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Oocyst formation in the coccidian parasite *Goussia carpelli*

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ABSTRACT: The ultrastructural features of sporogony of *Goussia carpelli* (Léger & Stankovich) are described from the intestine of laboratory-infected carp. Zygotes were encased by a bilayered membrane (about 45 nm thick), which could be taken for the outer cell boundary of the oocyst. No indications were found that the parasitophorous vacuole membrane of the host cell contributed to oocyst wall formation. The formation of 4 sporoblasts from the sporont appeared to occur simultaneously. Initially, the sporoblasts were enveloped by a unit membrane and later were covered by a fine bilayered envelope which then transformed into a 120 nm thick sporocyst wall. The sporocyst wall formed a bivalved shell; the valves were joined by a continuous suture visible in the scanning electron microscope as a slightly prominent longitudinal fold. The sporozoites started to develop at opposite sides of the residual body within the sporoblasts, and in more advanced stages 2 sporozoites lying side by side could be observed. The sporozoites were morphologically similar to sporozoites of other Coccidia. During sporulation, the host cell lost its integrity, deteriorated and turned to an almost structureless mass which appeared as the 'yellow bodies' known from light microscopy.

INTRODUCTION

Goussia (syn. *Eimeria*) *carpelli* (Léger & Stankovich, 1921) Dyková & Lom, 1983 is a coccidian parasite of intestinal epithelial cells of common carp. In hatchery populations, mainly juvenile fish have been found to be infected (Ivasik & Kulakovskaya 1959, Zaika & Kheisin 1959, Kocylowski et al. 1976), and information on biology and morphological features of developmental stages of *G. carpelli* has been given by Léger & Stankovitch (1921), Kent & Hedrick (1985) and Steinhagen et al. (1989), while notes on the ultrastucture of oocysts and sporocysts have been reported by Lom & Dyková (1982a).

In the present communication ultrastructural observations on sporogonic developmental stages of *Goussia carpelli* from laboratory-infected carp and from carp with spontaneous infections are presented, and the formation of 'yellow bodies' is discussed.

MATERIAL AND METHODS

Common carp *Cyprinus carpio* raised from eggs in the laboratory were infected with *Goussia carpelli* at the age of 3 to 4 mo by fecal contamination or by allowing them to feed on *Tubifex tubifex* infected with *G. carpelli*. On Days 6 to 10 post-infection (PI) (at 20 °C), fish were dissected and the first 5 mm of the intestine was removed and fixed for electron microscopy at 4 °C overnight with 2.5 % glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.2, postfixed with 1 % OsO_4 in the same buffer and then stained en bloc with uranyl acetate. After dehydration in a series of graded ethanol, the tissue was embedded in Spurr's resin. Additional samples from spontaneously infected carp fingerlings were fixed for 90 min at 4 °C in 0.1 M cacodylate-buffered 2 % OsO_4 and embedded in Epon-Araldite mixture. For scanning electron microscopy, samples of isolated sporocysts were fixed in 2 % osmic acid buffered with 0.1 M sodium cacodylate, critical-point dried and examined in a Tesla 400B scanning microscope. Ultrathin sections were stained with uranyl acetate and lead citrate and studied in Zeiss EM 10A, Jeol 100B and Phillips 420 electron microscopes.

RESULTS

Macrogametes and sporulating oocysts were observed in cells of the intestinal epithelium and the lamina propria 6 to 10 d PI. The process of fertilization

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itself could not be observed. However, microgametes were observed in the cytoplasm of the host cell harbouring the macrogamete (Fig. 1). The cytoplasm of macrogametes with a central nucleus contained numerous amylopectin granules and prominent osmiophilic material full of small lucent vesicles. In what appeared to be zygotes (Fig. 2), these aggregates became condensed, more uniformly opaque and later became reduced in size and less opaque. The parasitophorous vacuole was reduced to a narrow space.

In the zygote, the unit membrane at the cell surface (Fig. 2) transformed into or was replaced by a bilayered membrane, about 45 nm thick (Figs. 3 & 4). Beneath this membrane, stretches of opaque membrane started to appear which were contiguous with, or underlain by, cisternae of endoplasmic reticulum (Fig. 3). Later, these stretches fused into what could be taken for the outer cell boundary of the young oocyst; the narrow gap (about 70 to 260 nm) between this boundary and the bilayered membrane appeared to be filled with an amorphous substance. There was no indication that host cell membranes could participate in formation of oocyst boundary. In some instances (or at a certain stage of development?) the bilayered membrane was covered by another loosely undulating membrane (Fig. 5). In the course of later development, the bilayered membrane, which is the actual oocyst wall, became closely apposed to the host cell membrane, i.e. to the parasitophorous vacuole membrane. The host cell mostly formed a narrow band around the parasite (Figs. 4 & 6). Sometimes, however, the space between the oocyst wall and the membrane of the parasitophorous vacuole was filled with a dense mass of various vesicles derived from the host cell (Fig. 7), or just by an amorphous substance (Fig. 8).

Before the onset of sporulation, the sporont was covered by a simple cell membrane (Fig. 8). Formation of the 4 sporoblasts appeared to occur simultaneously (Fig. 7). After the division was completed, amylopectin granules, osmiophilic inclusions now of homogeneous appearance, endoplasmic reticulum and free ribosomes could be seen in the sporoblasts. The sporoblasts appeared enveloped by a cover formed by a unit membrane (Fig. 7), which started to form at the moment of cleaving of the sporont. Eventually, the sporoblasts each have their own membranous envelope; the space inside it may be filled with a foamy substance. Additional membranes delimit empty areas around the sporoblasts (Fig. 9), while the rest of the oocyst space is filled with a similar foamy material (Figs. 10 & 11). These membranes may still persist after the firm oocyst walls have been formed (Fig. 14). Next, the sporoblasts were covered by a fine bilayered envelope (Fig. 11), which transforms (Figs. 13 & 14) into the thick sporocyst wall.

The sporocyst wall consisted of a thick (about 63 nm) layer transversally striated in intervals of 12 nm; this layer grew wider along the sutural line. The striated layer is covered with a rather thin (15 nm) (Fig. 15) lucent layer subtending on opaque, coarsely granular coat.

Isolated sporocysts observed in the scanning electron microscope have a smooth wall with a slightly prominent longitudinal fold representing the suture, the line of dehiscence of the 2 sporocyst shell valves. No membranous structures are attached (Fig. 12).

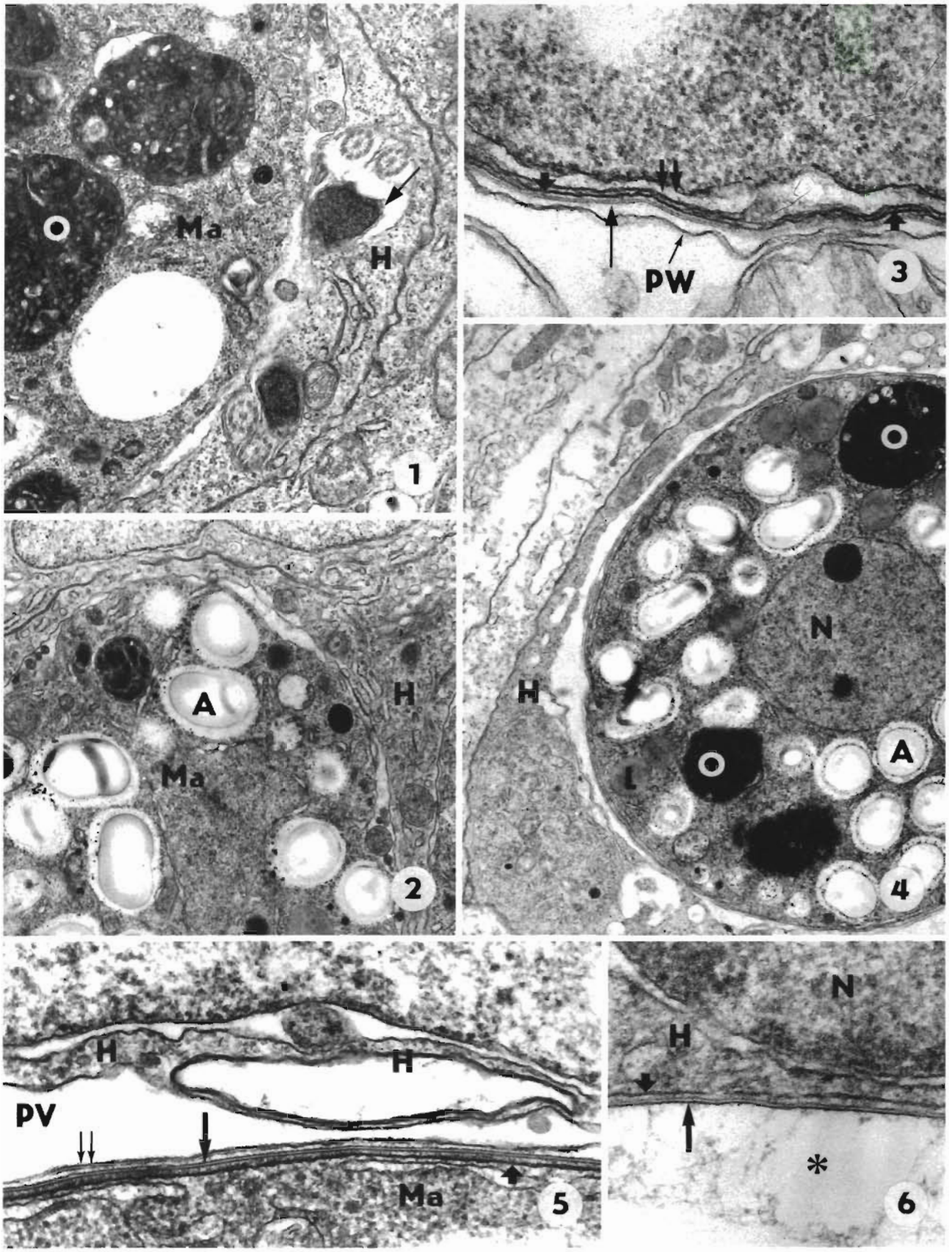
The sporoblast forms 2 sporozoites that split off from the remaining sporoblast material, which becomes a sporocyst residuum. Cross sections of early stages show 2 developing sporozoites at opposite sides of the sporoblast. In sections of more advanced stages, 2 sporozoites lying side by side are arranged head to tail. The sporozoites exhibit an apical complex with 2 polar rings, conoid, micronemes, rhoptries, dense bodies, refractile bodies, nucleus and amylopectin inclusions.

While the oocyst sporulated, the host cell, revealing at first almost normal structure (Fig. 16), gradually lost its integrity, and all its constituents deteriorated (Fig. 17). The cytoplasm condensed, turning eventually into an almost structureless opaque mass. Mature oocysts were then expelled together with the host-cell remains, which appear as the yellow bodies well known from light microscopy. There may be one to several oocysts within a yellow body (Fig. 18), which can originate from one or several host cells fused together.

In most infections, especially the heavier ones, one can observe in the cells of the host epithelium aggregations of amylopectin granules, usually together with

Figs. 1 to 6. Oocyst formation of *Goussia carpelli* infecting *Cyprinus carpio*. Fig. 1. Microgametes (arrow points to their nuclei) in the host-cell cytoplasm (H) next to a macrogamete. × 24 000. Fig. 2. A zygote enveloped by a simple cell membrane. × 13 600. Fig. 3. A zygote enveloped by a bilayered membrane (long arrow) underlain by a continuous opaque layer (short arrow) subtended by a cisterna of endoplasmic reticulum (double arrow). × 64 000. Fig. 4. A zygote in process of membrane formation, within a host cell forming a narrow band around the parasite. × 10 800. Fig. 5. Bilayered oocyst wall (long arrow) underlain by the opaque layer (short arrow) and covered by a slightly wavy double membrane (double arrow). The oocyst wall faces the host cell in form of a thin, vacuolated envelope. × 58 300. Fig. 6. Oocyst wall (long arrow) closely apposed to the parasitophorous vacuole membrane (short arrow). Asterisk designates the free space in the oocyst. × 45 600

Abbreviations used in figures. A: amylopectin granules; H: host cell; L: lipid inclusion; Ma: macrogamete; N: nucleus; O: osmiophil inclusion; PV: parasitophorous vacuole; PW: parasitophorous vacuole wall; S: sporoblast; W: oocyst wall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islands of deteriorated cytoplasm. These groups of granules may occur alone or associated with groups of oocysts, always intracellularly. Usually they lie within remains of a structure representing obviously a deteriorated zygote (Fig. 19); in some cases, nothing but a group of amylopectin granules is left.

DISCUSSION

Oocyst wall formation in *Goussia carpelli* seems to be relatively simple. A bilayered envelope is formed at the surface of the zygote. After a new sporont outer-shell membrane has been formed, evidently with participation of the subtending endoplasmic reticulum, the bilayered envelope is detached and forms the actual oocyst wall. This simplicity may be misleading, since actual formation of the bilayered structure could not be observed, and since there may be some variation in the structures – the uneven membrane connection, the bilayered structure, and 'filamentous material' similar to that of the parasitophorous vacuole observed by Paterson & Desser (1981) in *G. iroquoina* were only occasionally observed. None of the previous papers on oocyst wall formation in picsine coccidia could exactly describe their origin. That is probably why some investigators (e.g. Hawkins et al. 1983, Morrison & Poynton 1989) claimed that the outer layer of the oocyst wall originated from the host membrane of the parasitophorous vacuole. If it is true that the typically thin oocyst wall of piscine coccidia has a mixed origin from host and parasite cell membranes, such a unique feature should also be reflected in the taxonomy of these parasites. However, our observations on *G. carpelli* [and other species examined by the present authors (*G. gasterostei*; unpubl.)] could not prove such a construction of the oocyst wall. The proximity of the bilayered membrane and the parasitophorous membrane wall which was observed in some cases might perhaps even in *G. carpelli* produce the false impression of an oocyst wall of mixed origin.

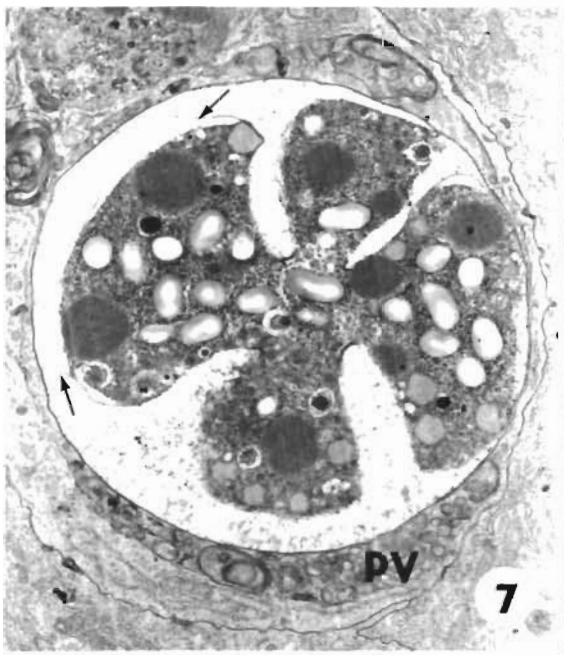
In other piscine coccidia, 1 or 2 envelopes were seen to enclose the zygotes; this is the case for *Goussia iroquoina* (Paterson & Desser 1984) and *Eimeria laureleus* (Desser & Li 1984), with 3 envelopes in *Goussia zarnowskii* (Jastrzebski & Komorowski 1990). In *G. cichlidarum*, there are 2 membranes covering the zygote (Paperna & Landsberg 1985, Paperna et al. 1986), which precede the appearance of a soft but thick oocyst wall.

The dark bodies in the zygotes of *Goussia carpelli* are reminiscent of inclusions which arise in *G. iroquoina* from the confluence of dense, membrane-bound vesicles (Paterson & Desser 1981). In this species, the oocyst wall is also underlain by endoplasmic reticulum cisternae, probably giving rise to sporont membrane as in *G. carpelli* and *G. zarnowskii* (Jastrzebski & Komorowski 1990).

Thus the pattern of zygote development in piscine coccidia – at least in several species – has similar features. One of these features is also the membranous cover of the sporoblasts, observed in sectioned *Goussia carpelli* oocysts in the process of sporulation. Such membranous envelopes were observed in other species (*G. caseosa*; Lom & Dyková 1982b). In *G. carpelli* they are obviously impermanent, since they were not observed in sectioned sporulated oocysts. Also, the scanning electron microscope did not reveal any of them on the surface of the sporocysts, and no membranous girdles were associated with the sporocyst suture. However, the sporocyst of *G. degustii* does bear such a membranous girdle along the line of dehiscence of its shell valves. Similar sutural veils were found to persist on *G. sinensis* sporocysts. The possible significance of the membranous covers in *G. carpelli*, if any, has to be elucidated.

Our observations unequivocally prove that the 'yellow bodies' harbouring oocysts shed from the epithelium are nothing but remains of deteriorated host cells, in which a process of lipofuscin formation took place as a result of deterioration of host-cell cytoplasm. Previous assumptions that they were cavities filled by tissue fluids or blood (Schäperclaus 1954) or degenerated host cell and coagulated tissue fluid (Molnár 1984) were not absolutely correct. Our interpretation is closer to that of Kent & Hedrick (1985), who saw the origin of yellow bodies in degenerated cell membranes and also suggested the presence of lipofuscin.

Figs. 7 to 14. *Goussia carpelli* infecting *Cyprinus carpio*. Fig. 7. Simultaneous formation of 4 sporoblasts by deep surface invaginations; they are enveloped by a thin pellicle (arrow). The parasitophorous vacuole is replete with a mass of moderately opaque vesicles. ×7300. Fig. 8. In some cases, the parasitophorous vacuole is filled with an amorphous substance; the young oocyst is covered by a simple membrane. ×37 800. Fig. 9. A membranous pellicle of the sporoblasts envelops a foamy substance (arrow); asterisk marks the empty space surrounded by another thin membrane. ×6200. Fig. 10. Enlarged central part of Fig. 9; membranous septa around the cell, membrane-bound sporoblasts and the foamy substance (asterisk); ×17 600. Fig. 11. More advanced sporoblasts with the initial membranous envelopes (long arrows) still present; short arrows point to the spore shell in formation. ×18 000. Fig. 12. Scanning electron microscope micrograph of a mature sporocyst. ×8700. Fig. 13. Sporoblasts with a still thin, bilayered shell covered with flocculent dense material and with cell membranes (arrows) still in close proximity. ×50 000. Fig. 14. Advanced formation of sporocyst shells, with the initial sporoblast envelopes still visible (arrow) between the 2 shells. ×95 000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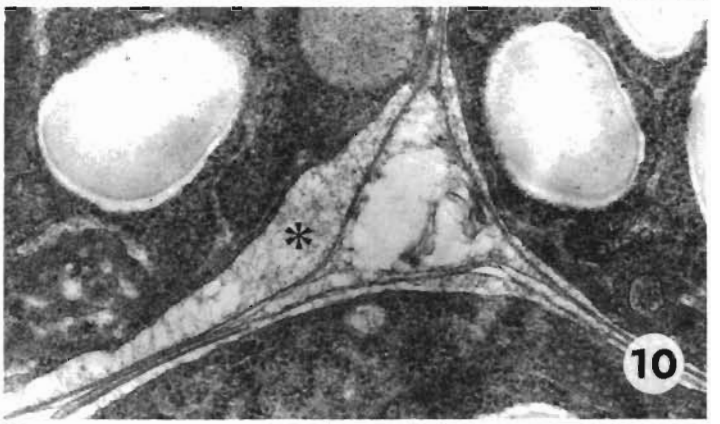
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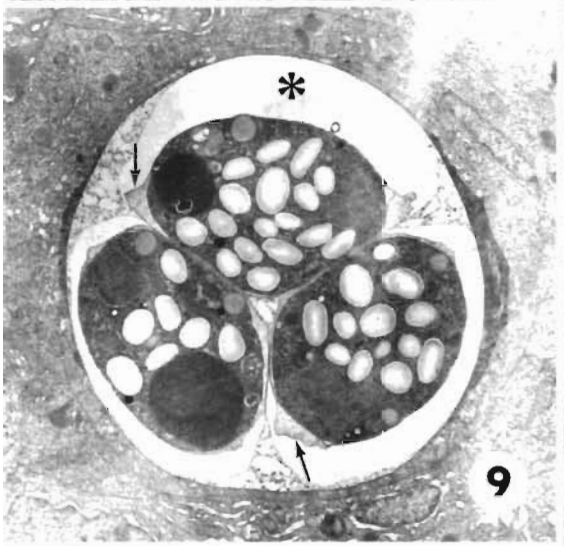
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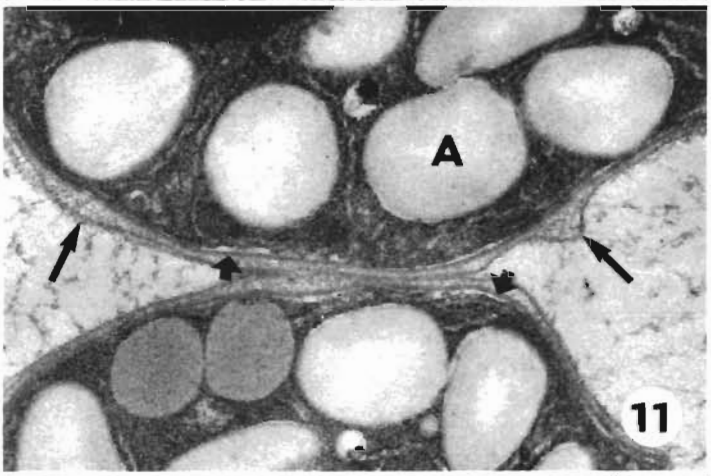
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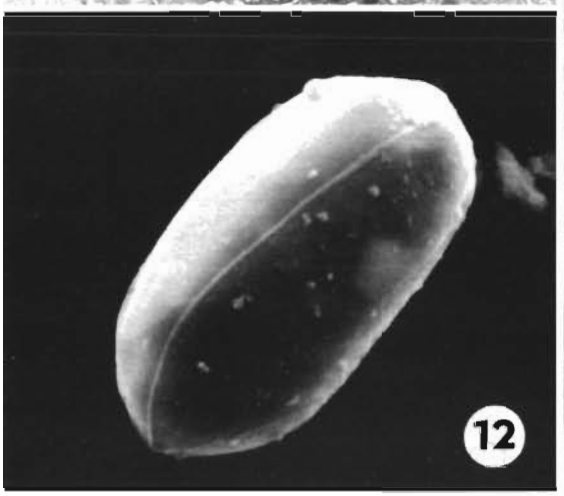
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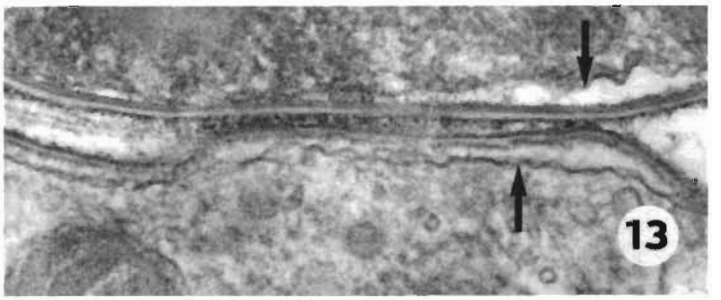
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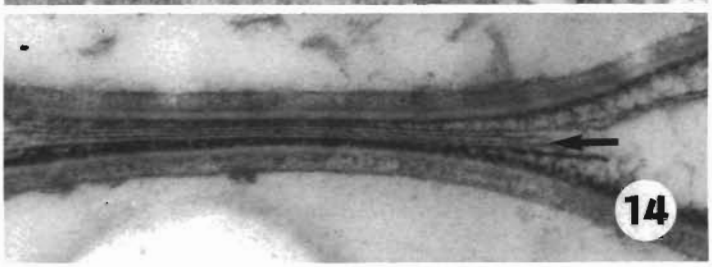
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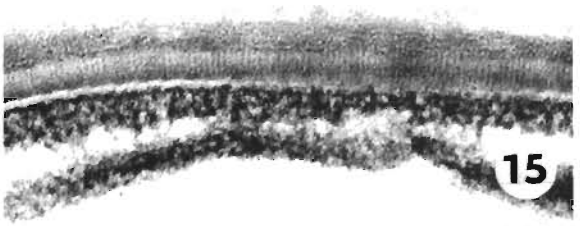
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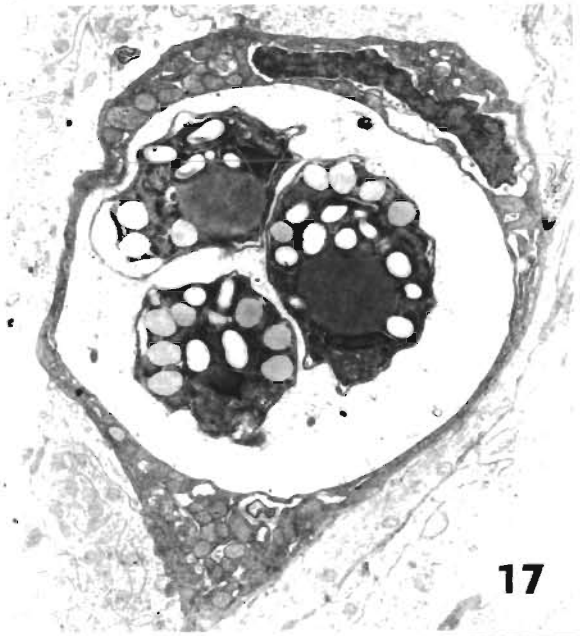
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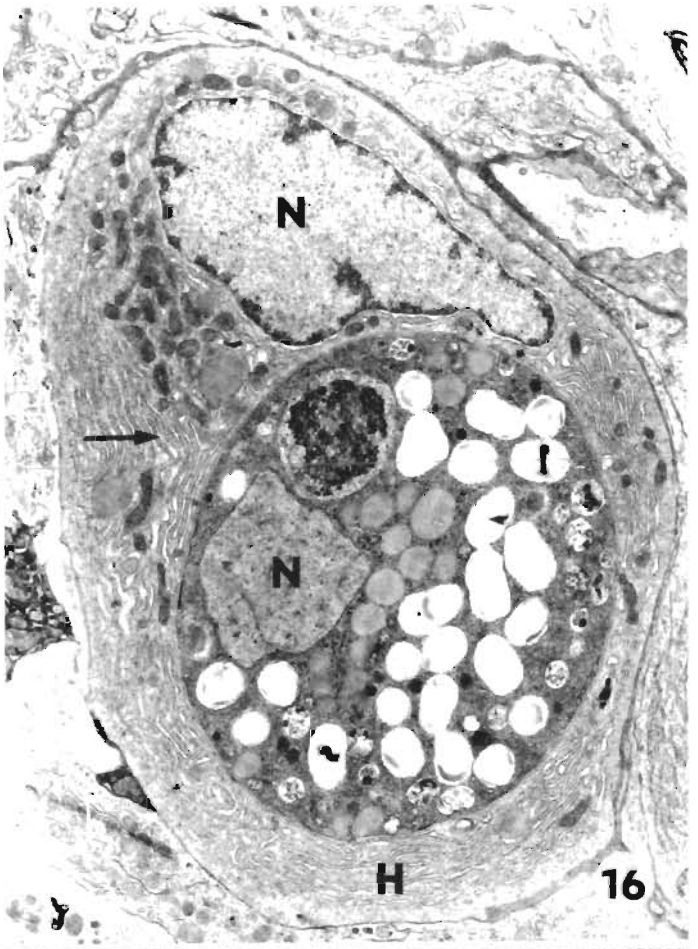
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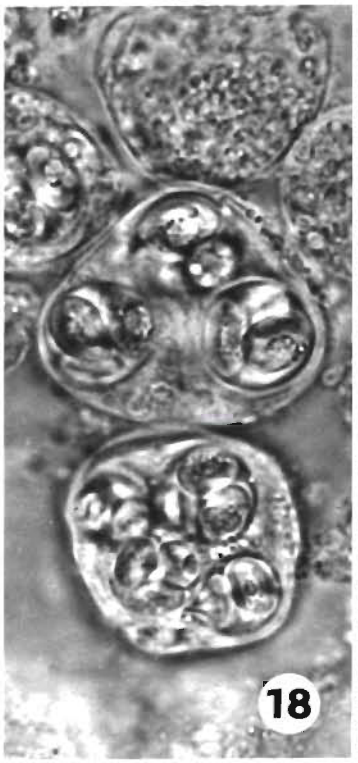
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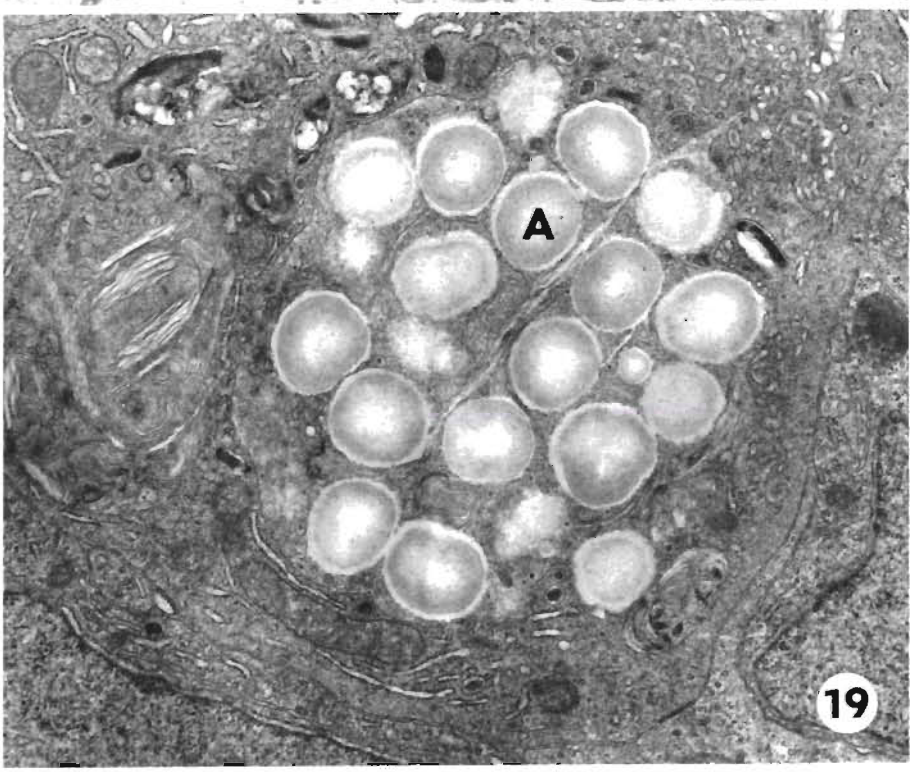
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Figs. 15 to 19. *Goussia carpelli* infecting *Cyprinus carpio*. Fig. 15. Wall of mature sporocyst. Inner layer exhibits periodic transversal striation. ×63 400. Figs. 16 to 18. Formation of yellow bodies. Fig. 16. Sporulating oocyst incorporated in a host cell with extended endoplasmic reticulum cisternae (arrow). ×4850. Fig. 17. Oocyst with sporoblasts in a deteriorated host cell. ×7600. Fig. 18. Several oocysts in deteriorated host cells expelled together, forming large aggregates (yellow bodies). Light micrograph, fresh preparation, ×1300. Fig. 19. Abortive development of oocysts: amylopectin granules and remains of a deteriorated zygote in a host cell. ×6700

Our observations indicate that large yellow bodies containing more than 1 or 2 oocysts can arise by fusion of 2 or more apposed parasitized cells. Yellow bodies are a common phenomenon in many epithelium-infecting *Goussia* and *Eimeria* species with smaller oocysts (e.g. *Goussia luciae, Eimeria ivanae, E. lairdi*). Species with larger and/or fragile oocysts, such as *Goussia subepithelialis* from common carp, shed their oocysts without yellow bodies, or only released sporocysts are discharged.

Findings on deteriorated zygotes of *Goussia carpelli*, in which further development was aborted, put in serious doubt an assumption of Baska & Molnár (1989) on the expulsion of a part of the zygote material from sporulating oocysts. This material – appearing as bodies, distinguished only by or consisting only of amylopectin granules – can be identified as the remains of aborted oocysts. It is by no means a phenomenon associated with normal zygote development. Abortive development is a phenomenon well known in other fish protozoan parasites, such as myxosporidians (Lom et al. 1983).

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LITERATURE CITED

Baska, F., Molnár, K. (1989). Ultrastrucutral observations on different developmental stages of *Goussia sinensis* (Chen, 1955), a parasite of the silver carp (*Hypophthalmichthys molibix* Valenciennes, 1844). Acta vet. hung. 37: 81–87

Desser, S. S., Li, L. (1984). Ultrastructural observations on the sexual stages and oocyst formation in *Eimeria laureleus* (Protozoa, Coccidia) of perch, *Perca flavescens*, from Lake Sasajewun, Ontario. Z. ParasitKde 70: 153–164

Hawkins, W. S., Fournie, J. W., Overstreet, R. M. (1983). Organization of sporulated oocysts of *Eimeria funduli* in the gulf killifish, *Fundulus grandis*. J. Parasit. 69: 496–503

Ivasik, V., Kulakovskaya, O. P. (1959). On carp coccidiosis. Zool. Zh. Ukr. 38: 1746–1750 (in Russian)

Jastrzebski, M., Komorowski, Z. (1990). Light and electron microscopic studies in *Goussia zarnowskii* (Jastrzebski, 1982): an intestinal coccidium parasitizing the three-spined stickleback. J. Fish Dis. 13: 1–24

Kent, M. L., Hedrick, R. P. (1985). The biology and associated pathology of *Goussia carpelli* (Léger and Stankovitch) in goldfish *Carassius auratus* (Linnaeus). Fish Path. 20: 485–494

Kocylowski, B., Zelazny, J., Antychowicz, J. (1976). Incidence of carp coccidiosis and its control. Bull. vet. Inst. Pulawy 20: 12–17

Léger, L., Stankovich, S. (1921). L'entérite coccidienne des alevins de carpe. Trav. Lab. Piscicult. Univ. Grenoble: 339–342

Lom, J., Dyková, I. (1982a). *Goussia carpelli* – structure of sporocysts and 'yellow bodies'. J. Protozool. 29: 508 (Abstract 139)

Lom, J., Dyková, I. (1982b). Some marine fish coccidia of the genera *Eimeria* Schneider, *Epieimeria* Dyková & Lom and *Goussia* Labbé. J. Fish. Dis. 5: 309–321

Lom, J., Dyková, I., Lhotáková S. (1983). *Kudoa lunata* n. sp. (Myxozoa, Myxosporea) and notes on the nature of muscular 'cysts' of the genus *Kudoa* Arch. Protistenk. 127: 387–397

Molnár, K. (1984). Some peculiarities of oocyst rejection of fish Coccidia. Symposia Biol. Hung. 23: 87–97

Morrison, C. M., Poynton, S. L. (1989). A new species of *Goussia* (Apicomplexa, Coccidia) in the kidney tubules of cod, *Gadus morhua* L. J. Fish Dis. 12: 533–560

Paperna, I., Landsberg, J. H. (1985). Ultrastructure of oogony and sporogony in *Goussia cichlidarum* Landsberg and Paperna, 1985, a coccidian parasite in the swimbladder of cichlid fish. Protistologica 21: 349–359

Paperna, I., Landsberg, J. H., Feinstein, N. (1986). Ultrastructure of the macrogamont of *Goussia cichlidarum* Landsberg and Paperna, 1985, a coccidian parasite in the swimbladder of cichlid fish. Annls Parasit. hum. comp. 61: 511–520

Paterson, W. B., Desser, S. S. (1981). Ultrastructure of macrogametogenesis, macrogametes and young oocysts of *Eimeria iroquoina* Molnar and Fernando, 1974 in experimentally infected fathead minnows (*Pimephales promelas*, Cyprinidae). J. Parasit. 67: 496–504

Paterson, W. B., Desser, S. S. (1984). Ultrastructural observations on fertilization and sporulation in *Goussia iroquoina* (Molnar and Fernando, 1974) in experimentally infected fathead minnows (*Pimephales promelas*, Cyprinidae). J. Parasit. 70: 703–711

Schäperclaus, W (1954). Fischkrankheiten, 3rd edn. Akademie Verlag, Berlin

Steinhagen, D., Körting, W., van Muiswinkel, W. B. (1989). Morphology and biology of *Goussia carpelli* (Protozoa: Apicomplexa) from the intestine of experimentally infected common carp *Cyprinus carpio*. Dis. aquat. Org. 6: 93–98

Zaika, V E., Kheisin, E. M. (1959). Carp coccidiosis on the Valdai fish farm. Bull. State Scient. Res. Inst. Lake and River Fisheries, 49: Parasites of freshwater fish and the biological basis of their control, Leningrad (Translated from Russian by Israel Prog. Scient. Transl., 2nd edn., 1965: p. 227–230)

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