

Hepatic microsporidiosis of juvenile grey mullet, *Chelon labrosus* (Risso), due to *Microgemma hepaticus* gen.nov. sp.nov.

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Abstract. *Microgemma hepaticus* gen.nov. sp.nov. is described from the liver of juvenile grey mullet, *Chelon labrosus* (Risso). Development occurs within xenomas (diameter 500 µm) which have microvillar surfaces, encircling bands of mitochondria and a reticulate hypertrophic nucleus. Vegetative developmental stages, meronts, are plasmodial and divided by plasmotomy. These stages are enclosed by host membranes. Sporogonic stages are free in the cytoplasm and divide by multiple exogenous budding. Uninucleate spores (2.4 µm × 4.2 µm) possess 7–9 coils of the polar filament and a lamellar polaroplast. Xenomas are associated with liver connective tissue, and cause necrosis of adjacent liver cells in certain circumstances. Host response to infection involves leucocyte infiltration and granuloma formation, with spores being destroyed by repeated macrophage phagocytosis and necrosis and resulting in gradual resolution of the lesion. Although juveniles apparently tolerate large parasite burdens there is some evidence of a contribution by the parasite to stress-related mortality. The transmission of the disease and its potentially high pathogenicity to larval fishes is discussed.

Introduction

Microsporidiosis of fishes has been shown to be of major importance both in the wild and under fish farming conditions. The potential danger to fish stocks in the wild has long been recognized (Fischthal 1944; Sandholzer, Nostrand & Young 1945; Haley 1954; Sindermann 1970; Hauck 1984), the decline of entire fisheries having been attributed to the disease. Reports of losses in fish farms due to microsporidiosis have been made by Summerfelt & Warner (1970), McVicar (1975) and MacKenzie, McVicar & Waddell (1976). The disease is likely to be of concern in fish farming owing to high density of stock. However, there is the possibility of control and treatment in these manageable waters provided fundamental information is available on the parasite concerned.

Sprague (1977a) recorded 76 species of microsporidia parasitic in fish hosts. However, relatively few of these were well known, and only a small number of ultrastructural studies of development had been made at that time. Subsequently, electron microscopic studies of type species have been reported (Canning & Nicholas 1980; Canning, Lom & Nicholas 1982; Canning & Hazard 1982) and a few earlier species have been redescribed at this level (Loubès, Maurand & Ormières 1979; Morrison & Sprague 1981b, 1983). New genera have been described at EM level by Matthews & Matthews (1980) and Morrison & Sprague (1981a). Despite this activity, most species remain poorly known, and in particular many of the species described by early workers and originally listed by Kudo

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(1924) await rediscovery and characterization in the light of modern developmental knowledge.

This present report deals with microsporidian disease caused in juvenile grey mullet by *Microgemma hepaticus* gen.nov. sp.nov., and contains an ultrastructural description of developmental features of the parasite and its interactions with host cells and organs.

Materials and methods

O-group *Chelon labrosus* (Risso) were collected by seine-netting at St John's lake, the Tamar Estuary, Cornwall, England during October to March, 1981 and 1982. Shoals of juvenile mullet were trapped in a tidal pool at low tide, and the seine net was passed around the pool. Fish were transferred live to the laboratory, where specimens were selected and killed by overdoses of ethyl-m-aminobenzoate (Sigma). On dissection, foci of infection were visible as white spots in the liver (Fig. 1a). Measurements of spores were made on fresh material. Pieces of infected liver were macerated in Young's teleost saline (Young 1933) and examined using phase-contrast microscopy. Filament extrusion was induced by irrigation of slides with iodine water or 5% hydrogen peroxide. All measurements were based on 20 observations. Histological studies were made on infected livers fixed in Bouin's fluid, embedded in wax, sectioned at 5 µm and stained with Mallory's triple stain. Material for transmission electron microscopy was dissected out of the liver, fixed in 3% glutaraldehyde in 0.1 m cacodylate buffer pH 7.2 at 4°C, post-fixed in 1% osmium tetroxide, and rinsed and dehydrated through an ethanol series. The tissue was infiltrated using concentrations of 25%, 50%, 75% and 100% Spurr's resin in propylene oxide at 12 h per step. Sections were cut on LKB Ultratome III and Reichert Ultracut ultramicrotomes, mounted on 200 mesh unsupported copper grids, stained with aqueous uranyl acetate and lead citrate (Reynolds 1963) and examined using Philips EM200 and EM300 electron microscopes.

Results

Microgemma hepaticus is found in the liver of *Chelon labrosus*, xenomas being associated with the walls of blood vessels and bile ducts, the host component of the capsule of the encysted metacercariae of *Bucephalus haimeanus* and the enclosing membrane of the liver itself (Fig. 1a-c). Typical microsporidian spores were present in squash preparations of infected liver. Approximately 38% of O-group fishes were infected with the parasite, and no infected fish were encountered in the occasional 1 and 2 group fishes captured during netting of the juveniles (15 specimens).

Microgemma hepaticus gen.nov. sp.nov.

Merogony. Merogonic stages were identified as multinucleate plasmodia of variable size and shape, ranging from spherical 5 µm in diameter (Fig. 2a) to elongated, measuring 15 µm × 5 µm (Fig. 2b). Nuclei were 1.5 µm in diameter and were occasionally paired, although not closely apposed as in the diplokaryotic state. Up to eight nuclei were observed in sections of large meronts. The cytoplasm was fibrillar in appearance with

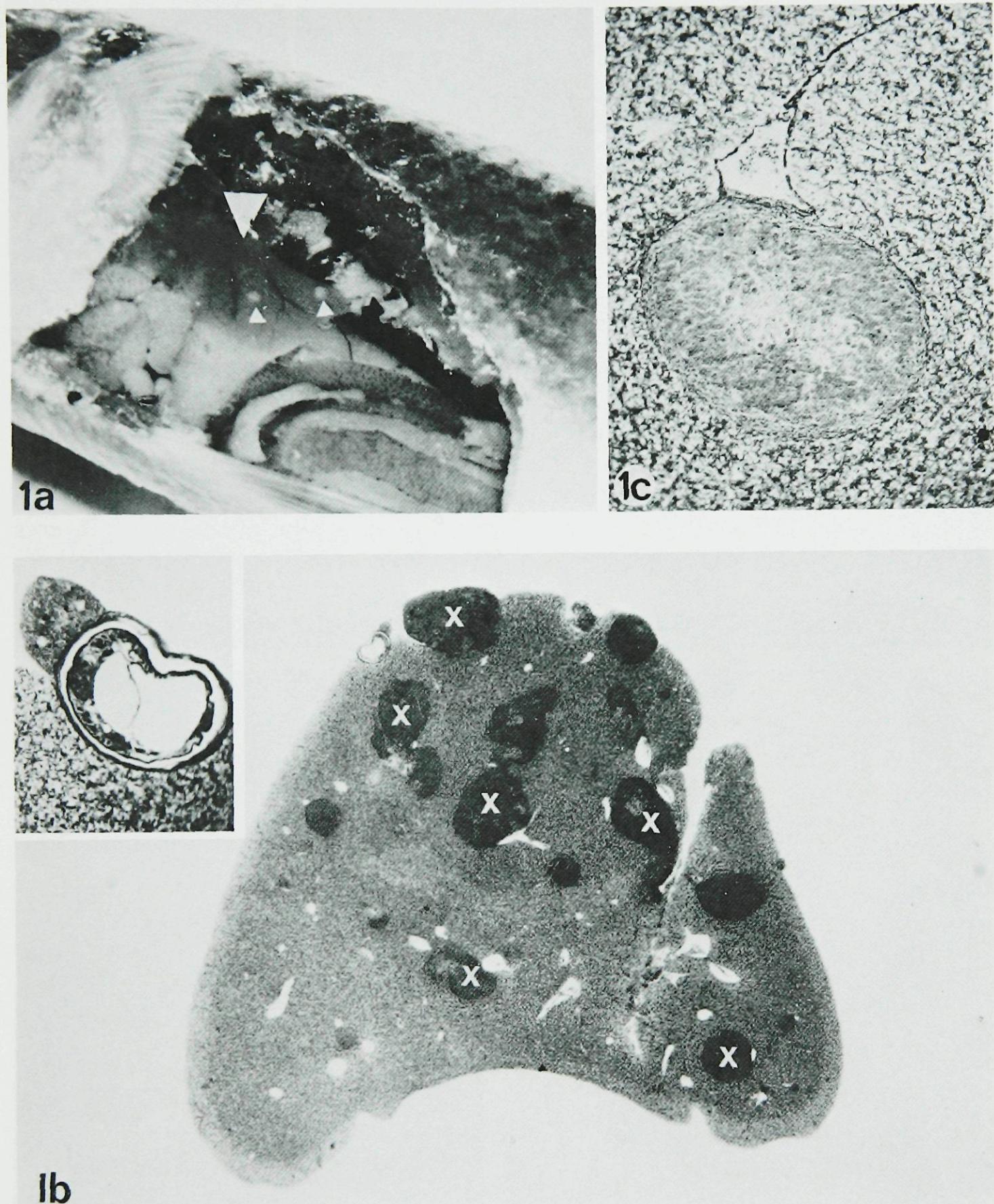


Figure 1. Superficial appearance and distribution of *M. hepaticus* infection. Fig. 1a. Left lobe of smelt liver (large arrow): light infection with *M. hepaticus*. Arrows—xenomas. Fig. 1b. Transverse section, whole heavily infected liver ($\times 8$). X—granulomas of *M. hepaticus* associated with various liver vessels. Inset: granuloma associated with host capsule of metacercaria of *Bucephalus haimeanus* ($\times 400$). Fig. 1c. Granuloma associated with hepatic portal vein branch ($\times 420$).

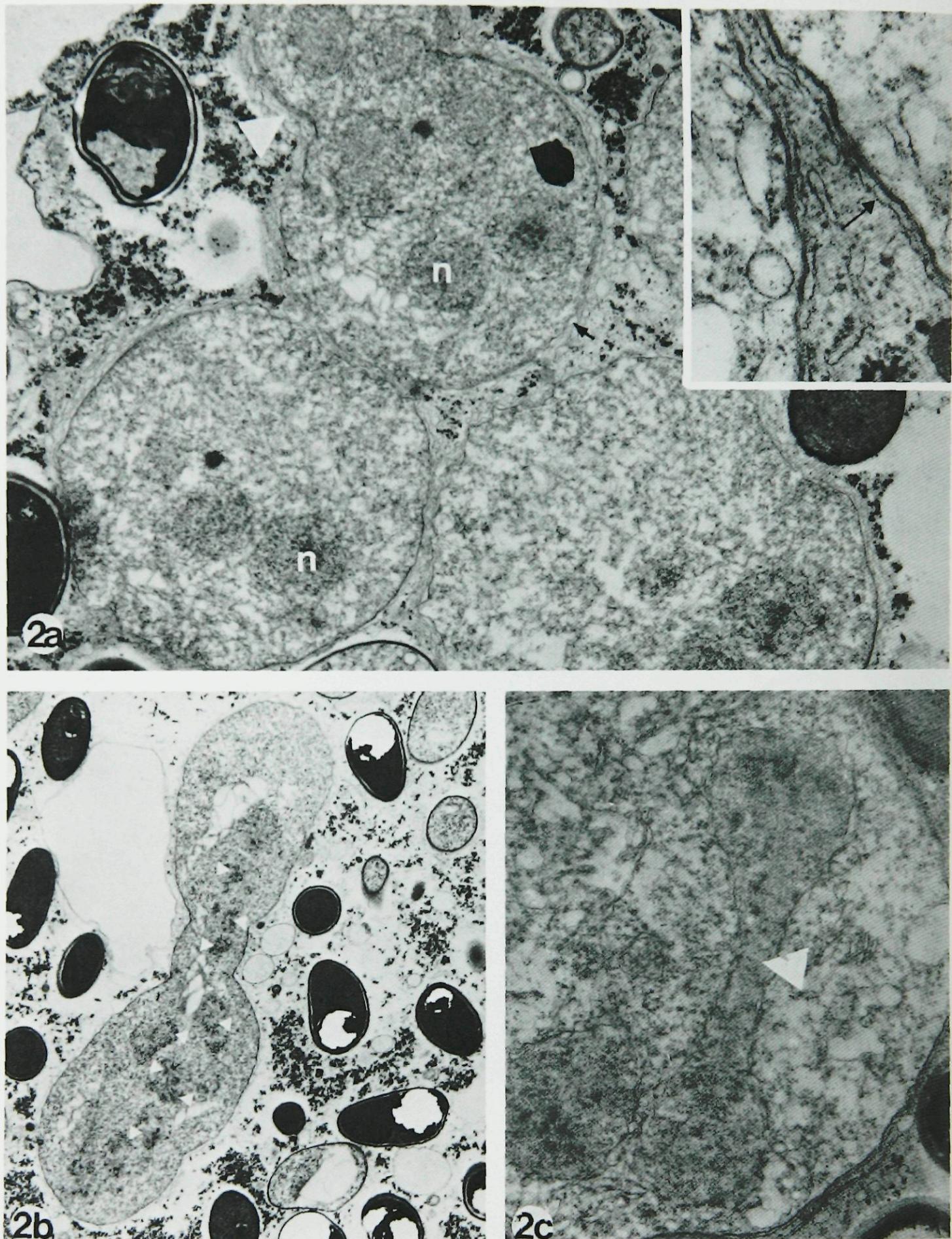


Figure 2. Merogony of *M. hepaticus*. Fig. 2a. Small, spherical meronts ($\times 9400$): n—nuclei; arrowhead—budding of small meront; small arrow—meront enclosing membrane. Inset: meront enclosing membrane (arrow) ($\times 38000$). Fig. 2b. Large, elongate meront in division ($\times 5000$): arrowheads—dividing nuclei with dense chromatin. Fig. 2c. Dumbbell-shaped telophase nucleus ($\times 15200$): arrowhead—spindle microtubules crossing isthmus.

rough endoplasmic reticulum and scattered groups of ribosomes. Nuclear division was synchronous within a meront. Dumbbell-shaped telophase nuclei were observed (Fig. 2c). Cell division was by plasmotomy and occurred in small and large meronts (Fig. 2a, b).

A notable feature of merogonic stages was their complete enclosure by host membrane. There appeared to be a single membrane around each parasite (Fig. 2a inset), and it resembled the membrane of the xenoma endoplasmic reticulum, being frequently associated with ribosomes. The meront-enclosing membrane was often surrounded by cisternae and vesicles of endoplasmic reticulum.

Sporogony. The onset of sporogony was recognized by the presence of electron-dense patches on the outer surface of the plasmalemma and by the absence of surrounding membrane systems (Fig. 3). Sporonts were more or less rounded cells measuring approximately $6\mu\text{m}$ in diameter and containing 6–8 nuclei. Sporont cytoplasm was similar to that of meronts, but with greater development of endoplasmic reticulum.

Sporogony proceeded by budding, the first stage being the production of single uninucleate buds (Fig. 4a). The process accelerated with the formation of multiple buds, resulting in a variety of divisional stages. These included several separate buds on the same sporont (Fig. 4b), multiple buds at a single site (Fig. 4c) and multiple buds on a cellular protrusion (Fig. 4d). This process eventually resulted in the complete division of sporogonial plasmodia into various combinations of buds, including pairs (Fig. 5a),

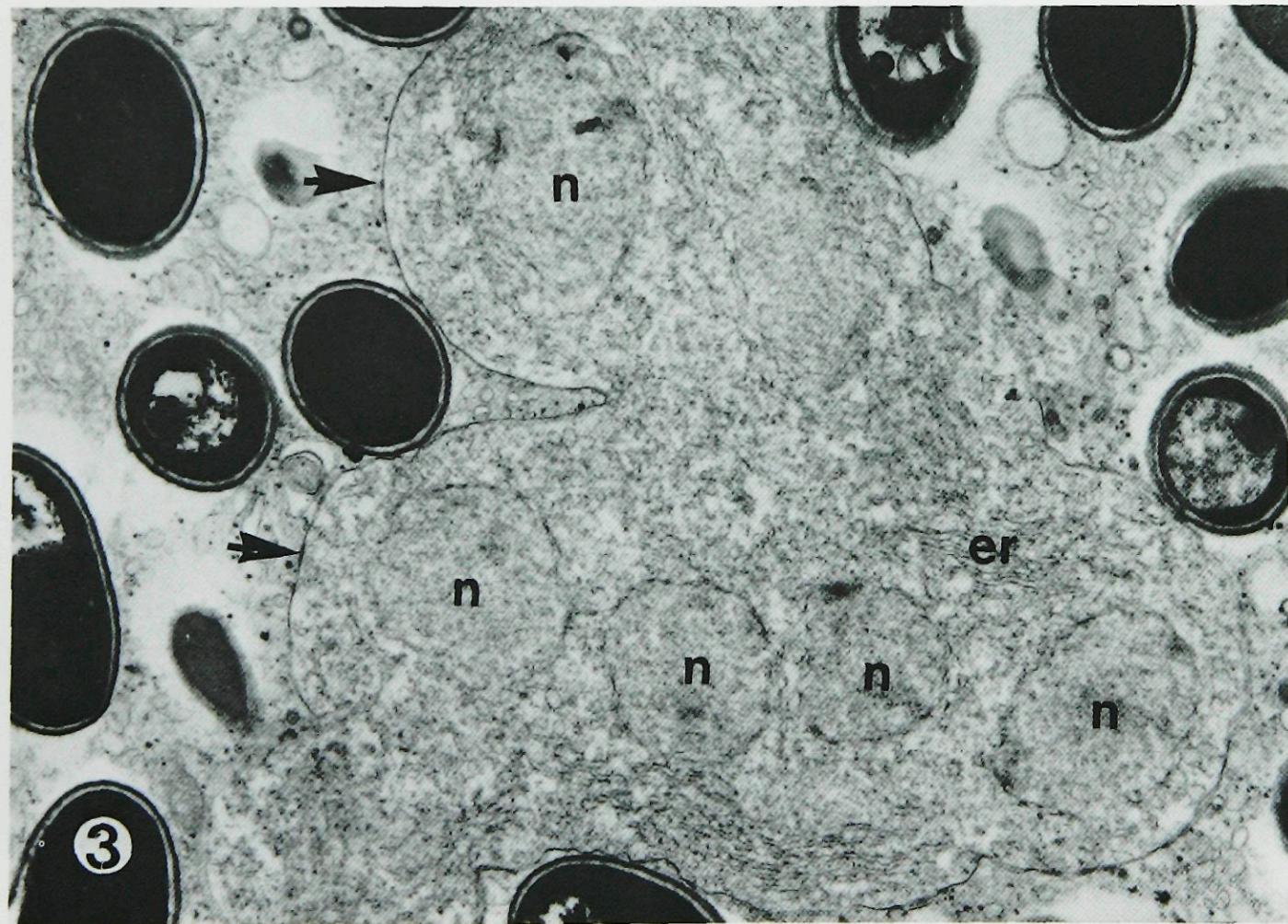


Figure 3. Early sporont of *M. hepaticus* ($\times 10000$): n—nuclei; er—endoplasmic reticulum; arrowed extramembranous thickenings and absence of host-derived enclosing membrane.

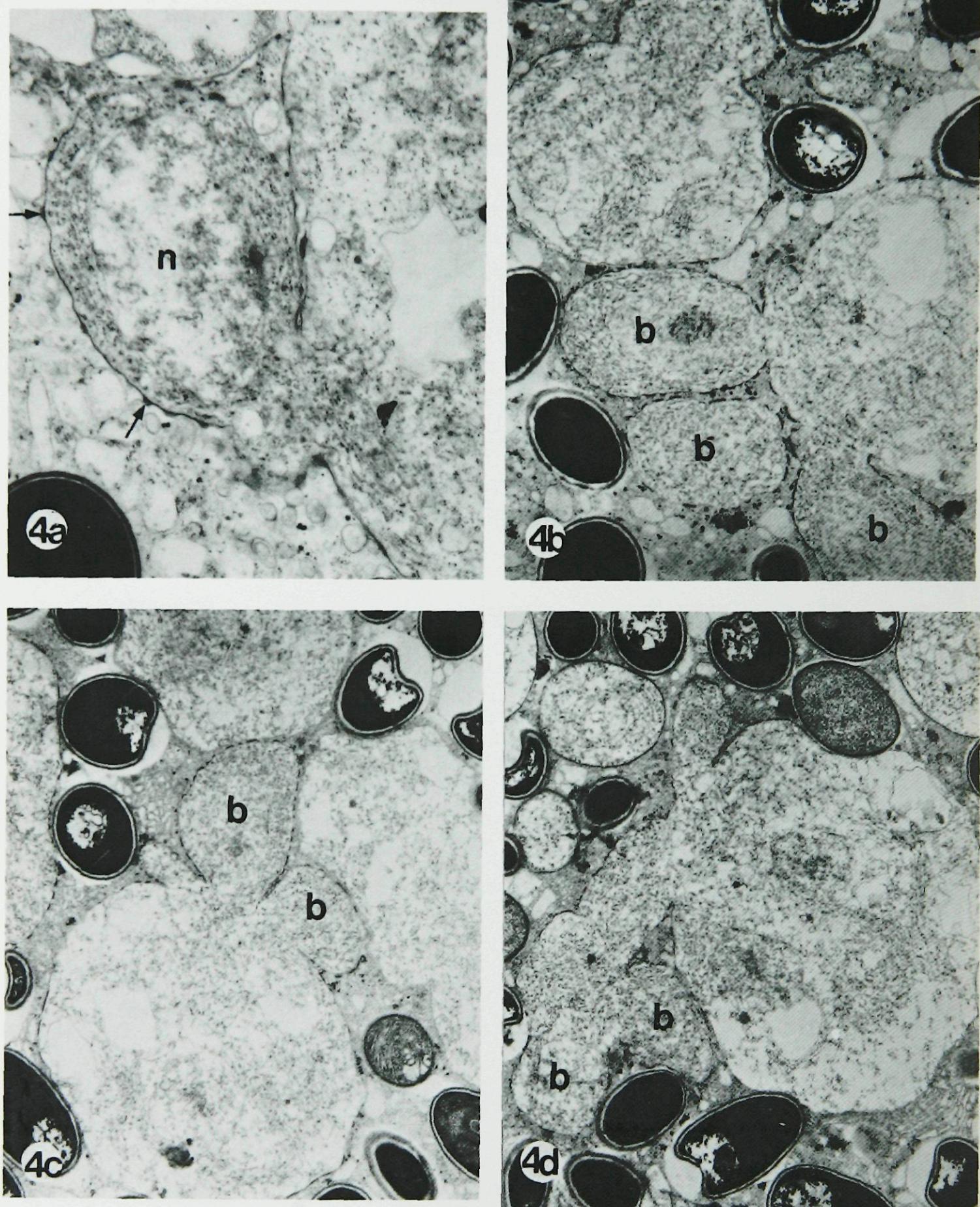


Fig. 4. Division stages of *M. hepaticus* sporonts. Fig. 4a. Single uninucleate bud ($\times 32\,400$): n—nucleus; arrows—extramembranous thickenings. Fig. 4b. Separate buds on a single sporont ($\times 17\,500$): b—buds. Fig. 4c. Multiple buds at a single site ($\times 8\,900$): b—buds. Fig. 4d. Buds at terminus of a cytoplasmic protrusion ($\times 7\,000$): b—buds.

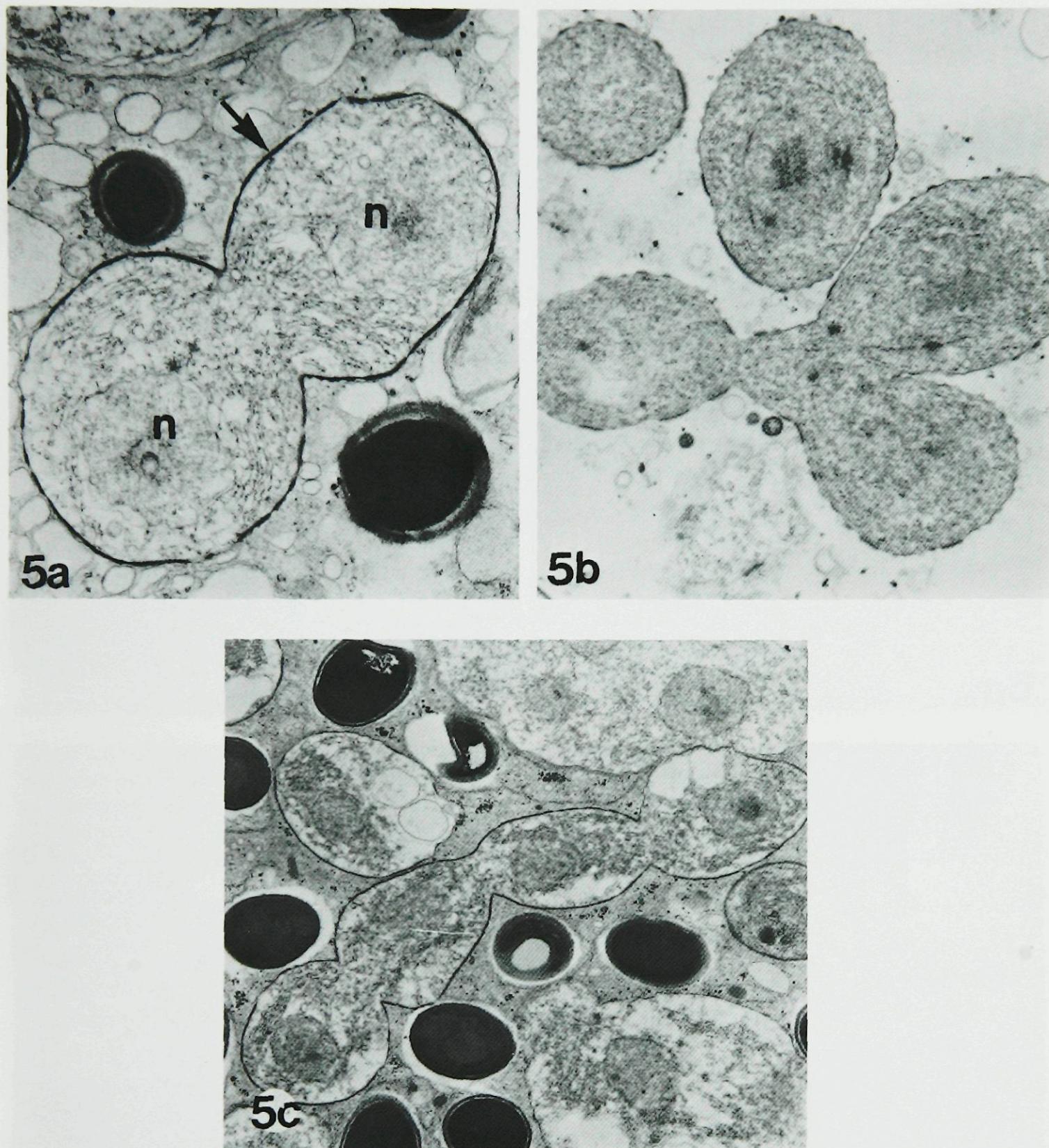


Figure 5. Final division stages of *M. hepaticus* sporonts. Fig. 5a. Paired sporoblasts ($\times 18\,100$): n—nucleus; arrow—almost complete extramembranous thickening. Fig. 5b. Rosette of sporoblasts ($\times 18\,000$). Fig. 5c. Chain of sporoblasts ($\times 7700$).

rosettes (Fig. 5b) and chains (Fig. 5c). Sporogonic division was concluded by the separation of these bud groups into individual uninucleate sporoblasts.

Sporogenesis began at the time of separation of the sporoblasts. The polar filament was formed at the periphery of a reticular Golgi apparatus (Fig. 6a, b). The polaroplast developed from expanded sacs positioned anteriorly around the polar filament; these

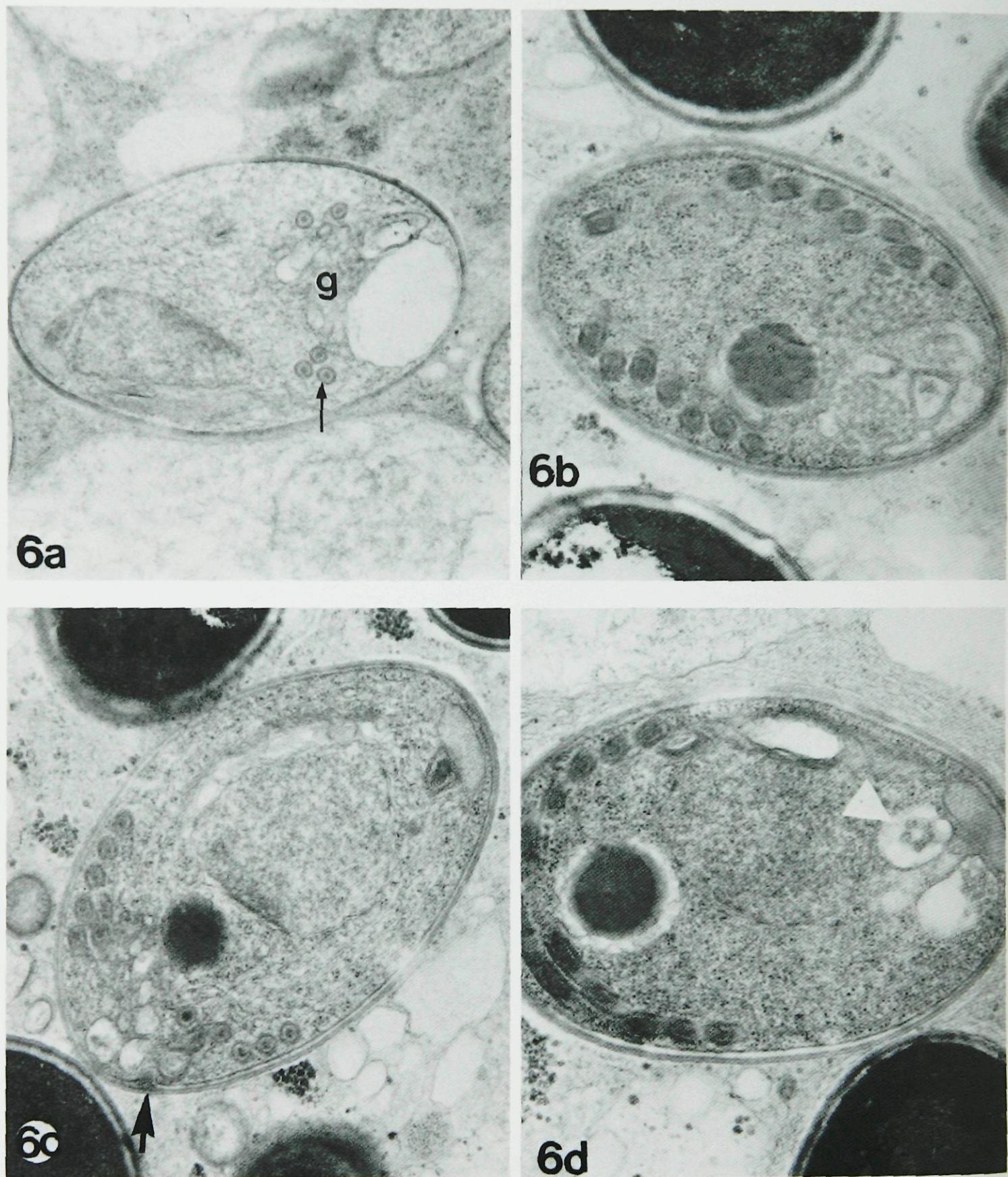


Figure 6. Stages in sporogenesis of *M. hepaticus*. Fig. 6a. Sporoblast showing polar filament assembly at periphery of Golgi reticulum ($\times 18\,100$): arrowed—polar filament; g—Golgi reticulum. Fig. 6b. Later stage of filament assembly ($\times 18\,100$). Fig. 6c. Sporoblast with tube connecting synthetic system with endospore (arrowed) ($\times 18\,100$). Fig. 6d. Early stage of polaroplast formation ($\times 20\,000$): arrowhead—expanded sacs of polaroplast.



Figure 7. Stages of polaroplast formation, and spore morphology of *M. hepaticus* ($\times 14\,000$): A—sporoblast with mainly saccate polaroplast (arrowhead); B—sporoblast with lamellar polaroplast anteriorly (solid arrowhead) and saccate posteriorly (open arrowhead); C—spore; pv—posterior vacuole; lp—lamellar polaroplast; n—nucleus.

increased in number and formed flattened cisternae as development progressed (Figs 6d, 7a, b). The exospore was derived from the extramembranous thickenings formed during sporogony and the endospore was thickened throughout the sporogenesis period, in connection with a tube-like structure linking it to the posterior part of the sporoblast cytoplasm (Fig. 6c). The cytoplasm became progressively denser, as a consequence of increasing numbers of ribosomes.

Spore. The spore was pyriform in shape, measuring $4.2\,\mu\text{m} \times 2.4\,\mu\text{m}$ in greatest breadth. The extruded filament measured $57\,\mu\text{m}$. The posterior third of the spore was occupied by the posterior vacuole. At the ultrastructural level (Fig. 7c), the polar filament was uniform in diameter with six to ten coils present in the posterior part of the spore. The polaroplast was entirely lamellar in nature, the saccate form of sporoblasts being lost. The membranes were arranged in groups of three with thin outer membranes and a thickened central membrane. The single spore nucleus, when visible, occupied the central region of the spore and was spherical (diameter $1.2\,\mu\text{m}$) or compressed into a concave shape between the posterior vacuole and polaroplast. The spore cytoplasm was of high electron density and contained large numbers of ribosomes.

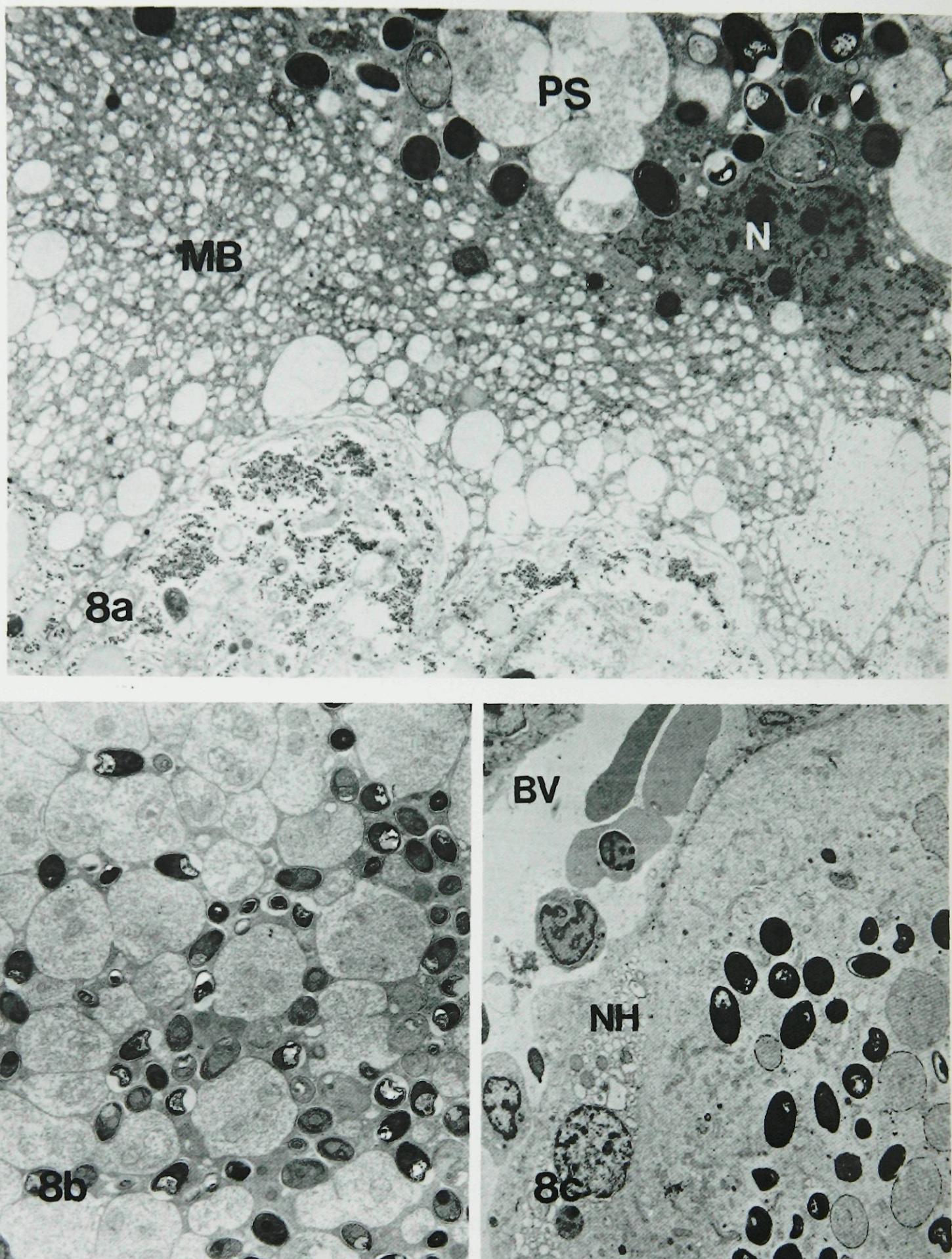


Figure 8. Features of the xenoma of *M. hepaticus*. Fig. 8a. Peripheral region of *M. hepaticus* xenoma ($\times 3300$): MB—mitochondria band; N—hypertrophic nucleus; PS—parasite stages. Fig. 8b. Mixed merogonic and sporogonic stages in centre of xenoma ($\times 2400$). Fig. 8c. Xenoma adjacent to blood vessel ($\times 5600$): BV—blood vessel; NH—necrotic hepatocyte between xenoma and vessel wall.

Effects on Chelon labrosus

Cell response. Infected cells underwent hypertrophy and formed a highly organized host/parasite complex, or xenoma, measuring up to 500 µm in diameter. Ultrastructural studies revealed that superficially the xenoma cytoplasm was thrown into microvillar processes which formed a region of variable thickness, ranging from 0.7 µm to 2.7 µm (Fig. 8a). Immediately beneath this layer was a band 20–25 µm thick consisting mainly of closely packed mitochondria. The host cell nucleus was hypertrophic and reticulate and was positioned at the inner part of the mitochondrial band, encircling most of the central part of the xenoma. The central part was occupied by spores and developmental stages of *M. hepaticus* along with host cell organelles, there being no particular orientation of stages within this zone (Fig. 8b).

Host response to infection

No host response was observed around active xenomas. However, degenerate xenomas resulted in a typical inflammatory tissue response leading to granuloma formation (Fig. 1b, c). In large foci of infection, necrosis was occasionally observed in adjacent liver tissue, particularly where bile ducts were involved in the infection. At the ultrastructural level, necrotic liver cells were only observed where they were pressed between the xenoma and a liver vessel (Fig. 8c). Spores were destroyed by repeated phagocytosis and cell necrosis, eventually resulting in the reduction of the xenoma to a region of phagocytic cells each containing a small number of spores in electron dense phagocytic vacuoles.

Discussion

The naming of a new genus, *Microgemma*, for this hepatic microsporidian from *Chelon labrosus* is clearly necessary on the basis of comparative morphology of features usually employed in the separation of genera (Vavra, Canning, Barker & Desportes 1981) and in comparison with genera described by Sprague (1977b), Weiser (1977), Loubes *et al.* (1979), Canning & Nicholas (1980), Matthews & Matthews (1980), Morrison & Sprague (1981a), Canning & Hazard (1982) and Canning, Lom & Nicholas (1982). Diagnostic features of *Microgemma* include: isolated nuclei at all stages of development; asexual developmental cycle; multinucleate plasmodial merogonic stages, which divide by plasmotomy and are enclosed within host cell membranes; sporogonic stages in direct contact with host cell cytoplasm; sporogony polysporoblastic, with the fragmentation of sporogonial plasmodia by an exogenous budding process; all development within a host cell hypertrophy tumour; members of the genus parasitic in marine fishes. The full generic and species diagnoses of *M. hepaticus* are presented at the end of this section.

Microsporidiosis due to *M. hepaticus* is primarily a disease of the juvenile population. Histopathological studies indicate that large areas of liver tissue can be occupied by the parasite xenomas, although high parasite burdens can be tolerated. However, it was noted that heavily infected individuals were among the first to die after capture, suggesting that the disease may be a contributory factor to juvenile mortality under stress situations. Liver damage, by pressure atrophy, was only caused where hepatocytes were squeezed

between xenomas and liver structures. This observation does have implications in the pathological mechanism of the disease; it would suggest that for a given liver volume there will be a critical intensity of infection, above which the number of xenomas results in massive pressure atrophy due to the trapping of hepatocytes between the xenomas themselves. This critical intensity would be greatly reduced with reduced liver size, and thus the infection of small larval and post-larval fishes is liable to be much more serious than in the juvenile population sampled here. Since transmission of *Microgemma hepaticus* is dependent upon the release of spores following host death, it would be of advantage to the parasite to infect the larval and juvenile populations owing to their natural high mortality rates. In addition, with the limited availability of such host stocks each year, it may be advantageous to cause the death of larval fishes to accelerate the transmission process.

The life cycle of *M. hepaticus* is divided into vegetative development (merogony) and sporogony, in common with most microsporidia (Vávra 1976a, b). Merogonic development was based entirely on plasmodial forms, having no clearly defined phases such as uninucleate or cylindrical cell populations, as occur in other fish-infecting species which form xenomas, such as *Glugea* species (Weissenberg 1911, 1968; Debaisieux 1920; Berrebi 1979; Canning *et al.* 1982) and *Tetramicra brevifilum* (Matthews & Matthews 1980). The diplokaryotic state is not considered to be a feature of *M. hepaticus*; the paired nuclei occasionally encountered in meronts lacked the characteristic flattened and thickened opposing membranes of the true diplokarya (Vávra 1965, 1976a; Weidner 1976; Spelling & Young 1983; Canning, Olson & Nicholas 1983). All stages recognized as meronts were clearly enclosed within vacuoles limited by a single host membrane. A similar situation has been described in *Tetramicra brevifilum* (Matthews & Matthews 1980) and in another hepatic microsporidium, of cottids, in the Plymouth area (Stubbs, personal communication). Although the origin of the membrane was not observed, it did resemble adjacent host endoplasmic reticulum. If this is the nature of the membrane, then the fact that it is a single membrane means that meronts must be contained within the endoplasmic reticulum, which would suggest a more intimate association with host cell organelles than previously suspected. Intimate association of endoplasmic reticulum with meronts has been shown in *Glugea* species (Berrebi 1979; Loubès, Maurand & Walzer 1981; Canning *et al.* 1982), where meronts are surrounded by cisternae of endoplasmic reticulum. However, in none of these reports does the relationship with endoplasmic reticulum reach the levels of that suspected with *M. hepaticus*. There are clear advantages to this site; firstly, the most rapidly growing stage of the parasite is placed in the host cell synthetic system and thus has a ready supply of materials; and secondly, the meronts are connected directly to the host cell nucleus, a link of possible major significance in the control of the host cell and xenoma formation.

Merogonic plasmodia passed directly to the sporogonic phase without a recognizable intermediate cell population. This is unusual among xenoma-forming microsporidia, as both *Glugea* and *Tetramicra* species have an intervening uninucleate cell population prior to the formation of sporogonic plasmodia (Weissenberg 1968; Debaisieux 1920; Berrebi 1979; Matthews & Matthews 1980; Loubès *et al.* 1981; Canning *et al.* 1982). The loss of the enclosing membrane of vegetative stages at the onset of sporogony is a distinctive feature of *M. hepaticus*, and may be the cause of the switch to the sporogonic phase of

development. Several authors have suggested that the trigger for sporogonic development is due to a change in environment within the host cell (Ishihara 1969; Sprague & Vernick 1971; Vávra, Bedrník & Činatl 1972; Maurand & Vey 1973; Vávra 1976b) and the loss of the host endoplasmic reticulum here would result in this. A reduced nutrient supply may well be the trigger for the production of resistant transmission stages. In certain species the onset of sporogony is triggered by a meiotic division, indicative of a sexual fusion at some stage of the life cycle (Loubès, Maurand & Rousset-Galangau 1976; Hazard, Andreadis, Joslyn & Ellis 1979; Loubès 1979; Vivares & Sprague 1979; Canning & Hazard 1982). Synaptonemal complexes, indicative of meiosis, were not observed here, suggesting that *Microgemma hepaticus* has an asexual life cycle. Cytological features of early sporonts were consistent with those of other species, with the presence of extramembranous material and increased endoplasmic reticulum (Vávra 1976a, b; Canning *et al.* 1979; Canning & Nicholas 1980). However, the polysporoblastic nature of sporogonic division is unlike other species, the main distinction being that random budding results in the formation of a great variety of forms. It is of interest that the various groupings of sporoblasts encountered in *M. hepaticus* have been individually described in various genera; rosettes of sporoblasts in *Tetramicra brevifilum* (Matthews & Matthews 1980) and *Nosema vivieri* (Vinckier, Devauchelle & Prensier 1970, 1971) and paired sporoblasts in numerous genera included *Encephalitozoon*, *Glugea* and *Nosema* (Sprague & Vernick 1971; Sprague 1977b). The sporogony process resulted in a single, monomorphic spore type; some fish-infecting species produce spores varying in size, e.g. *Pleistophora* (Canning, Hazard & Nicholas 1979; Canning & Nicholas 1980) or varying in development and morphology, e.g. *Spraguea lophii* (Loubès *et al.* 1979), although the significance of these in comparison with dimorphic microsporidia of insects is unknown (Hazard & Weiser 1968; Andreadis 1983).

Sporogenesis in general followed the pattern described for other species (Vávra 1976b). A unique observation was the association of the endospore with a tube-like structure, connecting the endospore, which is laid down throughout the sporogenesis period, with the cell synthetic systems. Liu & Davies (1972) detected vesicles associated with the spore membrane in freeze fracture preparations, but the present report is the first to indicate a connection between the endospore and cytoplasm in microsporidian sporoblasts. Spores were typical in structure, with the classical ultrastructural features of microsporidian spores (Vávra 1976a). Of particular note was the very high density of ribosomes, which apparently resulted in the high electron density of spores. It may be that these indicate a particularly high capacity for protein synthesis, of significance in the rapid take-over of host cells and the early stages of xenoma formation following sporoplasm injection.

The host cell response to infection with *M. hepaticus* results in massive hypertrophy and reorganization with the formation of the classical xenoma host-parasite complex (Chatton 1920; Weissenberg 1949). The xenoma of *M. hepaticus* had several notable features compared with others, including the microvillar surface layer, the mitochondrial band and the reticulate nucleus. Microvillar surfaces have been reported in '*Nosema cotti*' (Chatton & Courrier 1923: 'bordure en brosse'), *Mrazekia* from annelids (Janiszewska, Kassner & Madrzejewska 1978) and in *Tetramicra brevifilum* (Matthews & Matthews 1980), although not in *Glugea* species, where the cell is encapsulated. The microvilli presumably increase surface area for nutrient absorption; *Glugea* species, lacking these

structures, have massive peripheral pinocytic activity instead (Weidner 1976; Lom, Canning & Dyková 1979). The arrangement of mitochondria at the cell periphery is consistent with an active site for nutrient uptake, and in the absence of mitochondria in microsporidians must be fulfilling energy requirements of both host and parasite components of the xenoma. Similar concentrations occur in *Glugea* (Berrebi 1979; Lom *et al.* 1979; Weidner 1976), *Ichthyosporidium giganteum* (Sprague & Vernick 1974) and *Nosema herpobdellae* xenomas (Spelling & Young 1983); however, the zone in *M. hepaticus* xenomas is particularly well developed. The reticulate and hypertrophic host nucleus has been reported from the xenomas of several other species and Matthews & Matthews (1980) suggested that the arrangement provided scope for DNA replication and gave a high surface area for transfer of materials. While no studies have been performed on chromosomes of fish xenoma nuclei, Pavan, Perondini & Picard (1969) showed that alterations in microsporidian-infected dipteran salivary gland cells were due to chromosomal changes induced by the microsporidium. The nuclear alterations observed in *M. hepaticus* infection must be in response to the presence of the parasite, and may be involved in the control of the xenoma itself. The nature of the target cell for *M. hepaticus* infection was not established, but the association of xenomas with various liver structures and metacercarial capsules suggests a connective tissue cell rather than a specific liver cell.

The granulation response began when degeneration of the xenoma resulted in exposure of spores, as in other species (Dyková & Lom 1980). A notable feature was the absence of weakly reactive stages (Dyková & Lom 1980) to active xenomas. This is presumably due to the absence of pressure atrophy around xenomas, where they are not squeezing adjacent hepatocytes against liver structures; the spongy nature of liver tissue would tend to reduce pressure damage in most situations.

Seven species of microsporidia were listed by Sprague (1977a) as parasitic in fish livers, from ten hosts. These were placed in the genera *Pleistophora* and *Glugea*, and with one species each in the genera *Icthyosporidium*, *Octosporea* and the collective genus *Microsporidium*. A further species has subsequently been reported by Paperna, Sabnai & Castel (1978), *Pleistophora* sp., parasitic in the metacercarial cyst wall of *Heterophyes heterophyes* in the liver of the grey mullet, *Mugil cephalus*. On the basis of spore size, the only common feature quoted, *M. hepaticus* appears distinctly different from the previously reported species. Although comparison of spore size is treated with caution owing to differing preparatory techniques, it seems unlikely that *M. hepaticus* has previously been observed and assigned to an established genus. The possibility exists that the liver parasites have been wrongly placed in the genera *Pleistophora* and *Glugea*, there having been a tendency amongst early workers to assign species to a small number of genera on the basis of limited developmental information. More recent work indicates that *Pleistophora* species are muscle parasites (Schäperclaus 1941; Lom & Corliss 1967; Canning *et al.* 1979; Canning & Nicholas 1980; Canning & Hazard 1982) and *Glugea* species are primarily parasites of the connective tissue of the intestine (Weissenberg 1968; Stunkard & Lux 1965; Berrebi & Bouix 1978; Canning *et al.* 1982). It is unlikely, therefore, that members of these genera would be specific liver parasites. Rediscovery and investigation of these parasites for developmental features is necessary to determine their actual generic affiliation. Examination of other reports for affiliations of the genus *Microgemma* reveals two families whose characteristics are consistent with the develop-

ment of *M. hepaticus*. The family Tetramicriidae (Matthews & Matthews 1980), if the diagnosis is emended to replace 'tetrasporoblastic development' with 'polysporoblastic development', could contain *Microgemma*. In favour of this, the genera *Microgemma* and *Tetramicra* certainly have features of development, interaction with host cell, and environment in common. Alternatively, both could be placed in the family Unikaryonidae, Sprague (1977b), as both fulfil the requirements of Sprague's (1977b) diagnosis, namely sporulation in direct contact with host cell cytoplasm, polysporoblastic development and uninucleate sporoblasts. However, with the limited information available on the development of various marine microsporidia it seems advisable not to assign *Microgemma* to any particular family, in anticipation of further studies making the relationships between marine microsporidia more obvious.

Specific and generic diagnosis

Microgemma hepaticus gen.nov. sp.nov.

Locality. St Johns Lake, Tamar Estuary, Cornwall, England.

Host and site. *Chelon labrosus* (Risso, 1826) juveniles, liver.

Lesion. Spherical xenoma (granuloma in old infections) up to 500 µm in diameter, visible as white spots in the liver.

Vegetative stages. With characters of genus (see below).

Sporulation stages. With characters of genus (see below).

Spore. Pyriform, dimensions 4·2 µm × 2·4 µm at widest, posterior vacuole occupies posterior third of spore; polar filament isofilar, 7–10 coils, 57 µm long when extruded; polaroplast lamellar.

Microgemma gen.nov. Nuclei unpaired at all stages of merogony and sporogony; developmental cycle asexual; meronts are multinucleate plasmodia which divide by plasmotomy, enclosed within single host membranes; multinucleate meronts enter sporogonic phase directly, with no intermediate cell population; sporogony is in direct contact with host cell cytoplasm; polysporoblastic; division begins by single exogenous budding and accelerates by multiple budding and fragmentation of the plasmodium; development is within an xenoma host-parasite complex; parasitic in marine fishes.

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