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William L. Current
Targanta Therapeutics

John J. Janovy Jr.
University of Nebraska - Lincoln, jjanovy1@unl.edu

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ULTRASTRUCTURE OF INTERLAMELLAR *HENNEGUYA EXILIS* IN THE CHANNEL CATFISH

William L. Current and John Janovy, Jr.
School of Life Sciences and Cedar Point Biological Station, UN-L, Lincoln, Nebraska 68588

ABSTRACT: Ultrastructural aspects of interlamellar *Henneguya exilis* infections in channel catfish are reported. The plasmodium wall of this form differs from that of other species in that it is composed of two outer unit membranes which give rise to a zone of numerous pinocytic canals. Single-membraned canals appeared to be a stable feature of the wall while double-membraned canals are interpreted as those actively carrying out pinocytosis. Evidence suggests that host cellular cytoplasm as well as interstitial material is taken in by plasmodia. Plasmodium wall integrity, aggregation of parasite ectoplasmic components, numbers of pinocytic canals, and number of mitochondria proximal to the wall vary among different plasmodium profiles and may be related to plasmodium maturity. The parasite causes extensive hyperplasia of basal cells, which in turn replaces most other cell types found in noninfected gill filaments. Cytoarchitectural differences between basal cells of noninfected filaments and basal cells adjacent to plasmodia include significantly shorter microfilament bundles in the latter.

Seventeen species of *Henneguya* have been reported from several families of North American freshwater fishes (Hoffman, 1967). *Henneguya* species have been implicated as causative organisms of infectious disease resulting in significant losses to the catfish farming industry (McCraren et al., 1975). Seven different *Henneguya* disease manifestations in channel catfish are now recognized. One of these in which parasites begin their development among basal cells between gill lamellae, the interlamellar form, causes serious losses among immature fish (McCraren et al., 1975). Meyer (1969) reported epizootics of this form which resulted in losses of 95% of fingerlings. Additional concern over the interlamellar infection stems from reports that infected fish are less tolerant than healthy fish to handling and treatment with parasiticides. The interlamellar form is apparently spreading in the south-central United States due to unrestricted sale and transport of infected fish (McCraren et al., 1975).

This study concerns some ultrastructural aspects of the interlamellar form of *Henneguya exilis*. The plasmodium wall structure, interactions between the plasmodium and adjacent basal cells of the host, and apparent parasite-induced cytoarchitectural rearrangements of basal cells are reported. This is the first report of the ultrastructure of this form of *H. exilis* infection.

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MATERIALS AND METHODS

Infected fish were collected by gill netting from Lake Ogallala and Lake Keystone, Keith County, Nebraska, the lakes nearest the University of Nebraska Cedar Point Biological Station. Data presented in this paper were derived from a single infected channel catfish, *Ictalurus punctatus* (Rafinesque), about 6 lb in weight, collected 26 June 1975, in Lake Keystone.

Infected and uninfected gill filaments were excised, fixed for 2 hr at room temperature in 3% (v/v) glutaraldehyde in 0.1 M phosphate buffer (pH 7.4), and processed for electron microscopy according to the methods of Janovy et al. (1974). Silver-gray sections were cut with glass knives on a Porter-Blum MT-1 ultramicrotome, mounted on 150-mesh Formvar-coated grids, stained with uranyl acetate and lead citrate, and viewed and photographed with a Philips EM 201 electron microscope.

The distal portion of one filament was serially sectioned for light microscopy in order to determine the number and orientation of plasmodia within the filament. Epon sections 1 μm thick were taken at 12 μm intervals and stained with 0.5% toluidine blue in 1% sodium borate prior to examination.

The parasite was identified as *Henneguya exilis* Kudo 1929, based on spore measurements and morphology, geographical location, and host species. The infection type was established on the basis of criteria given in Minchew (1972) and McCraren et al. (1975). Basal cells were characterized ultrastructurally by their prominent bundles of elongate microfilaments, desmosomal junctions, and interdigitating pseudopodia.

Terminology used for life cycle stages in this study is in agreement with Lom and de Puytorac (1965) and Lom (1969). "Mature" spores were identified as those which exhibited very electron-dense cytoplasm surrounded by an extremely electron-dense shell and elongate polar capsules

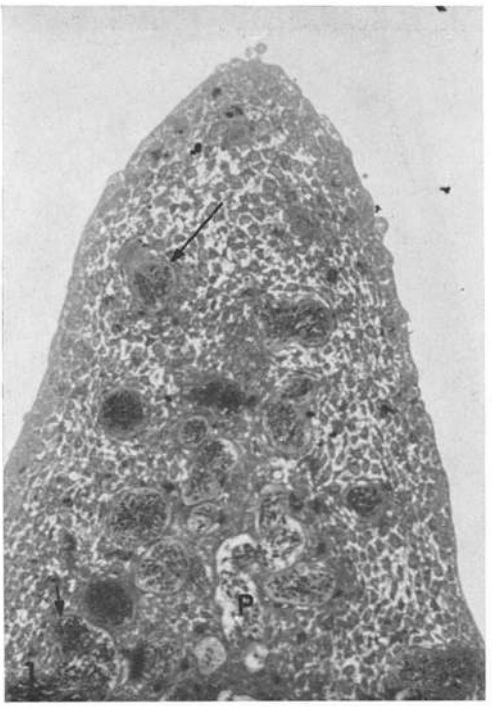

FIGURE 1. Light micrograph of 1 μm thick Epon section through the distal portion of a catfish gill filament infected with interlamellar *H. exilis*. Gill lamellae are completely absent as are the characteristic capillary beds and some cell types that characterize the noninfected filament. Profiles of plasmodia (P) are surrounded by compact layers of cells, while the remainder of the filament is filled with dendritic cells, probably derived by hyperplasia of basal cells which reside between lamellae of normal filaments. The long arrow points to a relatively "immature" plasmodium while the short arrow points to a "mature" plasmodium containing a large number of spores (see Observations and Discussion). Toluidine blue, × 150.

with a completely formed and identifiable polar filament (Lom and de Puytorac, 1965; Schubert, 1968; Lom, 1969). All other stages were considered "immature."

Measurements of 50 microfilament bundles were taken from 10 basal cells in infected filaments and from 10 basal cells in noninfected filaments, and Student's "*t*" test was used to compare the difference between their means. Measurements are reported as mean ± standard deviation. All measurements were taken from negatives of known magnification.

OBSERVATIONS

Infected gill filaments

In longitudinal sections the interlamellarly-infected gill filaments appeared swollen due to hyperplasia of some cell types also present in noninfected filaments. Parasites were seen as numerous irregularly shaped or subcircular profiles through various branches of different plasmodia (Fig. 1). Lamellae of infected filaments were fused beyond the point of structural recognition and some cell types characteristic of these structures, e.g., pillar cells and endothelial cells, were rarely observed. Branched plasmodia were contained primarily in the core of the filament, and profiles were rather uniformly distributed from the base to near the tip of the infected filament. At light microscope magnifications, fish cells appeared to be arranged in compact layers around the plasmodium profiles and the filament contained large numbers of loosely packed, dendritic cells, particularly distal to the parasite profiles themselves (Fig. 1). These light-level observations are consistent with those of Minchew's (1972) which describe the "most severe" interlamellar infections. Ultrastructural study revealed a number of specific features of not only the plasmodium wall but also of cells immediately adjacent to the wall.

Plasmodium wall

The plasmodium was delimited distally by two unit membranes separated by a 0.016 to 0.019 μm space, with much of the outermost membrane having a coat of fine granular ma-

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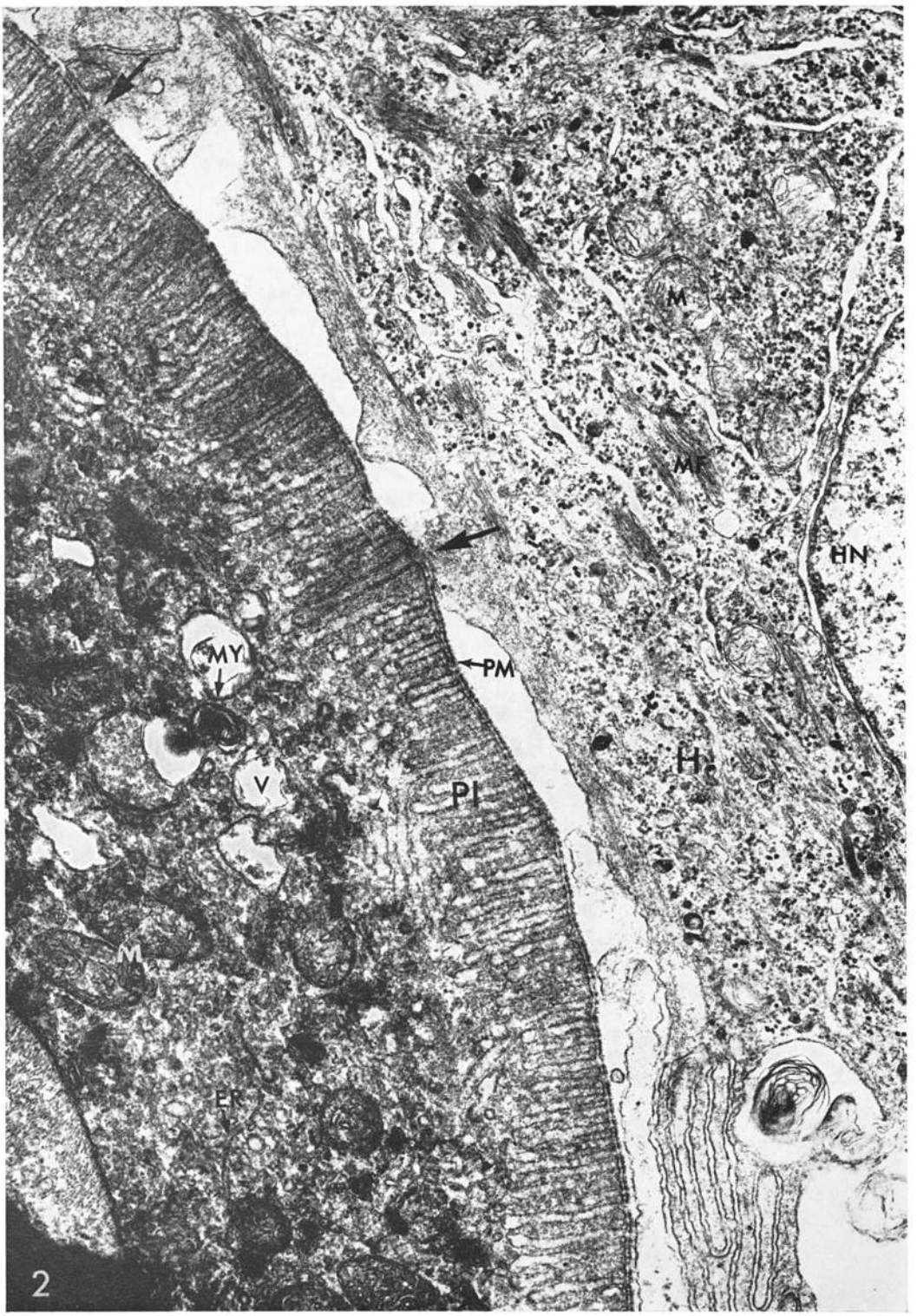
FIGURE 2. Transmission electron micrograph of the plasmodium wall of interlamellar *Henneguya exilis* and adjacent cells of the host. The plasmodium wall consists of two unit membranes (PM) and a zone of pinocytic canals (PI). Proximal to the zone of pinocytic canals are pinocytic vesicles and numerous mitochondria with branched, tubular cristae (M), vacuoles of various sizes (V), myelin figures (MY), and endoplasmic reticulum (ER). Arrows point to double-membraned pinocytic canals through which basal cell's cytoplasm is evidently passing. Within the basal cell (H) is seen the nucleus (HN), bundles of microfilaments (MF), and mitochondria (M). Host mitochondria contain saclike cristae. Interdigitated pseudopodia of basal cells are seen in the lower right corner. × 28,000.


M
MT
HN
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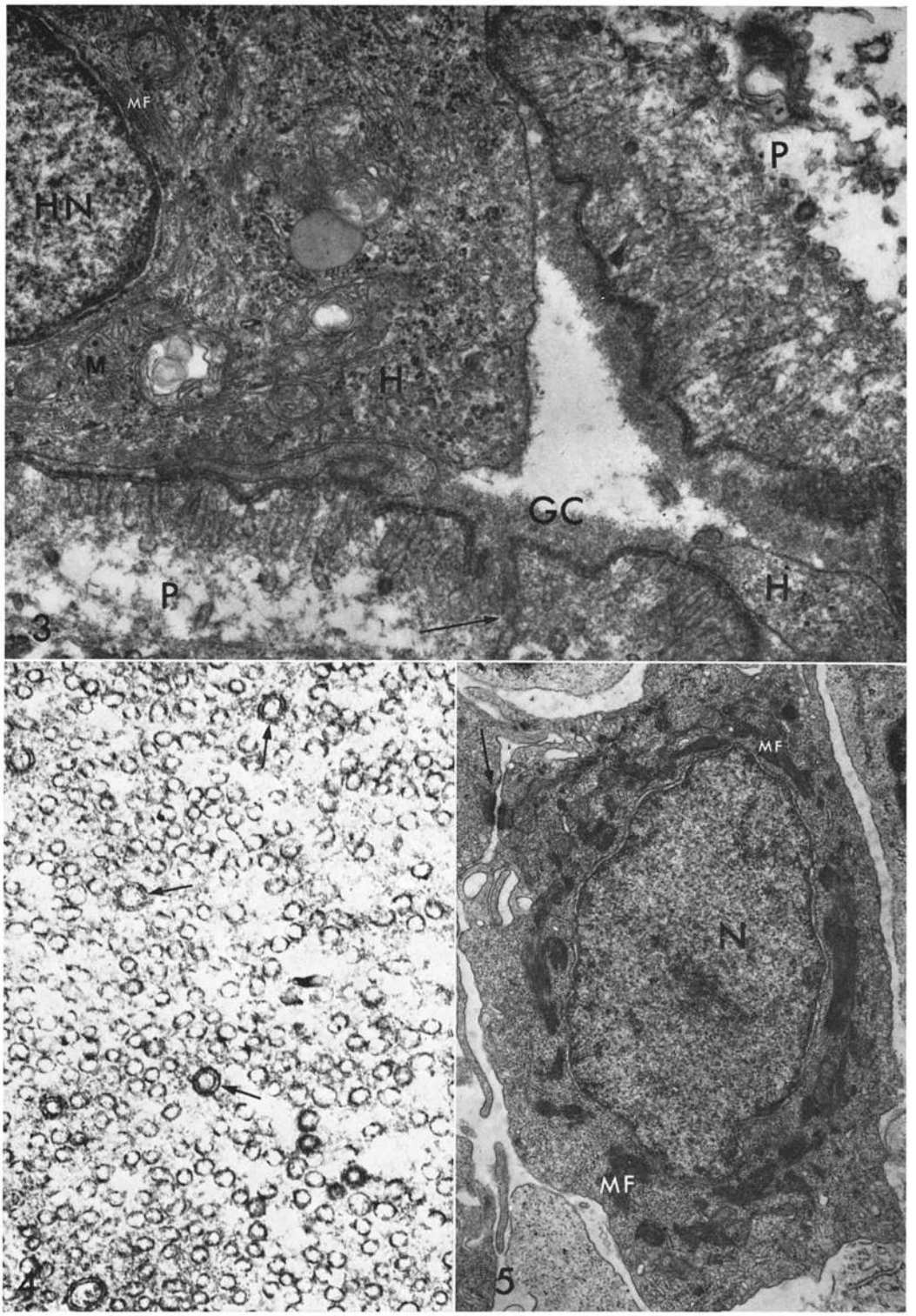
MF
HN
P
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MF
5

terial. The thickness of the coat varied (Figs. 2, 3). In some sections direct contact between parasite and fish cells was observed, and in these instances the outer parasite membrane appeared to be continuous with that of the adjacent fish cell (Fig. 2, arrows). Proximal to and derived from the two unit membranes was a 0.7 to 0.9 μm thick region of pinocytic canals, in which the numerous canals were arranged perpendicular to the plasmodium surface (Fig. 2). Most canals observed were composed of a single unit membrane, however, some canals were double-membraned (Figs. 2, 4). In tangential section these canals had a diameter of 0.06 to 0.16 μm, and in some regions of the plasmodium wall there were 8 to 12 canals per linear μm. In tangential sections up to 90 canals/μm^2 were present, however, most sections revealed 50 to 80 canals/μm^2. The two outer unit membranes along with the zone of pinocytic canals collectively comprise the plasmodium wall, previously referred to as the "cyst wall" in light microscopy (Minchew, 1972).

The plasmodium wall itself was the outermost limit of the plasmodium within which the spores develop. The plasmodium cytoplasm exhibited various structural features, depending on the particular profile or section, which we interpret as progressive developmental changes. Plasmodium profiles varied in the relative numbers of mature and immature spores they contained.

Profiles of plasmodia which contained relatively few mature spores exhibited a zone of pinocytic vesicles and numerous mitochondria proximal to the zone of pinocytic canals and showed a vesicular cytoplasm with a paucity of ribosome-sized particles (Fig. 2). Many of the pinocytic vesicles appeared to be double-membraned. A few small, relatively electron-dense Golgi profiles were seen. In addition, numerous membrane-bound vacuoles of a variety of sizes and with a variety of contents could be seen in the plasmodial cytoplasm proximal to the mitochondria (Fig. 2). Few profiles of endoplasmic reticulum, either smooth or rough, were seen in the plasmodia (Fig. 2).

In plasmodia whose profiles contained relatively large numbers of mature spores, there was an associated loss of integrity of the zone of pinocytic canals, aggregation or clearing of the ectoplasm, reduction in the number of membrane-bound vacuoles, and reduction in the number of mitochondria (Fig. 3).

Host tissue

Host's cells adjacent to parasites were evidently derived from basal cells, since they were ultrastructurally very similar to basal cells of noninfected gill tissue (cf. Figs. 2, 3, 5). Basal cells surrounding plasmodia were typically elongate with numerous cytoplasmic bundles of microfilaments oriented generally parallel to the plasmodium surface and contained randomly spaced cysternae of smooth endoplasmic reticulum. Nuclei of basal cells had few and small chromatin aggregations and a thin layer of chromatin directly beneath the nuclear envelope (Figs. 2, 3). Portions of basal cells were in direct contact with the plasmodium surface, and in those regions where there was no direct contact, accumulations of interstitial materials could be seen (Fig. 3). At some points of direct contact

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FIGURES 3–5. Transmission electron micrographs of the plasmodium wall of interlamellar *Henneguya exilis* and/or cells of the host. **3.** Wall of a plasmodium (P) characterized by a high ratio of mature/immature spores. A thick coat of granular material (GC) covers the plasmodium and some of this material is seen within a pinocytic canal (arrow). This granular material is also seen on the surface of adjacent basal cells (H). Compared with Figure 2, there is a reduction in numbers of pinocytic canals and the proximal layer of aggregated cytoplasm lacks numerous pinocytic vesicles and mitochondria. × 26,450. **4.** Tangential section through plasmodium wall, showing arrangement of pinocytic canals. Most canals are single-membraned, however, 2.3% are double-membraned (arrows point to three) in this particular section. × 38,750. **5.** Basal cell located between secondary lamellae of a noninfected gill filament. Numerous, large bundles of microfilaments (MF) surround the nucleus (N). Arrow points to desmosomal junction which was being formed with an adjacent basal cell. × 13,200.

between parasites and basal cells, the basal cells' cytoplasm appeared to be passing directly into double-membraned pinocytic canals of the plasmodium wall (Fig. 2). Myelin figures appeared within the cytoplasm of the plasmodium as well as within adjacent cells (Fig. 2). Basal cells adjacent to the plasmodium wall formed many regions of contact with one another, with interdigitated pseudopodia and desmosomal junctions (Fig. 2). Plasmodia were surrounded by a closely appressed layer of these cells (Fig. 1). Numerous microfilaments were associated with junctions between basal cells.

Cells surrounding plasmodia and basal cells from normal gill filaments contained similar bundles of microfilaments and formed similar desmosomal junctions. However, basal cells from infected tissue had significantly shorter bundles of microfilaments than basal cells from normal tissue. The mean length of microfilament bundles from infected gill tissue was 0.62 ± 0.24 μm compared to 1.75 ± 0.51 μm from basal cells in normal tissue, a difference significant at the 1% level when analyzed by Student's "*t*" test.

DISCUSSION

The major contributions of this paper are: (1) evidence that the interlamellar *Henneguya exilis* plasmodium wall differs ultrastructurally from that of other reported *Henneguya* species; (2) demonstration that the plasmodium wall contains both single- and double-membraned pinocytic canals; (3) evidence that differences in integrity of the plasmodium wall, number of pinocytic canals, number of mitochondria proximal to the wall, and extent of cytoplasmic aggregation may be due to plasmodium maturity; and (4) demonstration of cytoarchitectural differences between basal cells in infected and noninfected gill tissues.

Present data, when compared with observations of others, indicate that *Henneguya* species differ ultrastructurally in the nature of the plasmodium wall. The plasmodium of *H. psorospermica*, a "branchial" parasite of *Perca flavescens*, has a single outer membrane which gives rise to a "moderate" number of pinocytic canals (Lom and de Puytorac, 1965) and, according to these authors, corresponds to an interlamellar *H. exilis* infection. A single outer membrane also has been reported by Schubert (1968) for plasmodia of *H. pinnae* from the fins of *Ctenophora kingsleyae*. The *H. pinnae* outer membrane forms numerous microvilli-like projections that extend between host epithelial cells (Schubert, 1968). Whether these differences are entirely species differences or are related to such factors as infection site remain to be determined.

Structural evidence for extensive pinocytic activity has been reported for plasmodia of several myxosporidan genera (Lom and de Puytorac, 1965; Schubert, 1968; Lom, 1969; Spall, 1973). If such evidence can be accepted, then pinocytosis seems to be the principal means of feeding for *H. exilis*. The plasmodium wall of the interlamellar parasite is presented as a structurally and physiologically complex organelle which apparently transports nutrients via pinocytosis into the plasmodium. Single-membraned canals arise from the innermost of the two limiting membranes and appear to be a stable feature of the plasmodium wall during spore development. Pinocytosis is evidently initiated as the outermost membrane invaginates into one of these canals, carrying nutrient material with it, and terminates with the formation of pinocytic vesicles at the proximal end of the now double-membraned canal. If this interpretation is correct, each area of the outer membrane directly over a pinocytic canal represents a potential site of pinocytosis.

There seems to be strong evidence that pinocytosis enables the plasmodium to take in basal cells' cytoplasm (Fig. 2) as well as interstitial material adhering to the plasmodial wall (Fig. 3). This activity may contribute to the extreme pathogenicity of interlamellar infections, as plasmodia of less pathogenic myxosporida seem to have different mechanisms for pinocytosis and have been reported to take in interstitial material only (Lom and de Puytorac, 1965; Schubert, 1968; Lom, 1969; Spall, 1973). Schubert (1968) also has suggested that plasmodia of *H. pinnae* incorporate portions of host cells' cytoplasm via pinocytosis, however, the electron micrographs presented do not clearly show this.

Loss of plasmodium wall integrity, aggregation of cytoplasmic components, and reduction in the numbers of pinocytic vesicles,

mitochondria, and vacuoles proximal to the wall became apparent when plasmodium profiles containing relatively large numbers of mature spores were compared with those containing relatively few mature spores. Since all of the above structures are related, directly or indirectly, to the intake of nutrients via pinocytosis, there may be reduced metabolic activity in older plasmodia. After examination of many different plasmodium profiles, it appears that mitochondria proximal to the plasmodium wall begin to degenerate as spores mature, perhaps reducing the available energy necessary to maintain integrity of the wall. A similar mitochondrial degeneration has been noted in plasmodia of other myxosporida, including several *Henneguya* spp. (Lom and de Puytorac, 1965; Schubert, 1968). Release of mature spores may be enhanced by breakdown of the plasmodium wall.

Not only does interlamellar *H. exilis* in channel catfish appear to cause hyperplasia of basal cells that normally reside between lamellae (Minchew, 1972), but it may be responsible for the cytoarchitectural changes within these cells described above. Infected filaments are composed almost entirely of basal cells which replace almost all of the other cell-types found in normal filaments (Fig. 1). Infected filaments also are characterized by an almost complete absence of extravascular lymphocytes, mononuclear, and polymorphonuclear phagocytic cells generally associated with a host immune response. However, the exact role of the parasite in the production of these cellular changes, as well as the mechanisms by which they arise, has yet to be established.

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