Interaction of phagocytosed *Bonamia* sp. (Haplosporidia) with haemocytes of oysters *Tiostrea chilensis*

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ABSTRACT: The interaction between the haplosporidian pathogen *Bonamia* sp. and the haemocytes of oysters *Tiostrea chilensis* was studied ultrastructurally and using ultracytochemistry for the lysosomal hydrolases, β -glycerolphosphatase (β -GPase), cytidine monophosphatase (CMPase), thiamine pyrophosphatase (TPPase), and β -galactosidase. Although fine and coarse granulocytes, serous (brown) cells and hyalinocytes were present, agranular precursors of fine granulocytes (progranulocytes), degranulated coarse granulocytes, hyalinocytes and serous cells commonly phagocytosed *Bonamia* sp. A sequence of infection from initial occupation of tight phagosomes to formation of an enlarged phagosome or parasitophorous vacuole (PV), which sometimes broke down, occurred in all infected haemocytes. Enlargement of the phagosome membrane to eventually form a PV coincided with formation of bilaminar vesicles in the lipid bodies of parasites and their release from an indented area of the parasite surface. Too few infected granulocytes were observed to determine whether phagosome modification blocked lysosome-phagosome fusion. Vesicular CMPase and TPPase were produced by agranular haemocytes containing phagocytosed *Bonamia* sp. and passed into tight phagosomes. Despite some endocytosis of these enzymes by *Bonamia* sp., very few parasites appeared dense or moribund. From the orientation of lipid bodies toward the phagosome lumen, and release of β -GPase into the lumen from the lipid bodies, parasite hydrolytic enzymes in lipid bodies may function in parasite nutrition, by extracellular digestion of host haemocyte cytoplasm.

KEY WORDS: *Bonamia* sp. · Oyster haemocytes · Phagosome:lysosome interactions · Enzymes · Parasitophorous vacuole

INTRODUCTION

Of all forms of parasitism, the obligate intracellular parasitism of phagocytic haemocytes presents some of the greatest barriers to survival. Pathogens of mammals that utilise professional phagocytes (granulocytes, macrophages) during their life cycles, have to survive oxygen-dependent (H_2O_2 , O_2^-) and oxygen-independent (lymphokines, γ -interferon, complement) killing mechanisms, phagosome acidification, and destruction by lysosomal acid hydrolases. Despite this, diverse mammalian pathogens, such as *Mycobacteria* (Mor et al. 1988), *Toxoplasma gondii* (McLeod et al. 1991), microsporidians (Weidner & Sibley 1985), *Leishmania* spp. (Mauël 1990) and other trypanosomatids (McCabe & Mullins 1990), successfully survive and replicate in phagocytes. Survival is achieved by

blocking or evading phagocyte activation (McLeod et al. 1991), suppressing the respiratory burst (Sibley 1989, McCabe & Mullins 1990, McLeod et al. 1991), preventing phagosome-lysosome fusion (Nichols & O'Connor 1981, D'Arcy Hart et al. 1987, Sibley 1989, McLeod et al. 1991), and secreting substances that may form a protective barrier (Mauël 1990), modify the compartment occupied (de Carvalho & de Souza 1989), or create a parasitophorous vacuole (PV) (Joiner et al. 1990).

Bonamia spp. are obligate haplosporidian parasites of oysters (e.g. *Ostrea* spp., *Tiostrea chilensis*) (Pichot et al. 1979, Dinamani et al. 1987) that are phagocytosed by, and replicate in, haemocytes. In *Ostrea edulis*, *Bonamia ostreae* is primarily phagocytosed by granulocytes (Balouet et al. 1983, Balouet & Poder 1985, Chagot et al. 1992, Mourton et al. 1992), in which it appears to suppress the respiratory burst (Hervio et

al. 1988, Jean-Chagot 1989, Hervio 1992). The dense lipid bodies of *B. ostreae* contain cytidine monophosphatase (CMPase) and, as in *Leishmania* spp. (Mauël 1990), *B. ostreae* may use this acid phosphatase to protect itself from host haemocyte defence mechanisms (Hervio et al. 1991). The lipid bodies of *Bonamia* sp. in *T. chilensis* do not appear to contain CMPase, but may contain β -glycerophosphatase (β -GPase), another acid phosphatase (Hine & Wesney 1994, this issue).

This study was carried out to determine which haemocytes in *Tiostrea chilensis* phagocytose *Bonamia* sp., and how parasite lipid bodies function after haemocyte phagocytosis. As lysosomal enzymes are used by phagocytes to degrade endocytosed material, the distributions of 4 lysosomal enzymes (β -GPase, CMPase, thiamine pyrophosphatase, and β -galactosidase) were studied in haemocytes phagocytosing *Bonamia* sp.

MATERIAL AND METHODS

Infected oysters were opened, and the heart was removed and cut in two. Imprints were made with one half, and the other half was fixed for ultrastructural or enzyme cytochemical studies. Imprints were stained with Hemacolor™ (Merck), microscopically examined, and heavily infected oysters selected for further study.

Fine structure. Hearts were fixed in 2.5% glutaraldehyde in 0.22 μ m filtered seawater (FSW) for 1 to 2 h, washed twice in FSW, post-fixed in 1% OsO₄ for 1 h, stained en bloc with 5% uranyl acetate in 0.1 M sodium acetate buffer for 45 min, dehydrated in ascending (50 to 100%) ethyl alcohol, embedded in Araldite, sectioned, stained with 5% uranyl acetate for 10 min and 5% lead citrate for 5 to 6 min, and examined on a Philips 420ST transmission electron microscope (TEM).

Enzyme cytochemistry. The following ultracytochemical techniques were used: β -glycerophosphatase (β -GPase) (Robinson & Karnovsky 1983), cytidine monophosphatase (CMPase) (Hervio et al. 1991), thiamine pyrophosphatase (TPPase) (Robinson & Karnovsky 1983), and β -galactosidase (Rhodes & Blom 1986). Details of these techniques, and modifications to them, are given in Hine & Wesney (1994).

RESULTS

Haemocytes

Four haemocyte types were distinguished. (1) Fine granulocytes had a central or slightly eccentric round nucleus, with a uniform margin of heterochromatin, and circular to ovoid granules. Two forms were apparent; small cells (Sg) with a high nucleus to cytoplasm ratio, and large circular to ovoid granules (Fig. 1) that stained moderately to strongly for β -GPase and CMPase, and larger cells (Lg) with a lower nucleus to cytoplasm ratio that sometimes contained lipid bodies reactive for β -galactosidase, and had granules with a dark core or lacking a core (Fig. 2), that reacted weakly for CMPase, but were moderate or strong for β -GPase. Both types were frequently observed degranulating.

(2) Coarse granulocytes (Cg) had an eccentric nucleus, short profiles of rough endoplasmic reticulum (rER) and large irregular granules (Fig. 3) that showed patchy moderate, or rarely strong, reaction for β -GPase, CMPase and β -galactosidase. Cells lacking granules, but otherwise indistinguishable from Cg, were frequently observed. A few that appeared to lack phagosomes contained particulate CMPase and TPPase in cytoplasmic vesicles, and moderate (CMPase) to strong (TPPase) particulate reaction product was observed in cells with phagosomes containing necrotic debris.

(3) Large cells with an eccentric irregular nucleus bordered by a thin band of heterochromatin, and containing large irregular flecked masses (Fig. 4) were identified as serous or brown cells.

(4) Hyalinocytes were often irregular in shape, had an eccentric nucleus with mitochondria grouped nearby (Fig. 5), and contained a few cytoplasmic, sometimes dense, vesicles. Rarely, weak β -GPase was seen in vesicles.

Fine structure of host-parasite interaction

Bonamia sp. most often infected agranular cells resembling degranulated Cg (Fig. 6), agranular cells with lipid bodies and an ovoid nucleus with uniformly

Figs. 1 to 6. *Bonamia* sp. infecting *Tiostrea chilensis*. Haemocytes present. Fig. 1. Small cell showing central nucleus, uniform marginated heterochromatin, and spherical to ovoid granules. $\times 14\,540$. Fig. 2. Larger cell showing the ovoid to round nucleus with uniform marginated heterochromatin, and granules with a dense or missing core. CMPase unstained, $\times 10\,090$. Fig. 3. Coarse granulocytes, Cg, showing the eccentric irregular nucleus with prominent nucleolus and cytoplasm rich in ribonucleoside endoplasmic reticulum, rER. Note lack of clustering of granules around *Bonamia* sp. inside a developing parasitophorous vacuole, PV (arrow). β -GPase, $\times 9880$. Fig. 4. Serous cell showing the nucleus (N), characteristic large irregular flecked inclusions (*), and phagocytosed *Bonamia* sp. (B) in a developing PV (arrow). β -GPase control, $\times 11\,500$. Fig. 5. Hyalinocyte with characteristic perinuclear grouping of mitochondria. The *Bonamia* sp. is in a large phagosome and has a β -GPase-positive lipid body. β -GPase, $\times 11\,280$. Fig. 6. Agranular haemocyte with a nucleus similar to Cg (Fig. 3), a central Golgi complex, some rER, and containing 3 *Bonamia* sp. (B). TPPase unstained, $\times 12\,330$

Figs. 7 to 14. *Bonamia* sp.:haemocyte interaction in *Tiostrea chilensis*. Fig. 7. Agranular haemocyte showing the ovoid nucleus with uniform heterochromatin around most of the margin, ovoid β -galactosidase positive lipid inclusions (arrows), many rER profiles, and a *Bonamia* sp. (B) attached to the surface. β -Galactosidase unstained, $\times 9610$. Fig. 8. Granulocyte showing ovoid to round granules, some with light cores (arrow), a lipid inclusion (L), and 2 *Bonamia* sp. (B). β -GPase, $\times 13\,390$. Fig. 9. *Bonamia* sp. (B) dividing in a tight phagosome within an agranular haemocyte rich in rER. Note the uniformity of the space between the phagosome membrane (arrows) and the parasite. $\times 15\,900$. Fig. 10. Presence of bilaminar secretory vesicles within a *Bonamia* sp. (arrow). $\times 28\,900$. Fig. 11. Detail of the vesicles in Fig. 9. $\times 134\,000$. Fig. 12. Similar vesicles to those in Figs. 9 & 10 within a parasite lipid body. TPPase+L-tartrate as inhibitor, $\times 105\,000$. Fig. 13. Bilaminar secretory vesicles passing out of *Bonamia* sp., on the right. $\times 90\,130$. Fig. 14. Typical indentation of the parasite from which secretory vesicles are being shed, and which are confined within the phagosome membrane (arrows). $\times 22\,890$

marginated sparse heterochromatin (Fig. 7), hyalino-cytes (Fig. 5), serous cells (Fig. 4) and Cg (Fig. 3), and very rarely (<1% of infections) fine granulocytes (Fig. 8). A sequence of infection appeared to occur from initial binding of the parasite to the phagocyte surface (Fig. 7) to presence of *Bonamia* sp. in unmodified tight phagosomes (Fig. 9), and subsequently formation of PVs containing parasites. In unmodified, tight phagosomes there was a gap of 25 nm between the parasite and host membranes (Fig. 9). This space enlarged when ovoid bilaminar vesicular particles, 110×90 nm with 5 nm between the membranes, formed inside the parasite (Figs. 10 & 11) apparently within the lipid bodies (Fig. 12), and were released from its surface (Fig. 13). In >90% of *Bonamia* sp., at all developmental stages, particles were released from an indented or concave region of the parasite surface (Figs. 14 & 15). Particle release appeared to coincide with host phagosome membrane enlargement, forming a PV around the parasite (Fig. 16).

When 2 or more parasites occurred together, the PV around each was joined to form a common vacuole with the parasites around its periphery (Fig. 16). The host membrane could be seen between and around the parasites, which often had their lipid bodies oriented toward the lumen of the PV (Fig. 17). The lipid bodies were often at the parasite surface and either appeared to protrude from it (Fig. 18), or were occasionally seen outside the parasite (Fig. 17). In the few granule-containing haemocytes with phagocytosed *Bonamia* sp., haemocyte granules did not fuse with the phagosome membrane (Fig. 3). Sometimes the PV membrane appeared to break and much of the haemocyte cytoplasm was replaced by parasites amid disintegrating host granules and parasite lipid bodies, haemocyte organelles, and disorganized membranes.

Enzyme cytochemistry

Parasites were rarely seen within granular cells, and uninfected cells lacking membrane-bound granules showed little cytoplasmic enzyme activity. However,

lipid bodies in agranular cells were positive for β -galactosidase (Fig. 7). When haemocyte granules lay near a disintegrating PV, their β -GPase did not surround the parasites (Fig. 8). Lipoid bodies in *Bonamia* sp. were weakly to moderately positive for β -GPase, and when lipid bodies protruded from the parasite surface some β -GPase was released into the PV (Fig. 15). Extracellular β -GPase was not observed to coat the parasite surface.

In tight phagosomes of agranular cells CMPase coated the parasite surface and appeared to derive from haemocyte vesicles fusing with the unmodified phagosomes (Fig. 19). Haemocyte vesicles did not have distinct membranes and reaction product often appeared particulate (Fig. 20). Similar deposits were occasionally seen, apparently endocytosed, within the parasites (Fig. 21). CMPase was also observed in tight phagosomes of serous cells (Fig. 22). Parasites in the PV were not coated with CMPase.

TPPase showed similar distribution to CMPase, being present in tight phagosomes and as loose particulate reaction product in disorganized masses where the phagosome was more voluminous (Fig. 23). TPPase occurred in laminated structures within vesicles (Fig. 24), and similar configurations occurred near the parasite surface (Fig. 23). Similar reaction product was observed as intracytoplasmic deposits (Fig. 23), which only occurred in parasites surrounded by reaction product, and may therefore have been endocytosed. Many parasites showed partial loss of lipid body content (Figs. 6, 15 & 20) which may have included weak TPPase activity (Figs. 12 & 25), and occasionally lipid body contents appeared to pass into the phagosome (Fig. 25). Most parasites appeared unaffected by TPPase, and *Bonamia* sp. was frequently observed leaving haemocytes (Fig. 26). No reaction product was observed in sections incubated without substrate.

The lipid bodies of some agranular haemocytes contained β -galactosidase (Figs. 7 & 27). These lipid bodies were not membrane bound, and although they appeared to coalesce, they did not fuse with the phagosome membrane (Fig. 27).

Figs 15 to 18 *Bonamia* sp. haemocyte interaction in *Tiostrongia chilensis* Fig. 15. Agranular haemocyte rich in rER with a phagocytosed *Bonamia* sp. Note the site of parasite surface indentation and vesicle shedding in relation to a dense body reacted for β -GPase. Some enzyme is on the surface of the parasite (arrow) β -GPase, $\times 23\,330$ Fig. 16. Agranular haemocyte rich in rER containing 3 *Bonamia* sp. in a well-formed PV $\times 8210$ Fig. 17. Agranular haemocyte showing the cell nucleus (N), and 5 *Bonamia* sp. joined by a common intact phagosome membrane. Note the *Bonamia* sp. lipid bodies are orientated toward, and 2 are in (arrows), the PV lumen. β -GPase, $\times 8370$ Fig. 18. Lipid body protruding from, and breaking the parasite surface, with loss of content on luminal side $\times 27\,620$

DISCUSSION

The Lg of *Tiostrea chilensis* are similar to those reported as granulocytes from *Ostrea edulis* by Auffret (1989) and Jean-Chagot (1989). The Sg resemble the hyalinocyte of *O. edulis* in Auffret (1989), and the small hyalinocyte of Jean-Chagot (1989). However, the 'electron-dense vesicles' described by Auffret (1989) are granules, and in nucleus:cytoplasm ratio, nuclear position and shape, and β -GPase staining in this study, the Sg resembles the granulocyte of *Crassostrea gigas* illustrated in Fig. 2e in Auffret (1989). Also, the hyalinocyte described here resembles the hyalinocyte of *C. gigas*, not the hyalinocyte of *O. edulis*, reported by Auffret (1989). The Cg of *T. chilensis* may be a more developed form of the small granulocyte; however, as this cell was frequently observed and was never seen with the uniform granules of small granulocytes, it is considered a separate cell type. With the exception of Sg, which sometimes reacted strongly for CMPase, observations made here support Jean-Chagot's (1989) suggestion that β -GPase is stronger in granulocytes than CMPase.

Most haemocytes phagocytosing *Bonamia* sp. were agranular, rich in short profiles of rER, with or without lipid bodies; their ontogeny is uncertain. On the basis of perinuclear grouping of mitochondria, some appeared to be hyalinocytes. The lipid bodies of others contained β -galactosidase, as do lipid bodies sometimes seen in developing Lg (Hine unpubl. data), and on the basis of nuclear morphology these cells may be granulocyte precursors (progranulocytes). It is unlikely that they are degranulated Lg as typical Lg granules were never observed in them. Also, despite the presence of many Lg in oysters co-infected with *Bonamia* sp. and bacterial infections, Lg very rarely (<1%) phagocytosed *Bonamia* sp. Other agranular cells may be degranulated Cg. Those with particulate CMPase and TPPase resemble haemocytes in *Crassostrea virginica* which are phagocytic in systemic *Haplosporidium nelsoni* (MSX) infections (Fig. 19 in Scro & Ford 1990). *In vitro* studies on *C. virginica* haemocyte interaction with MSX plasmodia have shown that granulocytes and agranular cells only phagocytosed damaged or killed plasmodia, and that granulocytes retreated rapidly after contact with plasmodia (Ford et al. 1993a). The haemocytes of *C. virginica* probably function more in wound repair than MSX phagocytosis (Ford et al. 1993b). *Tiostrea chilensis* agranular haemocytes containing necrotic debris or phagocytosed *Bonamia* sp. had more particulate CMPase and TPPase reaction product than those that were non-phagocytic, and may also function in wound repair.

The haemocytic response of *Tiostrea chilensis* to *Bonamia* sp. differs markedly from the response of oysters to *B. ostreae*. In *Crassostrea gigas* and *Ostrea*

edulis, *B. ostreae* infects every haemocyte type (Chagot et al. 1992), but, even allowing for change in composition and increase in granulocytes over time (Mourton et al. 1992), the primary phagocytes of *B. ostreae* are fine granulocytes (Balouet et al. 1983, Balouet & Poder 1985, Chagot et al. 1992, Mourton et al. 1992). Similarly in winter mortality of rock oysters (*Crassostrea commercialis*), caused by another haplosporidian, *Mikrocytos roughleyi*, only granulocytes are involved (Balouet et al. 1983). The apparent difference of haemocyte type involved may be partly due to nomenclature, as some cells phagocytosing *B. ostreae* resemble cells here called progranulocytes (Fig. 1 in Balouet et al. 1983). However, haemocytes regarded as granulocytes in both *T. chilensis* and in *O. edulis* (Fig. 3A in Mourton et al. 1992), were very rarely observed to phagocytose *Bonamia* sp. in this study. As *Bonamia* sp. also infects *O. angasi* in Australia, and the *O. angasi* haemocyte response is granulocytic (Hine unpubl. obs.), a comparative study of this pathogen in both oyster species may clarify oyster haemocyte function.

Phagocytosis of *Bonamia* sp. was not observed herein, but probably resembled phagocytosis of *B. ostreae* by *Ostrea edulis* haemocytes which actively endocytose *Bonamia* sp., rather than the parasite invading the haemocytes (Chagot et al. 1992). Lack of observation of phagocytosis may be due to the speed (<30 min) with which infection occurs (Mourton et al. 1992). Parasites were seen in tight phagosomes, but with the release of bilaminar particles the phagosome membrane enlarged to form a space between the parasite and host. Other obligate parasites of phagocytes release similar particles (Sibley 1989, McLeod et al. 1991, Pimenta et al. 1991) that vary in appearance with parasite species and strain (Sibley 1989). Purified bilaminar vesicles from *Toxoplasma gondii* (Fig. 8 in Sibley et al. 1986) which resemble those observed herein, may derive from rhoptries (Nichols et al. 1983), dense granules (Leriche & Dubremetz 1990) or the parasite surface (Sibley & Krahenbuhl 1988). They are inserted into the host phagosome membrane to form the PV (Joiner et al. 1990), and may prevent phagosome-lysosome fusion (Nichols et al. 1983, Joiner et al. 1990) or may be used to process and transport host metabolites necessary for parasite nutrition (Sibley 1989).

The similarity in production of bilaminar vesicles coinciding with enlargement of the host membrane to form a PV in *Bonamia* sp. and *Toxoplasma gondii* suggests a similar mechanism of PV formation. *B. ostreae* also occupies PVs containing vesicular and membranous inclusions (Fig. 69 in Jean-Chagot 1989), and it is often kidney-shaped, with a concave surface, in haemocytes (Montes et al. 1994). However,

Figs 19 to 25. *Bonamia* sp. and haemocyte cytochemistry in *Tiostrea chilensis*. Fig. 19. CMPase-positive vesicles in the cytoplasm, and along a tight phagosome (arrow), in a rER rich agranular haemocyte. CMPase, $\times 19\,800$. Fig. 20. Particulate (arrow) and vesicular CMPase reaction around a phagocytosed *Bonamia* sp. in a rER rich agranular haemocyte. Note loss of parasite lipid body content. CMPase unstained, $\times 23\,170$. Fig. 21. *Bonamia* sp. in a rER rich granulocyte showing CMPase positive material (arrows) that has probably been endocytosed. CMPase unstained, $\times 28\,460$. Fig. 22. *Bonamia* sp. in a serous cell showing CMPase within the tight phagosome around the parasite. CMPase, $\times 14\,840$. Fig. 23. Particulate TPPase around 2 *Bonamia* sp. showing the lamellar reaction product (arrow) and endocytosed (*) reaction product. TPPase unstained, $\times 42\,880$. Fig. 24. Lamellar TPPase reaction product (arrow) in cytoplasmic vesicular aggregate. TPPase unstained, $\times 52\,380$. Fig. 25. Lipoid body showing loss of TPPase positive content near the parasite surface, and reaction product around the parasite (arrow). TPPase, $\times 62\,000$

T. gondii forms large PVs if it actively invades the cell, but only lies in small vacuoles if antibody coated and passively endocytosed using the Fc receptor (Silva et al. 1982, de Carvelho & de Souza 1989, McLeod et al. 1991). *Bonamia* sp. is passively taken up (Chagot et al. 1992) and may form a large PV.

In *Toxoplasma gondii* the formation of a PV after active invasion results in inhibition of phagosome-lysosome fusion (McLeod et al. 1991), and the lack of lysosome-phagosome seen herein (Figs. 3 & 8) suggests fusion may be similarly inhibited. When *T. gondii* is passively endocytosed into small vacuoles, phagosome-lysosome fusion is not inhibited (Joiner et al. 1990, McLeod et al. 1991). In our study, CMPase and TPPase were observed coating parasites in tight phagosomes. Although it was unclear whether this enzyme derived from parasite lipid bodies, or from haemocytes, fusion of CMPase-containing granules with phagosomes apparently occurred (Figs. 19 & 20). Like *Toxoplasma gondii*, *Bonamia* sp. is endocytosed by different mechanisms that affect the ability of

haemocyte lysosomes to fuse with phagosomes. However, observations on *B. ostreae* (Jean-Chagot 1989, Chagot et al. 1992) do not suggest different endocytotic mechanisms. Alternatively, lysosome fusion may occur with unmodified tight phagosomes, before bilaminar vesicle modification of the phagosome membrane, but secretion of bilaminar vesicles modifies the phagosome to prevent lysosome fusion. Haemocyte lipid bodies do not fuse with phagosomes (Fig. 27) because of the lack of a bounding unit membrane.

The orientation of lipid bodies toward the PV lumen (Fig. 17), and their protrusion (Fig. 18), exocytosis (Fig. 17) and loss of content (Figs. 6, 15, 20 & 25) suggests they are functional at this stage of infection. They may perform 1 or more of 3 functions. Firstly they may release acid phosphatase to coat the parasite surface (see Hervio et al. 1991), as in *Leishmania* spp., to block host superoxide anion production (Maul 1990). However, this role is now attributed to lipophosphoglycan (LPG), which may be formed from acid phosphatase by addition of a carbohydrate structure in the Golgi com-

Figs. 26 & 27. *Bonamia* sp. infecting *Tiostrea chilensis*. Fig. 26. *Bonamia* sp. egressing from a degenerating haemocyte. TPPase, $\times 25\,120$. Fig. 27. Lipid bodies in a haemocyte around a parasite in a developing PV. β -Galactosidase, $\times 16\,010$

partment (Bates et al. 1990). LPG attenuates (McNeely & Turco 1990) or depresses (El-On et al. 1990) the host respiratory burst, by inhibiting protein kinase C, a modulator of macrophage oxidative metabolism (Mauël 1990). There was no evidence of β -GPase or CMPase being used to coat the parasite surface.

Secondly, *Bonamia* sp. lipid bodies are rich in unsaturated lipids (Hine & Wesney 1994) and may be involved in membrane traffic. Bilaminar membraneous vesicles are shed from near the lipid bodies. *Bonamia* sp. endocytoses membranes and appears to synthesise lipids through the Golgi and endoplasmic reticulum compartments to form lipid bodies (Hine & Wesney 1994).

Thirdly, lipid bodies contain β -GPase, and possibly TPPase, which appeared to pass from lipid bodies into the surrounding medium (Fig. 15), and lipid bodies were orientated toward the area of disorganized haemocyte cytoplasm (Fig. 17). Therefore, as *Bonamia* sp. grows and divides (Fig. 9) inside haemocytes, a possible role for the lysosomal enzymes is in nutrition, as suggested for *Leishmania mexicana* lipid bodies by Hassan & Coombs (1987).

In conclusion, *Bonamia* sp. is mainly phagocytosed by agranular haemocytes that lack a lysosomal system capable of injuring or destroying the parasite. Lysosome-phagosome fusion was not observed in infected granulocytes. Lack of fusion may be due to membraneous vesicles excreted by the parasite modifying tight phagosomes to form a PV and prevent lysosome fusion. Membraneous vesicles appear to derive from lipid bodies, which may also function in parasite nutrition.

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