegetative, development of myxosporeans multinuclear plasmodia are formed, in which vegetative and generative nuclei can be differentiated. The vegetative nuclei are situated freely in the cytoplasm of the plasmodium, whereas the generative nuclei are surrounded by their own cytoplasm and cell membrane and appear as independent cells. In the opinion of Schulman (1966), the plasmodia are multinuclear, polyenergetic organisms whose vegetative nuclei perform a trophic function, whereas the generative cells are involved in spore formation. According to some authors (Lom & Puytorac 1965; Schubert 1968; Lom 1969; Current 1979), spore formation, i.e. the development of the pansporoblasts, starts with the approach of two generative cells, one of which surrounds the other and thus becomes a pericyte, while the cell so enclosed divides further and forms the sporoblast and the spores. Other authors (Georgévitsch 1935; Schulman 1966; Uspenskaya 1981) consider that sporogony starts with the division of a single cell: one of the two daughter cells becomes the pericyte, and the other the sporoblast. The latter view is supported by experimental results obtained by Siau (1977) and electron-microscopic observations made by Hulbert, Komourdjian, Moon & Fenwick (1979) and Desser *et al.* (1983b).

*S. renicola* does not form a genuine plasmodium and no vegetative nuclei of trophic function occur. All nuclei participate in reproduction. Sporogony starts with the unequal division of a single cell, and this process is preceded neither by the coming together of two cells nor by the enclosure of one cell by the other. This observation seems to support those authors who consider that the pansporoblast arises by the unequal division of a single cell.

In the development of *S. renicola* multiple internal cleavage takes place before sporogony, when progeny cells are formed by unequal division of the nucleus and separation of the cytoplasm, within the cytoplasm of the primary and secondary cells developing in the swimbladder. A similar process takes place during the development of the C-blood-protozoan which is, in all probability, also a developmental stage of *S. renicola* (Bucsek & Csaba 1984). Internal cleavage does not appear to be a unique phenomenon typical of *S. renicola*: more thorough examinations would probably show similar processes in other myxosporeans.

This hypothesis is supported by the development of *Hoferellus cyprini* where Lom & Dyková (1985) have observed secondary, tertiary and even quaternary cells within the primary cells.

According to Seagrave, Bucke & Alderman (1980), internal cleavage also takes place in the development of the PKX organisms, the causative agents of proliferative kidney disease. These organisms probably correspond to early *Sphaerospora* developmental stages (Kent & Hedrick 1985; Fischer-Scherl, El-Matbouli & Hoffmann 1986).

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## References

Bucsek J.M. & Csaba G. (1984) Ultrastructural observations on a carp blood parasite of uncertain taxonomic position. *Symposia Biologica Hungarica* **23**, 137–147.

Csaba G. (1976) An unidentifiable extracellular sporozoon parasite from the blood of the carp. *Parasitologica Hungarica* **9**, 21–24.

Csaba G., Kovács-Gayer É., Békési L., Bucsek M., Szakolczai J. & Molnár K. (1984) Studies into the possible protozoan aetiology of swimbladder inflammation in carp fry. *Journal of Fish Diseases* **7**, 39–56.

Current W.L. (1979) *Henneguya adiposa* Minchew (Myxosporida) in the channel catfish: ultrastructure of the plasmodium wall and sporogenesis. *Journal of Protozoology* **26**, 209–217.

Desser S.S., Molnár K. & Horváth I. (1983a) An ultrastructural study of the myxosporeans, *Sphaerospora angulata* and *Sphaerospora carassii*, in the common carp, *Cyprinus carpio* L. *Journal of Protozoology* **30**, 415–422.

Desser S.S., Molnár K. & Weller I. (1983b) Ultrastructure of sporogenesis of *Thelohanellus nikolskii* Achmerov, 1955 Myxozoa, Myxosporea from the common carp, *Cyprinus carpio*. *Journal of Parasitology* **69**, 504–518.

Dyková I. & Lom J. (1982) *Sphaerospora renicola* n. sp., a myxosporean from carp kidney and its pathogenicity. *Zeitschrift für Parasitenkunde* **68**, 259–268.

Fischer-Scherl T., El-Matbouli M. & Hoffmann R. (1986) A new *Sphaerospora* sp. in brown trout *(Salmo trutta* m. *fario)* in Germany. *Bulletin of the European Association of Fish Pathologists* **6**, 16–19.

Georgévitsch J. (1935) Über Diplo- und Haplophase in Entwicklungsreise der Myxosporidien. *Archiv für Protistenkunde* **84**, 419–428.

Ivancheva G., Dyková I. & Lom J. (1985) Seasonal fluctuation in the prevalence of *Sphaerospora renicola* and myxosporean bloodstream stages in carp fingerlings in Bulgaria. *Folia Parasitologica (Praha)* **32**, 193–203.

Hulbert W.C., Komourdjian M.P., Moon T.W. & Fenwick J.C. (1979) The fine structure of sporogony in *Myxidium zealandicum* (Protozoa: Myxosporidia). *Canadian Journal of Zoology* **55**, 438–447.

Kent M.L. & Hedrick R.P. (1985) PKX, the causative agent of proliferative kidney disease (PKD) in Pacific salmonid fishes and its affinities with Myxozoa. *Journal of Protozoology* **32**, 254–260.

Kovács-Gayer É., Csaba G., Békési L., Bucsek M., Szakolczai J. & Molnár K. (1982) Studies on the protozoan etiology of swimbladder inflammation in common carp fry. *Bulletin of the European Association of Fish Pathologists* **2**, 22–24.

Körting W. (1982) Protozoan parasites associated with swimbladder inflammation (SBI) in young carp. *Bulletin of the European Association of Fish Pathologists* **2**, 25–28.

Lom J. (1969) Notes on the ultrastructure and sporoblast development in fish parasitizing myxosporidian of the genus *Sphaeromyxa*. *Zeitschrift für Zellforschung* **97**, 416–437.

Lom J. & Dyková I. (1985) *Hoferellus cyprini* Doflein, 1898 from carp kidney: well established myxosporean species or a sequence in the development cycle of *Sphaerospora renicola* Dyková and Lom, 1982? *Protistologica* **21**, 195–206.

Lom J., Dyková I. & Lhoták ová S. (1983) Ultrastructure of *Sphaerospora renicola* Dyková and Lom, 1982 a myxosporean from carp kidney and comment on the origin of pansporoblast. *Protistologica* **18**, 489–502.

...m J. & Puytorac P. (1965) Studies on the myxosporidian ultrastructure and polar capsule development. *Protistologica* **1**, 53–65.

Molnár K. (1980a) Renal sphaerosporosis in the common carp, *Cyprinus carpio* L. *Journal of Fish Diseases* **3**, 11–19.

Molnár K. (1980b) Sphaerosporosis, a new kidney disease of the common carp. In: *Fish Diseases, Third COPRAQ-session* (ed. by W. Ahne), pp. 156–164. Springer Verlag, Berlin.

Molnár K. (1984) Experimental evidence that protozoans causing swimbladder inflammation in common carp (*Cyprinus carpio* L.) are stages of *Sphaerospora renicola*. *Bulletin of the European Association of Fish Pathologists* **4**, 14–15.

Molnár K. & Kovács-Gayer É. (1986) Experimental induction of *Sphaerospora renicola* (Myxosporea) infection in common carp (*Cyprinus carpio*) by transmission of SB-protozoans. *Journal of Applied Ichthyology* **2**, 86–94.

Seagrave C., Bucke D. & Alderman D. (1980) The causative agent of proliferative kidney disease may be a member of Haplosporidia. In: *Fish Diseases, Third COPRAQ-Session* (ed. by W. Ahne), pp. 174–181. Springer Verlag, Berlin.

Siau Y. (1977) Premiers dates du développement expérimental en cultures de spores de la Myxosporidie *Myxobolus exiguus* Thélohan, 1895. *Compte Rendu Hebdomadaire des Séances de l'Académie des Sciences (Paris)* **285**, 681.

Schubert G. (1968) Elektronenmikroskopische Untersuchungen zur Sporenentwicklung von *Henneguya pinnae* Schubert (Sporozoa, Myxosporidea, Myxobolidae). *Zeitschrift für Parasitenkunde* **30**, 57–77.

Schulman S.S. (1966) *Myxosporidians in the Fauna of the USSR*. Nauka, Moscow. (In Russian.)

Uspenskaya A.V. (1981) [Comparison of cytophotometric and electron microscopic results of sporogenesis investigation in Myxosporidia belonging to various taxonomic groups.] *Tsitologiya* **23**, 570–580. (In Russian.)