Journal of Fish Diseases 1984, **7**, 39–56

Studies into the possible protozoan aetiology of swimbladder inflammation in carp fry

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Abstract. Laboratory examinations of pond-reared common carp, *Cyprinus carpio* L., revealed a close correlation between the prevalence of swimbladder inflammation (SBI), renal sphaerosporosis and infection by C-blood-protozoan among the carp fry. In impression smears as well as light and electron microscopic preparations we detected developmental stages of intercellular protozoa, mainly in the loose fibrous tissue of the swimbladder. The parasites multiplied by internal budding so that 20–46 secondary cells were formed in each primary cell and two tertiary cells were formed in each secondary cell. The final stage of development was a unit consisting of a secondary cell enclosing two tertiary cells, i.e. a so-called triple formation, which bore a close resemblance to the early sporogonic stages of the renal sphaerosporan *Sphaerospora angulata* Fujita, 1912. Certain morphological similarities and the frequent simultaneous presence of the swimbladder protozoan, C-blood-protozoan, and *S. angulata* in hosts with clinical SBI have led us to postulate that the former two parasites could represent the hitherto unknown presporogonic stages of *S. angulata*. In view of the pathological changes caused by the parasites in the hosts with clinical SBI, and negative bacteriological and virological findings we have postulated that the swimbladder protozoan is the primary cause of SBI in carp fry.

Introduction

Swimbladder inflammation (SBI) is a common, economically important disease of the common carp, *Cyprinus carpio* L. First observed in Hungary by Szakolczai (1967), among three-summer carp, it had long been known in neighbouring countries (Hofer 1904; Roth 1922).

There are divergent opinions on the aetiology of SBI. Certain authors have regarded it as a viral disease (Arshanica 1969; Ahne 1973; Bachmann & Ahne 1973), while others (Neciporenko, Rudenko, Karpenko & Advosev 1963; Kanaev, Lobuncov & Naumova 1967; Szakolczai 1967; Markiewicz 1966; Mattheis & Kulow 1967; Kocylowski, Antychowicz & Zelazny 1970) have advocated the primary aetiological responsibility of bacteria, because of the isolations of many bacterial strains from carp with SBI. Szakol-

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czai (1967) detected bodies reminiscent of unicellular parasites (Coccidia, *Pleistophora*) in squash preparations of affected swimbladder and suggested they might be the causal agent of the disease. Kanaev & Kuzmin's (1970) report on experimental reproduction of SBI by feeding carp organs containing *Myxobolus* spores agreed well with Szakolczai's protozoan hypothesis. Otte (1966) implied that the blood flagellate *Cryptobia cyprini* may be responsible for SBI. Molnár (1980a) observed during studies on carp renal sphaerosporosis that the latter condition was frequently developed by fish with clinical SBI, and he postulated a relationship between the prevalence of SBI, renal sphaerosporosis and the condition caused by the C-blood-protozoan originally described by Csaba (1976). Waluga & Budzynska (1980) detected the developmental stages of *Sphaerospora carassii*, the causal agent of gill sphaerosporosis in carp, in visceral blood vessels, but did not pursue the possible identity of these stages with the C-blood-protozoan. Indirect proof of a relationship between SBI and renal sphaerosporosis has emerged from Grishcenko's (1967) observation that hosts with SBI harboured spore-like bodies in the renal tubules, and from a photograph published by Schäperclaus (1979) as the image of the swimbladder wall, although it actually shows a renal tubule packed with sphaerospores.

Kovács-Gayer has regularly detected developmental stages of parasitic protozoa in the wall of the swimbladder of carp fry with SBI (Kovacs-Gayer, personal communication, in Molnár 1980b; Kovács-Gayer, Csaba, Békési, Bucsek, Szakolczai & Molnár 1982). In this paper we discuss the possible involvement of these protozoa in the aetiology of SBI and their possible identity with the C-blood-protozoan and *Sphaerospora angulata* Fujita, 1912.

Materials and methods

Our studies, conducted in 1979–81, were based partly on routine diagnostic examinations of common carp submitted for veterinary inspection and partly on experimental studies of carp fry made available for laboratory research. Detailed investigation into the relationship between the prevalence of SBI, sphaerosporosis and C-blood-protozoan were performed in 1981, when we screened the carp fry in three pond farms (two in western Hungary and one in eastern Hungary) at two week intervals from the first month after spawning until the autumn.

Carp fry used in two other experiments were also studied. We examined between mid-October and mid-December 59 fish from a population used in a feeding experiment, in which the fry received a pelleted feed and were kept at 20–22°C. We also examined carp fry hatched by the end of August, reared in a filtered recycling system and fed initially on *Artemia*, and then on *Tubifex* obtained from natural habitats; these fish were six weeks old when examined.

As fish were sacrificed, blood samples were taken and examined fresh for C-blood-protozoans. The external sac of the swimbladder was then peeled off to ascertain the presence and stage of SBI. The kidneys were examined for sphaerosporosis in unstained squash preparations and impression smears. Smears were also obtained from the altered

regions of the inner sac; renal impression smears were also prepared. Impression smears from the swimbladder and kidney, and blood smears were stained with Giemsa.

All organs exhibiting gross changes were examined for microscopic lesions. Egg albumen was injected into the swimbladder to preserve its shape before fixation. Material was fixed in 10% formol or Bouin's solution and after processing was stained with haematoxylin and eosin (H&E) or Giemsa.

For electron microscopy swimbladder tissue was fixed in pH 7·4 Na cacodylate-buffered 2·5% glutaraldehyde solution for 2 h, post-fixed in 1% OsO_4 solution for 1 h, dehydrated in step-graded ethanol and embedded in Durcupan ACM. The ultra-thin sections were counterstained with uranyl acetate and lead citrate, and were examined in a Philips 201 CS electron microscope.

For bacteriological examination, inocula from the liver, kidney and swimbladder of each fish with clinical or suspected SBI, and from two apparently healthy fish in each lot, were transferred to blood agar plates and Anacker–Ordal media (Anacker & Ordal 1959). The bacterial isolates were identified according to Cowan (1975) and Macfaddin (1978).

For virological examination, the swimbladder and other organs of fish with acute SBI were homogenized in antibiotic-containing PBS. The organ suspensions were sterilized by ultra-centrifugation and were inoculated on to FHM, EPC, or occasionally, primary carp ovarian cell cultures. The tube cultures were incubated at 18 °C.

Figures on the prevalence of SBI and protozoa presented here are based on the examination of 289 carp fry, although about 1000 fish were examined during this study. Descriptions of parasite stages and changes in the host are based on observations from carp fry only.

Results

The frequency of occurrence of SBI varied between seasons and carp farms. The disease first appeared in June among 4- to 6-week-old carp fry. Clinical changes were frequently absent in the initial stages, and lesions were found only on the inner sac of the swimbladder. In such cases the normally transparent wall of the inner sac (Fig. 1a) was cloudy (Fig. 1b), with minor haemorrhages and dilated, injected vessels (Fig. 1c). Occasionally the haemorrhages were visible directly on the outside of the swimbladder, without removal of the outer sac; in such cases there was considerable mural thickening (to 2–3 mm) of the swimbladder. The posterior sac of the swimbladder was rarely involved.

During August acute haemorrhages were no longer seen in the swimbladder, only mural thickening remained in some cases. However, in some apparently normal swimbladders removal of the outer sac revealed a thin deposition of an emerald green-coloured material, presumably fibrin, which adhered to the inner sac, and exhibited in places brownish spots (residues of previous haemorrhages, see Fig. 1d). The prevalence of SBI tended to decrease considerably during September.

The sequence of pathogenesis described above applied only to pond farm populations; in experimental conditions we observed a different pathogenesis.

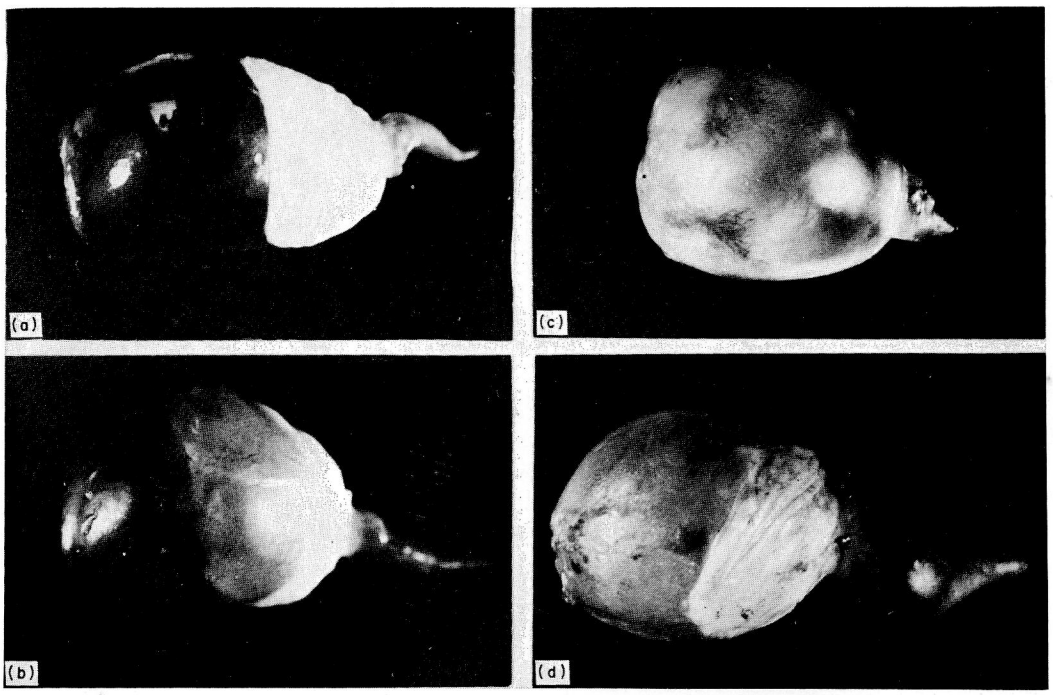


Figure 1. Swimbladders of healthy and SBI-affected carp fry. (a) Normal swimbladder. Note glass-like transparency of the inner sac. (b) Swimbladder of a host with acute SBI. Note cloudy opacity of the inner sac. (c) Haemorrhages and branched pattern of injected vessels in the inner sac of the swimbladder. (d) Advanced SBI. Note residues of earlier mural haemorrhages in the inner and outer sac of the swimbladder, and the clumped fibrinous deposition on the surface of the thickened inner sac (all × 3).

Parasitological examination of the swimbladder

In fry with acute SBI, we detected many unknown protozoan organisms in histological sections and impression smears obtained from the anterior sac of the swimbladder. In no case were protozoa found in the posterior sac. In histological sections the protozoa were frequently localized in the fibrous tissue of the tunica interna, but most of them were found extravasally, in the richly vascularized loose connective tissue. The parasites were most easily visible at the border between haemorrhagic and intact areas of the capillary-rich tissue, lined up along the vascular walls (Fig. 2). Most stages appeared to be conglomerations of large (3–4 μm) nuclei, 15–20 in number, but less often stages 1–2 μm long, suggestive of karyorrhexis, could also be seen (Fig. 3).

The protozoan nature of the stages seen in sections was substantiated by Giemsa staining of impression smears. The majority of the protozoans seen in the impression smears were 17–30 μm in size, and occurred within aggregations of several primary cells (Fig. 4a). The primary mononuclear envelope cells contained up to 46 independently dividing secondary cells, with their own cytoplasm (Fig. 4b). The secondary cells were 3–4 μm in diameter and within a few, presumably more developed individuals, two

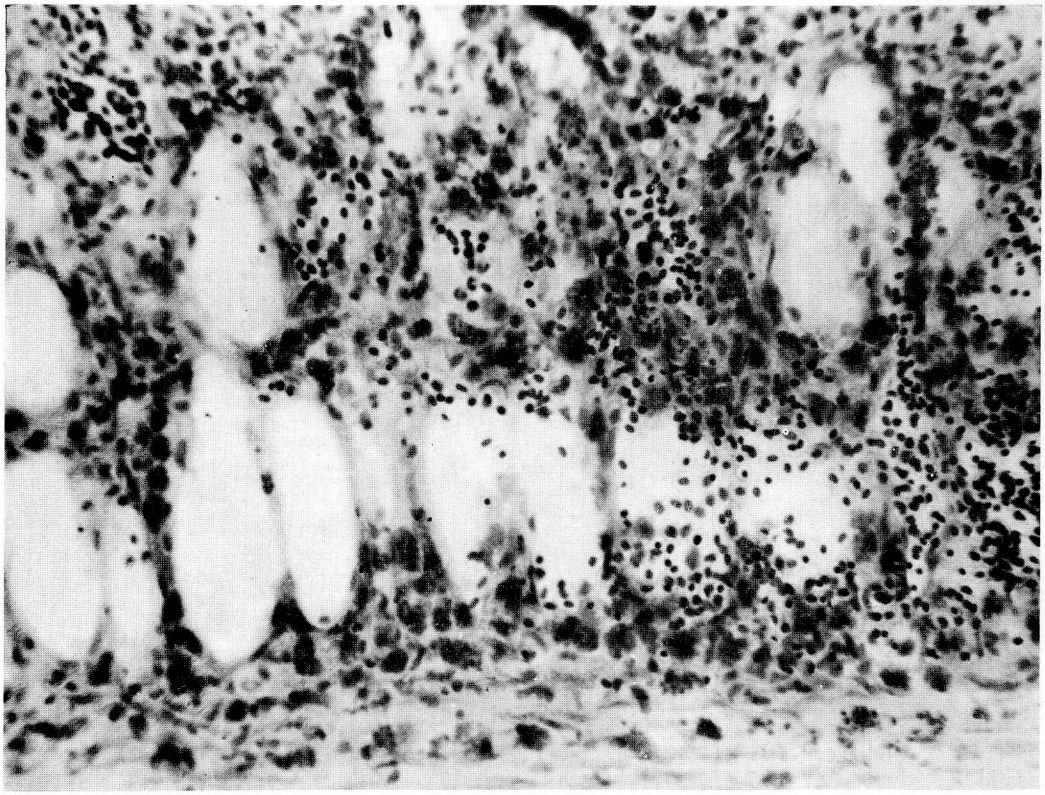


Figure 2. Section of swimbladder wall from a fish with acute SBI. Many developing protozoa can be seen at the border between haemorrhagic and healthy tissue in the richly vascularized layer of the thickened swimbladder wall (H&E, × 232).

tertiary cells also appeared. The two tertiary cells and the considerably larger nucleus of the secondary cell made a characteristic triple formation (Fig. 4c). With Giemsa the nucleus of the primary cell was lightly eosinophilic with, mainly in units containing numerous secondary cells, a distinct reticular structure (Fig. 4g, h). The cytoplasm of the primary cell stained pale blue. The secondary cells were for the most part roundish or spindle-shaped, their nuclei assumed a darker eosinophilic shade than those of the primary cells and their cytoplasm took on a darker blue stain. The cytoplasmic margin of the tertiary cells was hardly visible but their nuclei were intensively eosinophilic.

Electron microscopy revealed that the shape of the protozoan is irregular, its membrane is thicker, and its cytoplasm is darker compared to the surrounding host cells. The primary cell encloses a varying number of secondary cells. The nuclei of the former are roughly of the same size as the secondary cells, and contain a prominent round nucleolus and a relatively pale chromatin substance. The secondary cells are delimited by a simple or double membrane, are more electron dense than the primary cells and enclose occasionally still denser tertiary cells. The mitochondria of the parasite are vesicular, and frequently many microtubules are also present in the cytoplasm (Figs 5 & 6).

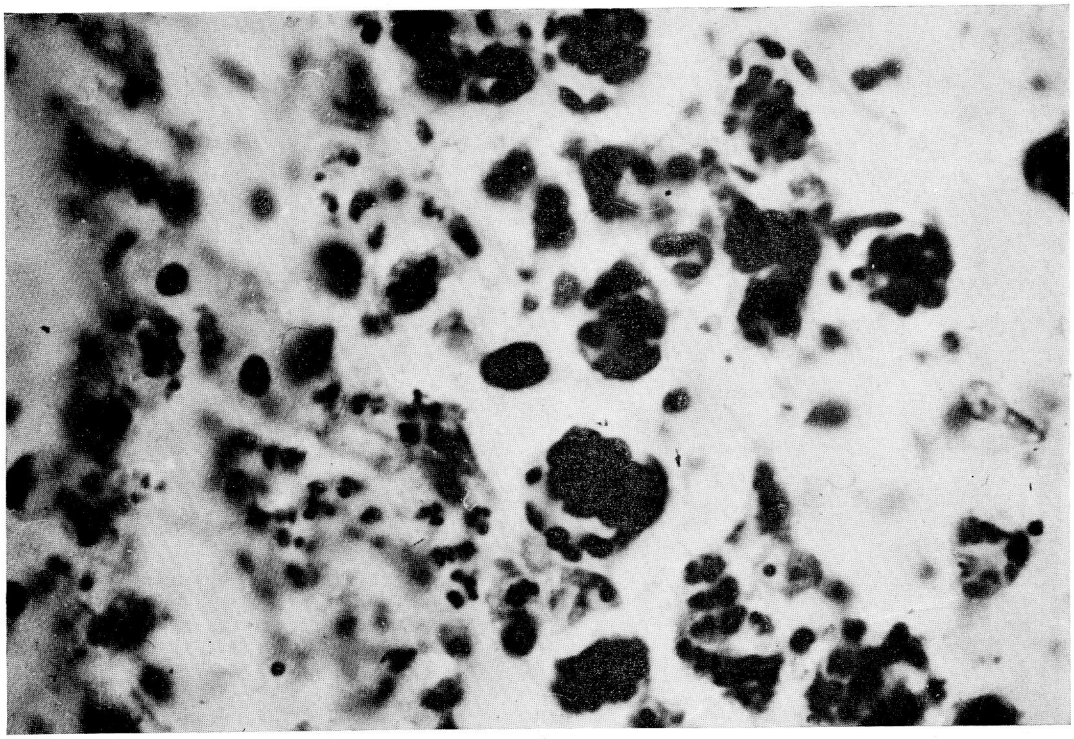
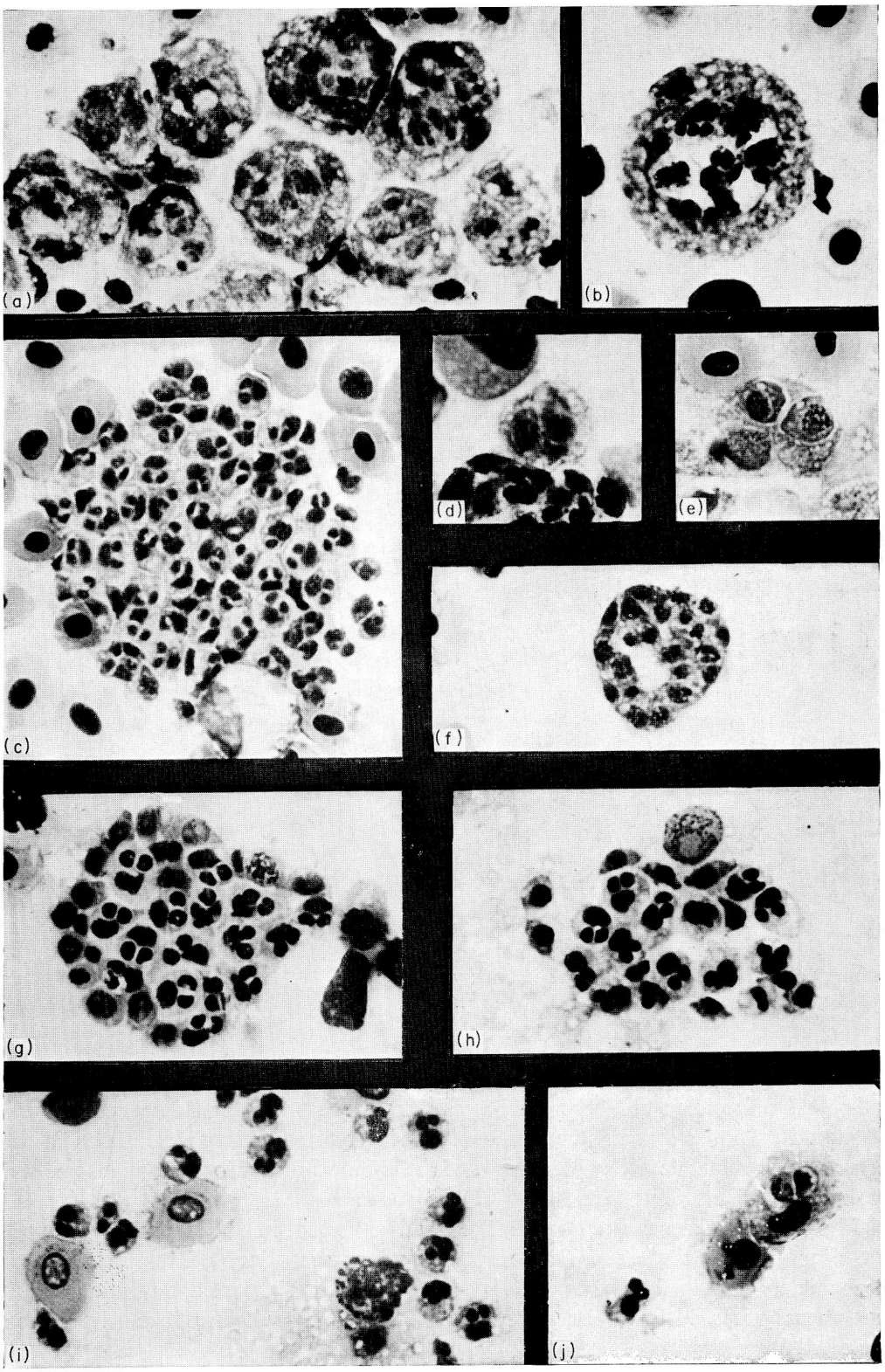


Figure 3. Parasite aggregations in the wall of the swimbladder. Scattered solitary stages can also be seen in the tissues (H&E, × 740).

The developmental stages of the parasite can be arranged in a logical sequence. The earliest stages were obviously the cells enclosing two nuclei (Fig. 4d), and those formed by one primary cell with two secondary cells within it (Fig. 4e). The size of the parasite seems to increase parallel with the multiple division of the secondary cells, which finally number 20–46 (Fig. 4f). A further slight increase in size of the primary cells takes place during the development of tertiary cells within the secondary ones. Tertiary cell development is not synchronous within the different secondary cells (Fig. 4g). The pale nucleus of the primary cell was still visible (Fig. 4h) during tertiary development, but later disintegration of the primary cell lead to release of the characteristic triple formations (Fig. 4i). The latter structures seem to have been responsible for the rhexis-like changes seen in sections (Fig. 3).

Figure 4. Swimbladder parasites from impression smears stained with Giemsa. (a) Aggregations of protozoa (× 930). (b) Mononuclear primary cell with a pale cytoplasm, enclosing secondary and tertiary cells (× 1116). (c) Aggregated secondary units; two tertiary units are present in most (× 1116). (d) Early binucleated primary cell (× 1116). (e) Early primary cell, enclosing two secondary cells (× 1116). (f) Conglomeration of early secondary cells (× 1116). (g) Conglomeration of early secondary cells; note appearance of tertiary cells in the centrally placed cells (× 1116). (h) Squash of parasite aggregation. The pale nucleus of the primary cell is clearly visible (× 1116). (i) Dispersed secondary cells (× 1116). (j) Triple formations composed of one primary cell plus two secondary cells (right), and one secondary cell plus two tertiary cells (left) respectively (× 1116).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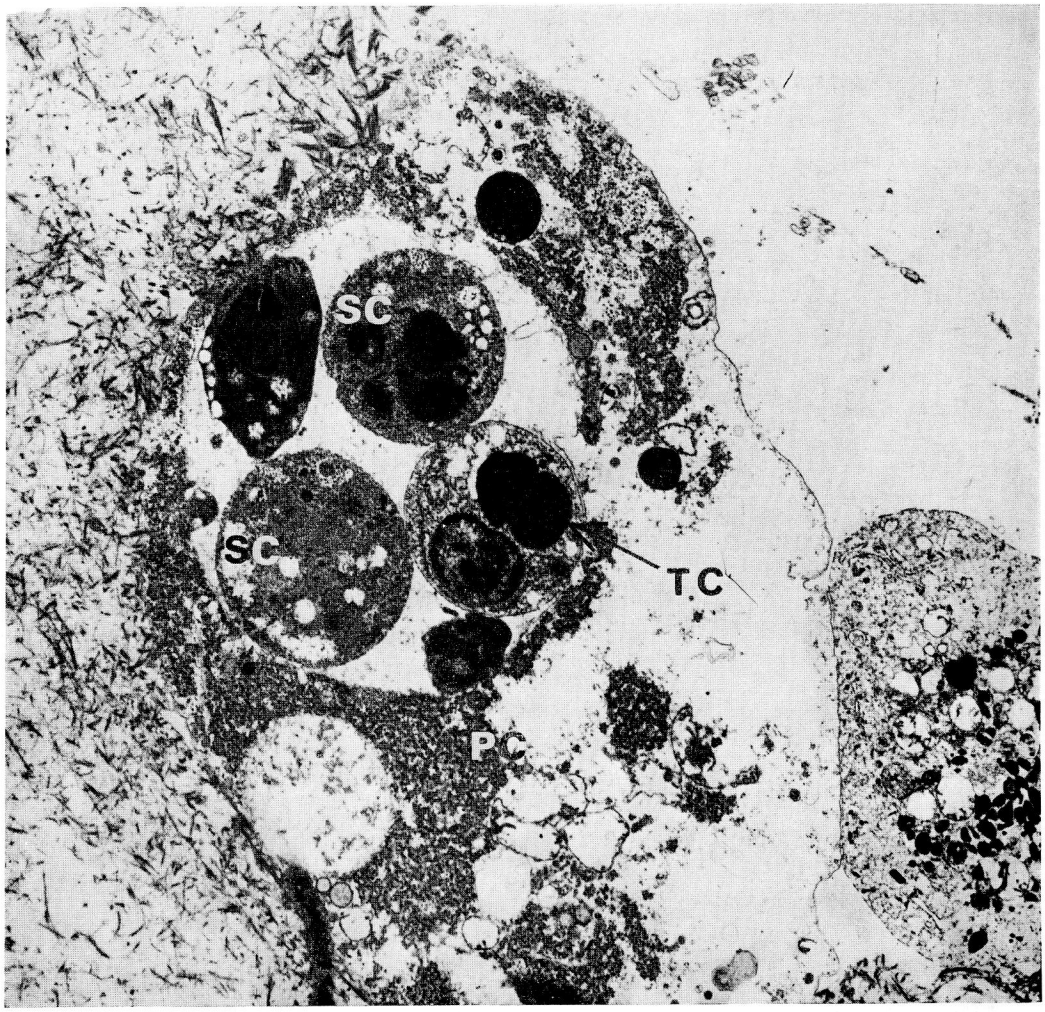

Figure 5. Electron micrograph of disintegrating primary cell (PC) enclosing four secondary cells (SC); a tertiary unit (TC) can be seen in one secondary cell (× 7300).

The triple formations enclosing tertiary cells can be easily differentiated by cellular and nuclear size from the larger primary cells enclosing two secondary cells (Fig. 4j). (It should be noted that the early stage enclosing two secondary cells is considerably larger than the final developmental stage of the C-blood-protozoan, which also contains two nuclei.)

Mononuclear stages were not infrequently also present in our preparations, but it is not clear whether these represent early stages, or secondary cells released from a mechanically damaged parasite. The latter possibility is supported by the observation that smears prepared by a relatively robust method contained almost exclusively mononucleated stages.

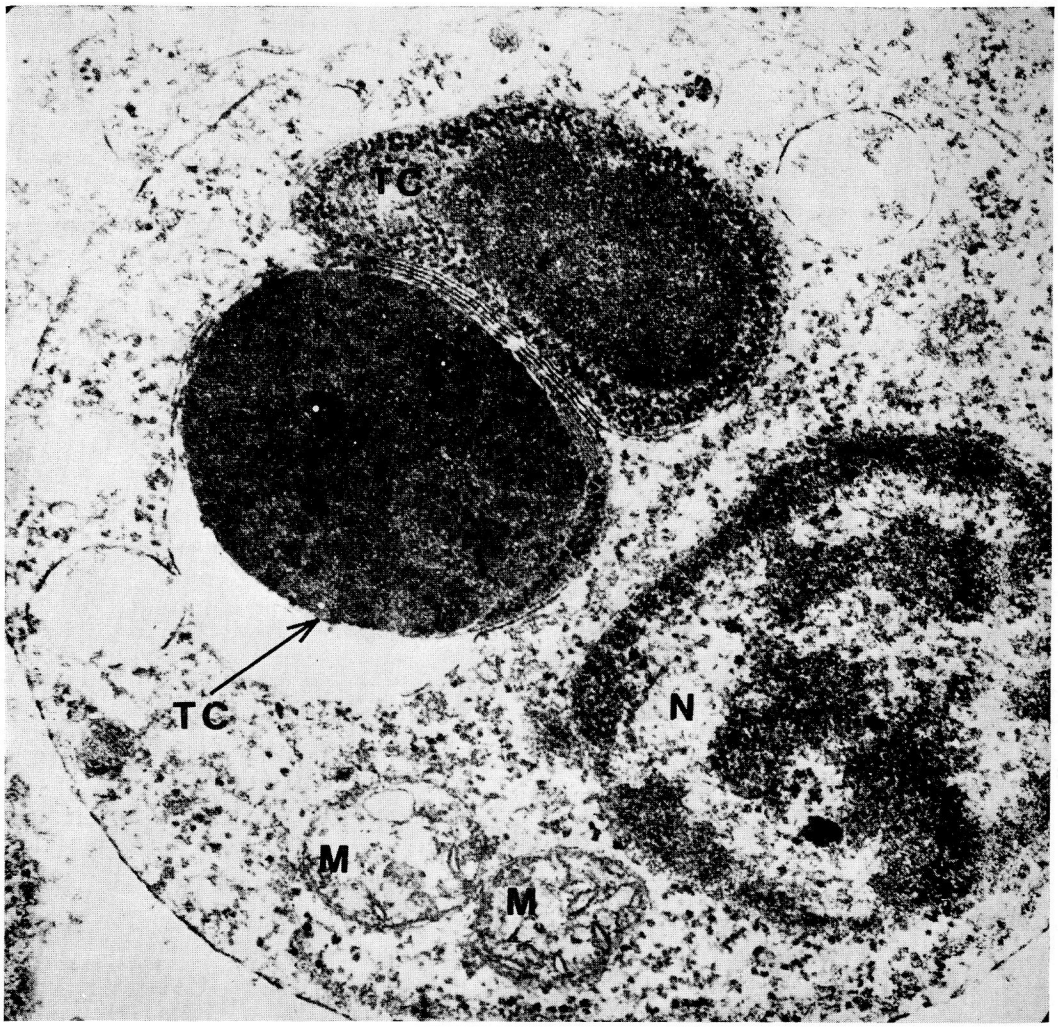

Figure 6. Ultrastructure of a secondary cell delimited by a simple membrane. N, nucleus; M, mitochondria; TC, tertiary cells delimited by a double membrane (× 25 080).

Interrelationships between SBI, and infections by C-blood-protozoan and S. angulata

C-blood-protozoa first appeared in the blood, and developmental stages of *Sphaerospora angulata* in the kidney were also detectable from the first part of July; this was also the period when swimbladder protozoa were first found. The prevalence of all three protozoans increased until the end of July, after which it tended to decrease gradually, until only a sporadic occurrence was seen in November.

During acute stages of SBI, we detected *Sphaerospora* in the kidneys of all diseased fish (Table 1).

In August the swimbladder protozoan was demonstrable only in fish with more acute SBI. The renal tubules of most diseased fry were packed with sphaerospores at

Table 1. A comparison of the occurrence of swimbladder inflammation, renal sphaerosporosis and C-blood-protozoan in carp fry

Periods of investigation	July				August				September			
Number of fish examined	85				118				86			
Swimbladder inflammation	+ 20		− 65		+ 51		− 67		+ 22		− 64	
	+	−	+	−	+	−	+	−	+	−	+	−
Renal sphaerosporosis	20	0	2	63	40	11	7	60	20	2	14	50
	100%		3%		78%		10%		91%		22%	
C-blood protozoan*	14	0	2	21	30	8	3	41	9	2	13	47
	8/6	0/0	1/1	6/15	9/21	2/6	1/2	7/34	3/6	1/1	5/8	7/40
	57%	—	50%	28%	30%	25%	33%	17%	33%	50%	38%	15%

* Blood was examined from a part of the sample only.

this time, but the intensity of infection was non-uniform. *Sphaerospora*-infected specimens were also found in both healthy carp populations and those with chronic SBI (Table 1). A similar pattern of *Sphaerospora* infection was found in September (Table 1).

No appreciable correlation was demonstrable between SBI and the prevalence of the C-blood-protozoan on the one hand, and sphaerosporosis and C-blood-protozoan infection on the other, in any period of study. However, it must be noted that subsequently we observed that fish not harbouring C-blood-protozoa in the peripheral blood did have masses of them in the renal and swimbladder capillaries. The C-blood-protozoan was present in all populations examined at all periods covered in our study.

Swimbladder protozoa were characteristically present only during the acute stage of SBI, and were rarely detected in pond farm populations after August. However, the parasites did persist for a longer period in the two experimental populations studied. In the population kept at high water temperatures the number of fry with SBI was still high by the end of September and the protozoa were observed in the changed swimbladder until October. In the other experimental population reared in a recycling system severe clinical SBI and intensive infection with swimbladder protozoa and renal sphaerosporosis was found in 5- to 6-week-old fish. In some fry C-blood-protozoans were also found.

Life cycle of Sphaerospora angulata

The development of *S. angulata* was observed in the renal tubular lumen (Fig. 7), in which sporogony takes place; no developmental stage was detected in the tubular epithelium. The earliest stage identified was a unit composed of a mother cell and two daughter cells, i.e. two sporonts enveloped by the pansporoblast's envelope

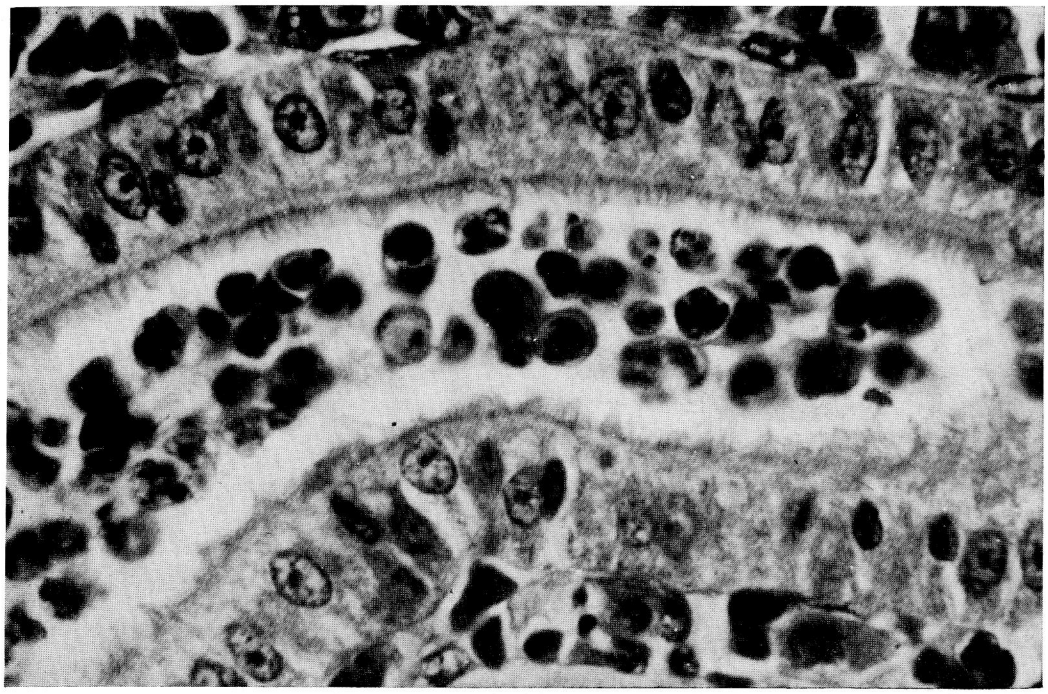

Figure 7. *Sphaerospora angulata* spores and pansporoblasts in renal tubular lumen of a carp (H&E, × 684).

cell. At that stage the larger envelope cell nucleus and the smaller daughter cell nuclei were reminiscent of the triple formation stage of the swimbladder protozoan (Fig. 8a). The nuclei of the sporonts grow larger (Fig. 8b) and divide several times (Fig. 8c), finally giving rise to a new stage with 13 nuclei (Fig. 8d, e; the original single envelope cell nucleus plus six nuclei from each two sporoblasts). The cells of the sporoblast undergo a functional differentiation: two each transform to valvogen and capsulogen cells, respectively, and the third pair of nuclei gives rise to the binucleated generative cell.

In the final stage of development the capsulogenic cells give rise to the distinctly eosinophilic polar capsules; these were frequently lost during processing, leaving vacuole-like empty spaces (Fig. 8) in the stained impression smears.

An ultrastructural study of sporogony will be presented in a subsequent paper.

Life cycle of the C-blood-protozoan

This protozoan was originally described by Csaba (1976). The parasite is easily visible in both stained and unstained blood smears and occurs in large numbers within the capillary vessels of different organs and of the swimbladder, and also in renal vessels. In fresh preparations the protozoa are recognized as vigorously moving, large bodies, 3–15 μm in size. In stained preparations the primary cells are seen to enclose 1–8 spindle-shaped or roundish, independent secondary cells. The secondary cells multiply

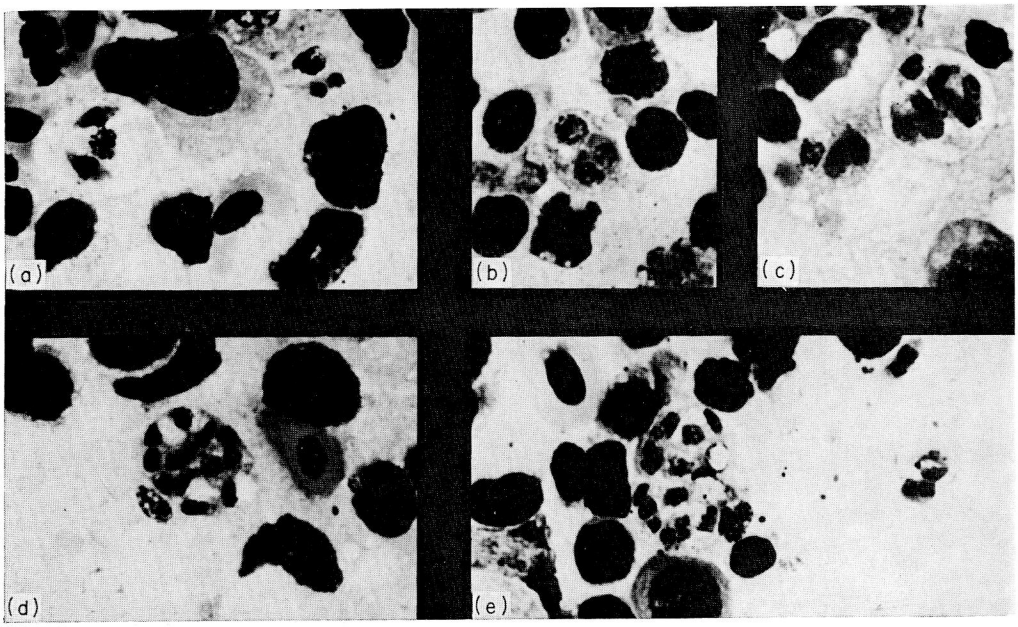

Figure 8. Developmental stages of *S. angulata* in a renal impression smear stained with Giemsa (× 1200). (a) Triple formations composed of an envelope cell nucleus and two sporoblasts; note close resemblance to the triple formations of the SB-protozoan. (b) Developing sporoblasts within a pansporoblast. (c) Developmental stage composed of two multinucleated sporoblasts and an envelope cell nucleus (right). (d) Hexanucleated early sporoblasts within a pansporoblast. (e) Early spores with polar capsules within a pansporoblast. Note early triple formation on right.

by division inside the primary cell and assume a variety of shapes during division (Fig. 9). The 6–8 secondary units formed by division are roundish rather than spindle-shaped, and two, three and four minute nucleoli, taking on an intensive eosin stain, appear within them in sequence (Fig. 9p, q, r). In these cases a nucleolus, also eosinophilic, is additionally formed in the secondary cells; it presumably represents a tertiary cell.

The ultrastructure of the C-blood-protozoan has been described in another paper (Bucsek & Csaba 1981).

Other investigations

Attempts at isolation of bacteria from acute cases of SBI failed. In chronic cases bacteria of the *Aeromonas hydrophila-punctata* group, and less often *Flavobacter* and *Pseudomonas* spp. were isolated from the swimbladder. These bacteria were also occasionally isolated from other organs (kidney, liver) of fish with chronic SBI.

Virological examinations were negative. In no case was a cytopathic agent isolated, and electron microscopic investigation also failed to detect virus-like particles in the swimbladder.

Parasitological examination resulted in detection of a great variety of parasites in different organs of the hosts but, apart from *S. angulata*, only the rare occurrence of the

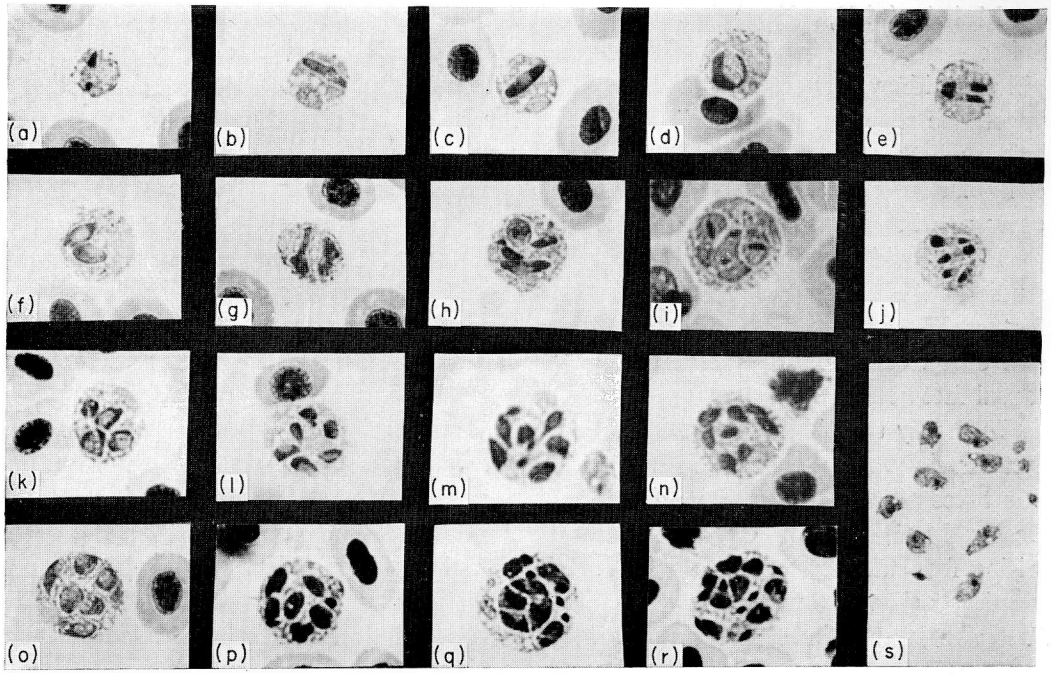

Figure 9. Development of the C-blood-protozoan, as followed in blood smears stained with Giemsa (× 1200). (a–d) Stages composed of one primary and one secondary cell. (e–g) Stages composed of one primary cell and two secondary cells (the nucleus of the primary cell stains pale). (h–k) Stages composed of four secondary cells. (l) Six-cell stage. (m–n) Eight-cell stages. (o) Six-cell stage with tertiary cells also present. (p–r) Eight-cell stage with tertiary cells also present; note eosinophilic clumps. (s) Eight secondary cells and four eosinophilic clumps released from a disintegrated primary cell.

myxozoan fin parasite *Thelohanellus nikolskii* and gill parasite *Sphaerospora carassii* deserve note.

Discussion

The present studies have shown a remarkable coincidence between the occurrence of SBI and renal sphaerosporosis in carp fry. A nearly 100% concurrence was observed during peak prevalence of the two conditions in July (Table 1) suggesting the involvement of *S. angulata* in clinical SBI. The presence of the unicellular parasites in the swimbladder supports this hypothesis.

The swimbladder protozoan is probably a myxozoan as judged by its light and electron microscopic structure. The sporogonic process of the swimbladder protozoan remains for the time being as obscure as that of the C-blood-protozoan described by Csaba (1976). The ultrastructural features of the latter parasite (Bucsek & Csaba 1981) are in many respects similar to those of the swimbladder protozoan discussed earlier in this report.

Molnár (1979, 1980b) has postulated that the presporogonic stages of the gill sphaerospore *S. carassii*, and of the renal sphaerospore *S. angulata*, develop outside the gill and kidney, respectively. Waluga & Budzynska (1980) claimed to have detected the vegetative stages of *S. carassii* throughout the organs of carp fry. The question thus arises whether the C-blood-protozoan, known only by its vegetative stages, and the swimbladder parasite, which also seems to represent a presporogonic stage, could correspond to an early developmental stage of a *Sphaerospora* sp.

Direct evidence for or against this implication does not emerge from the present studies, but evaluation of prevalence rates, and certain morphological and developmental features support the hypothesis that the protozoan parasites found in the swimbladder and blood could equally represent presporogonic stages of *S. angulata* and thus be causal factors of SBI.

In theory the C-blood-protozoan could be a stage of other myxozoan species. The cyst-forming *Myxobolus* and *Thelohanellus* spp. can be ruled out, since their multiplication takes place inside cysts, and if they have precursor stages at all, as postulated by Desser, Molnár & Weller (in press), these cannot be more numerous than the cysts arising from them. From the epizootiological point of view, identity with a non-cyst-forming *Sphaerospora* sp. is a possibility; in this respect, the developmental stages of *S. carassii*, although of minor importance in the populations studied, may also occur in the blood.

A common feature of the parasite stages found in the kidney, swimbladder and blood is that they exist extracellularly, and give rise to secondary cells, which reach their final number by endogenous division until, at least in the case of the swimbladder and blood parasites, tertiary cells appear in them (Fig. 10). This course of development closely resembles the endogenous proliferation observed in the *Marteilia* infection of mussels (Perkins 1976). All secondary cells have a well-defined independent cytoplasm, whereas the cytoplasmic margin of the tertiary cells is indistinct (Figs 4c; 9p, q, r; 10). In the case of the renal parasite the primary cell represents the envelope cell of the pansporoblast.

However, considerable differences also exist between the renal, swimbladder and blood protozoa. The C-blood-protozoan separates after serial divisions inside the primary cells into eight secondary units, each of which encloses two nucleii; of these, the larger one belongs to the secondary cell, and the smaller one to the tertiary cell, whose narrow cytoplasmic margin is scarcely visible. In the primary cell of the swimbladder protozoan more than 40 secondary cells are formed, and each separates into a trinucleated unit in which the large nucleus belongs to the secondary cell, and the two small nuclei belong to the two tertiary cells, whose narrow cytoplasmic margin is again scarcely visible.

The renal parasite *S. angulata* gives rise during its sporogonic process to 12 functionally different cells which develop within the envelope cell of the pansporoblast, and form two spores at the end of the process.

There is no direct evidence of a continuity in the development of the protozoan parasites found in the three different host sites. They appear practically simultaneously in their respective localizations. *Sphaerospora angulata* can certainly be regarded as a

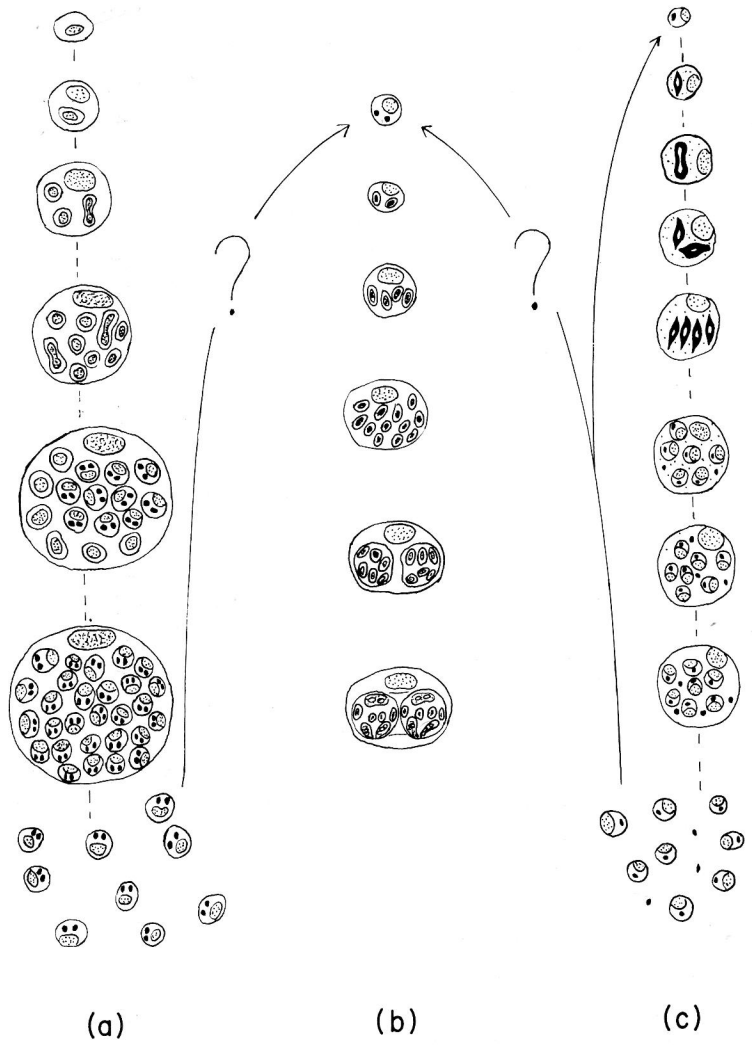

Figure 10. Diagram of possible relationships between the development of the swimbladder protozoan, C-blood-protozoan and *Sphaerospora angulata*. (a) Development of the swimbladder protozoan. (b) Sporogony of *S. angulata*. (c) Development of the C-blood-protozoan.

terminal, i.e. sporogonic, stage of development in the renal tubule, but there is no definite proof whether the swimbladder or the blood-dwelling parasites represent its presporogonic stages.

The presence of the C-blood-protozoan throughout the fry, as indicated by squash preparations, is due to its haematogenic spread and therefore it is no proof of any relationship with the life cycle of *S. angulata*.

The striking similarity between the triple formations found in the swimbladder (Fig. 4i) and renal convoluted tubules (Fig. 6a, b) suggests that *S. angulata* and the swimbladder parasite represent the same, or at least related species. Trinucleated units

arising in a primary cell are characteristic of the presporoblastic development of Myxozoa (Shulman 1966). A detailed description of triple formation development was given by Debaissieux (1925) for *Myxobolus* (*Thelohanellus*) *notatus*. Seagrave, Bucke & Alderman (1980) found parasites resembling the swimbladder protozoan in the kidneys of rainbow trout, *Salmo gairdneri* Richardson, affected by proliferative kidney disease, and considered them to be a species closely related to *Marteilia* spp.

Since we found the SB protozoan in the swimbladder of most fish with SBI, and could not isolate bacteria from the swimbladder during the early stage of SBI, we postulate that the initial changes of opacity and thickening of the swimbladder wall are due exclusively to swimbladder protozoa. The pathogenic effect of swimbladder protozoa, and their possible identity with *S. angulata* could explain the striking concurrence of SBI and renal sphaerosporosis in the diseased fry populations. The precise role of the C-blood-protozoan remains obscure, although masses of it would occasionally appear in the capillary vessels of the swimbladder. We believe that some of the parasites mutiplying in the swimbladder gain access to the kidney at a very early stage, and account for the onset of renal sphaerosporosis simultaneously with SBI. Some individuals may even enter the kidney before clinical SBI develops, which would explain the presence of renal sphaerosporosis in carp fry with apparently intact swimbladders (Table 1), as occasionally occurred.

The concurrence of SBI and sphaerosporosis was less conspicuous during August and September. Sphaerospores were always detected in fish with clinical SBI, but occasionally parasites, especially spores, were found in the renal tubules of fish recovered from SBI. At the same time, sphaerospores were occasionally absent in fish with SBI in August or September (Table 1). We believe that in the latter cases, clinical SBI was maintained by a secondary bacterial infection supervening on the swimbladder parasitosis, rather than by parasites themselves.

The high prevalence of SBI among carp fry used in the warm-water experiment, and presence of swimbladder, blood and renal protozoa in these fish, suggests that ambient temperature may play an important role in the development of SBI.

Pathogenic agents larger than bacteria can only be introduced into recycling systems with the fish feed. That carp fry kept in an experimental recycling system had SBI and all three parasites when examined at about five weeks of age, suggests that the latter were introduced with the tubificids used for feeding, possibly in the intestinal contents, i.e. mud; alternatively the possibility of an intraovarian infection by sphaerospores, as postulated by Waluga & Budzynska (1980) cannot be ruled out.

In summary, we believe that the swimbladder protozoan is not necessarily the sole causal factor of SBI but that it bears the primary aetiological responsibility for the characteristic SBI of carp fry, as described in this report. Other causal agents are probably also involved in the similar disease of two- or three-summer carp. The swimbladder protozoan causes opacity and thickening of the swimbladder wall and minor mural haemorrhages, which may heal spontaneously if no secondary bacterial infection supervenes. During healing, mural hyperaemia and thickening tends to decrease until the wall becomes thin and transparent again and only clumps of haemosiderin remain to indicate the mural haemorrhages. If a secondary bacterial infection occurs further

haemorrhages appear, mural thickening is aggravated, exudate fills the lumen of the anterior sac and, infrequently, that of the posterior sac. We believe that all severe cases of SBI among carp fry are due to a secondary bacterial infection.

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