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Occurrence of a New Microsporidan: *Enterocytozoon bieneusi* n. g., n. sp., in the Enterocytes of a Human Patient with AIDS[1]

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ABSTRACT. A new microsporidium is reported infesting the enterocytes of a Haitian patient with AIDS. The stages observed were diplokaryotic cells, sporogonial plasmodia, unikaryotic sporoblasts, and spores. Neither a sporophorous vesicle (pansporoblastic membrane) nor parasitophorous vacuole were differentiated around the developmental stages, which were in direct contact with the host cell cytoplasm. The polar tube (5–6 coils) was differentiated before fission of the sporogonial plasmodium. The mature spores measured 1.5 μm × 0.5 μm. The spore wall was very thin as the endospore was absent or poorly differentiated. The organism is named *Enterocytozoon bieneusi* n. g., n. sp. and is assigned to the suborder Apansporoblastina.

DIARRHEA has been recently recognized as a frequent symptom in the acquired immune deficiency syndrome (AIDS), especially in Haitian patients (5, 11). Protozoa that have already been reported from the intestine of patients with AIDS include *Cryptosporidium, Giardia,* and *Isospora* (3). The case reported here deals with a 29-year-old, non-homosexual, Haitian patient with AIDS. The diagnosis of AIDS was based upon a decrease in OKT_4/OKT_8 lymphocyte ratio to less than 0.1 (n = 2–3) and multiple opportunistic infections (9). The patient was suffering severe diarrhea associated with the presence of *Giardia lamblia* in the stool. Furthermore, electron microscopic studies of duodeno-jejunal and ileal biopsies demonstrated the presence of developmental stages of a parasite resembling a microsporidan.

MATERIALS AND METHODS

Fragments of duodeno-jejunal and ileal mucosa taken by aspiration biopsy were fixed in 3% (v/v) glutaraldehyde in 0.2 M cacodylate buffer (pH 7.2–7.4) for 2 h at 4°C, rinsed in buffer, then post-fixed in 1% (w/v) OsO_4 in buffer for 1 h at 4°C. After ethanolic dehydration, the material was embedded in Epon resin. The sections, stained with uranyl acetate and lead citrate, were observed with TEM Philips EM 300.

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RESULTS

All the developmental stages of the microsporidan were observed in the enterocytes (Fig. 1). The younger stages are spherical cells of 3–4 μm mean diameter, characterized by a pale cytoplasm containing ribosomes and some flattened cisternae of the endoplasmic reticulum (ER). Two nuclei in diplokaryotic arrangement were usually observed (Fig. 3). Cytoplasmic vesicles of the host-cell (Fig. 3) and occasionally mitochondria (Fig. 2) lie closely appressed to the single cell membrane of the parasite, thus giving the appearance of two membranes at the parasite surface in places. These stages grow into multinucleate plasmodia of 6 μm mean diameter (Figs. 1, 2). The plasmodia come to contain numerous bodies consisting of heterogeneous material contained within a dark cortical layer. These bodies correspond to sections of several polar tubes, which differentiate simultaneously (Fig. 2). The anterior (basal) part of each polar tube, its anchoring disc and the vesicular and lamellar parts of the polaroplast are in close association with a plasmodial nucleus (Fig. 4). All these structures—anchoring disc, polar tube, and polaroplast—are components of the extrusion apparatus characterizing the spores in the order Microsporida (4).

At a later stage, the cytoplasm of the sporogonial plasmodium becomes denser; an electron-lucent vesicle and two sets of closely packed circular sections corresponding to 4–5 coils of the polar tube may be seen in the vicinity of each plasmodial nucleus (Fig. 5).

When the extrusion apparatus is fully differentiated, the spo-

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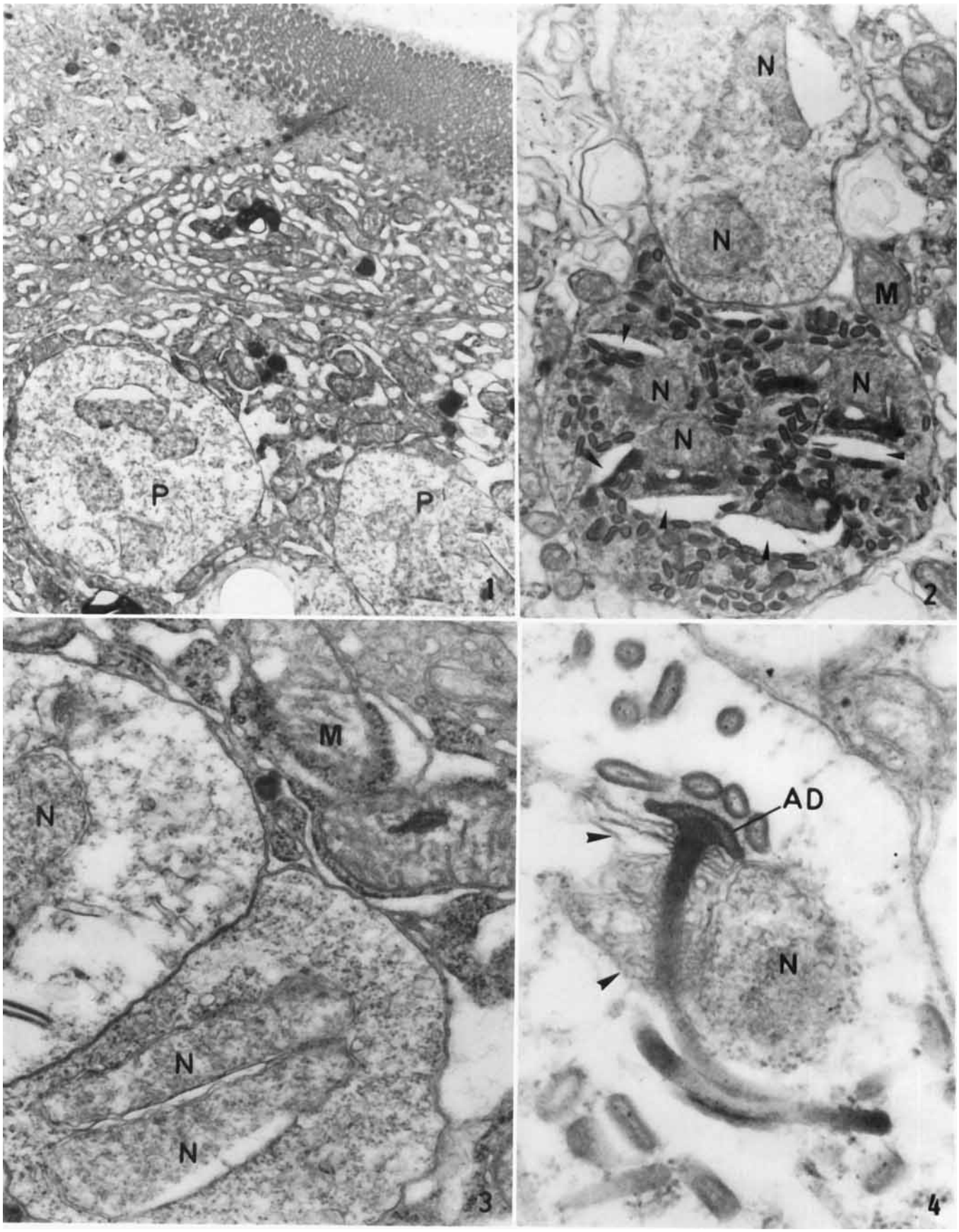
Figs. 1–4. Developmental stages of *Enterocytozoon bieneusi* n. g., n. sp. **1.** Two plasmodial stages within the enterocytes. P, plasmodia. ×7000. **2.** Two sporogonial plasmodia. One (top) at the beginning of the sporogony (nuclear multiplication), the other (bottom) during the formation of the polar tubes. The dark bodies correspond to sections of the latter. The electron-lucent vesicles are indicated by arrowheads. N, sections of nuclei. ×23,000. **3.** Young stages, one of them with two sections of nuclei in a diplokaryotic arrangement. N, nucleus, M, mitochondrion of the host cell. ×32,000. **4.** Longitudinal section of a differentiating extrusion apparatus. The anchoring disc (AD) and the anterior part of the polar tube are formed. The vesicular and lamellar parts of the polaroplast (arrowheads) are apparently originating from a reticular Golgi and ER issued from the envelope of the plasmodial nucleus (N). ×65,000.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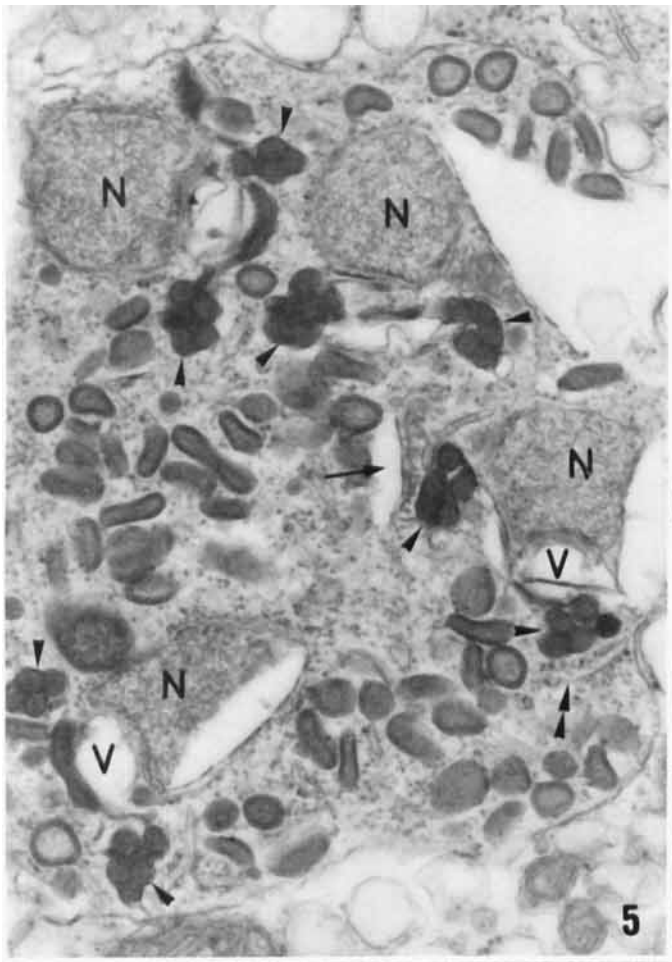

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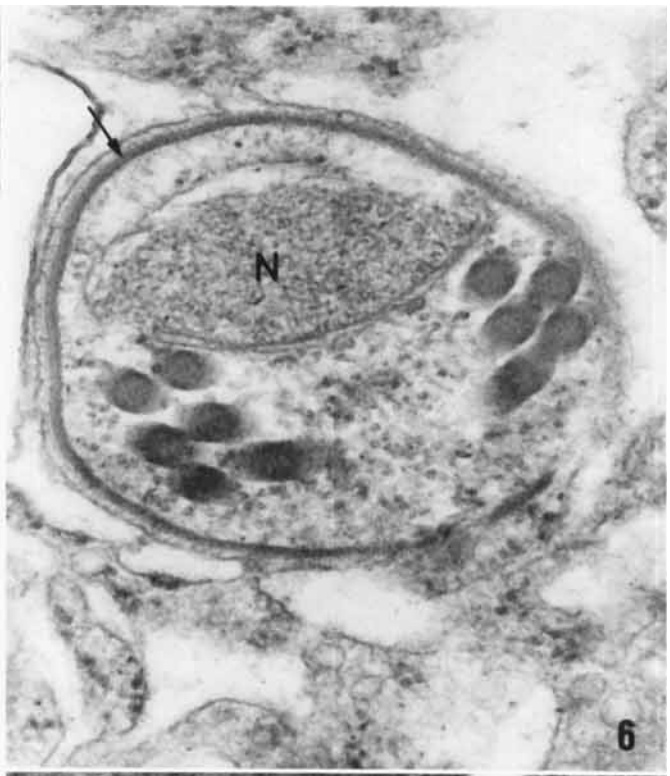

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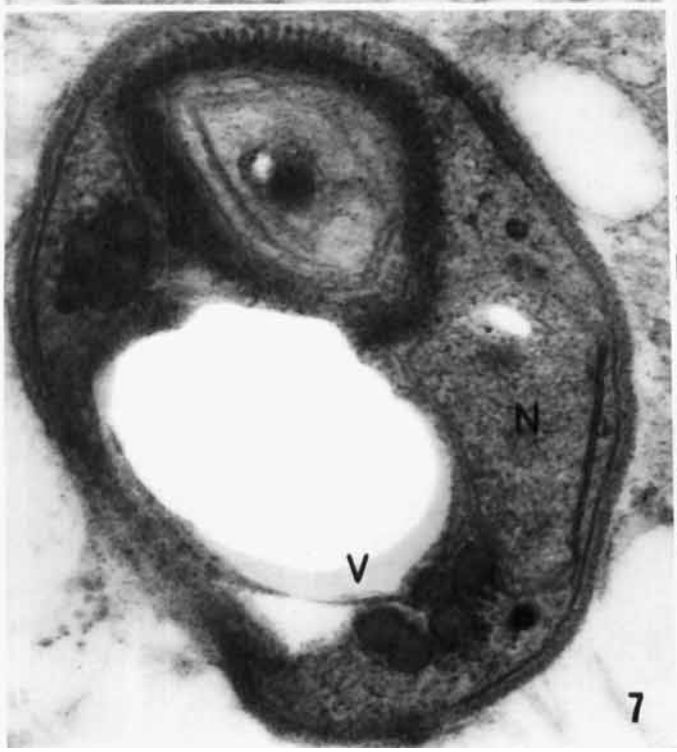

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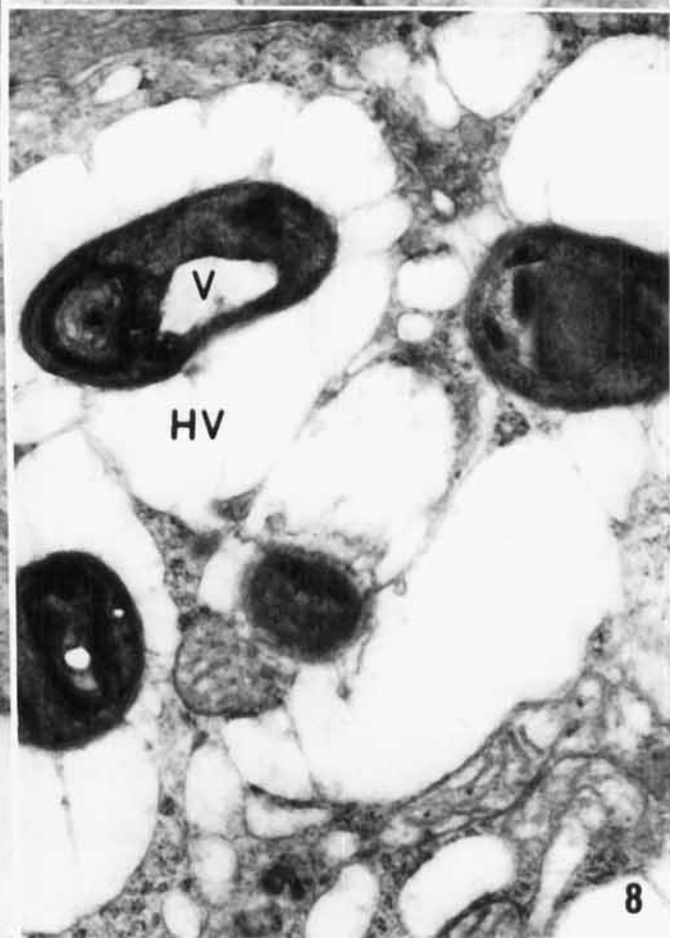

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rogonial plasmodium divides into about eight sporoblasts, each containing a nucleus and the corresponding extrusion apparatus (Fig. 6). The fission of the sporogonial plasmodium was not observed and probably occurs very rapidly. It may result from the enlargement and fusion of flattened vesicles, which appeared during the differentiation of the polar tube (Figs. 2, 5).

After the fission, the sporoblasts are in direct contact with the host cytoplasm, surrounded by the highly developed vacuolar system of the enterocyte. These vacuoles correspond to the dilated ER vesicles of the latter cell. The sporoblasts are bounded by a single membrane, originating partly from the plasmodial membrane and possibly partly from elements of the ER. The sporoblast membrane is covered with a layer of dense material (Fig. 6). The latter corresponds probably to the outer proteinaceous layer of the spore wall, i.e. the exospore (4), which is the layer first differentiated in the microsporidan spores.

The transformation of the sporoblasts into mature spores results from the retraction and condensation of their cytoplasm and nucleus. The dense spores measure 1.5 μm × 0.5 μm (Fig. 7). They exhibit the typical structures of the microsporidan spores, i.e. the vesicular and lamellar polaroplast and a polar tube, forming a coil (4); however, the inner layer of the spore wall, i.e. the endospore, is less well developed than in other genera in the order Microsporida (Figs. 7, 8). Alongside the nucleus a lucent vacuole is frequently observed, which probably corresponds to the vesicle previously differentiated in the sporogonial plasmodia and closely associated with each nucleus (Fig. 4). It is noteworthy that the retraction of the maturing spores results in the enlargement of the peripheral vacuolar system of the host cell (Fig. 8).

DISCUSSION

Two microsporidan genera have been reported in man: *Nosema* with the species *N. connori* (7, 14) and *Encephalitozoon* (1, 2, 6, 8, 12, 16, 18). The latter genus is sometimes confused with *Nosema* (10) and *Toxoplasma* (2, 16, 18), especially when they give rise to encephalitis. *Nosema* is characterized by the diplokaryotic arrangement of the nuclei during all the life cycle (14). In *Encephalitozoon,* the developmental stages are monokaryotic and sporulation occurs in a large parasitophorous vacuole (10). In both genera, the sporont divides to produce two spores and the sporulation sequence occurs without the formation of a sporophorous vesicle (pansporoblastic membrane). They are therefore included in the suborder Apansporoblastina (4, 15, 17).

The parasite reported here does not exhibit the diplokaryotic nuclei in the sporulation sequence characteristic of the genus *Nosema.* It does not develop within the parasitophorous vacuole characteristic of *Encephalitozoon.* Furthermore, the sporont is represented by a multinucleate sporogonial plasmodium giving rise to about eight spores rather than the two spores as in these other genera. The absence of the pansporoblastic membrane suggests, however, that it may be placed in the suborder Apansporoblastina.

Seven families were distinguished within the latter suborder according to differences in their development (15). The family Pereziidae exhibits a development somewhat similar to that of the microsporidan of the enterocytes: 1) The developmental stages are in direct contact with the host cytoplasm. 2) The parasites are diplokaryotic at the beginning of the life cycle and monokaryotic during the sporulation sequence. 3) Sporogony is polysporoblastic—probably octosporoblastic—by multiple fission of sporogonial plasmodia.

The parasite of the enterocytes differs from those of the family Pereziidae by the following characters: 1) Their sporogonial plasmodia are spherical whereas those of Pereziidae are moniliform. 2) The spore wall is very thin, the endospore being absent or poorly differentiated. 3) The extrusion apparatus is differentiated before the division of the sporogonial plasmodia into sporoblasts. This character has not been reported previously and therefore justifies the creation of a new genus and species for the parasite of the enterocytes. The name *Enterocytozoon bieneusi* is proposed in reference to the localization in the host of the microsporidan and in memory of the patient.

Microsporidan spores have been reported in the enterocytes of the monkey *Callicebus moloch* (13). These spores were larger than those described here and seven cross sections of coiled polar tube were demonstrated instead of four or five as in the present species; however, they exhibit similar features such as the elongate shape, the inner vacuole, and a rather thin spore wall. It would be tempting to suppose that the microsporidan infestation of our immuno-deficient patient was of animal origin. If so, *E. bieneusi* might be related to the species from the *Callicebus* monkey; however, this interpretation remains purely speculative in the absence of other documented stages in this report (13).

TAXONOMY

Diagnosis of Enterocytozoon *n. g.*

Developmental stages in direct contact with host cytoplasm. Proliferative diplokaryotic cells develop into sporogonial plasmodia which produce (probably eight) sporoblasts by multiple fission. The extrusion apparatus is formed before fission of the sporogonial plasmodia. Spores lack a well developed endospore and contain an electron-lucent vacuole.

Type species *Enterocytozoon bieneusi* n. sp. with spore measuring 1.5 μm × 0.5 μm (in sections) and 4–5 turns of the polar tube coil. In enterocytes of a man with AIDS.

Enterocytozoon bieneusi is placed in the suborder Apansporoblastina Tuzet et al. (order Microsporida, class Microsporea, phylum Microspora) (4, 15). The family has not yet been determined despite resemblances to the life cycle of the Pereziidae, species of which are known from marine invertebrates (15).

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Figs. 5–8. Formation of the spores of *E. bieneusi* n. g., n. sp. **5.** The sporogonial plasmodium before the fission. Each nucleus (N) is associated with a vesicle (V) and two sets of sections of the 4–5 coils of the corresponding polar tube (arrowheads). Granular cisternae of the ER (double arrowhead) associated in some cases with a vesicle (arrow) are seen in the vicinity of each differentiating sporoblast. ×40,000. **6.** Sporoblasts after the fission of the sporogonial plasmodium. This stage is characterized by the occurrence of the spore wall (exospore) (arrow). ×84,000. Fig. 7. Section of a mature spore showing the nucleus (N), the lucent vacuole (V), five sections of the polar tube coils (bottom) and the lamellar polaroplast (top) with a peripheral layer of ribosomes. ×84,000. **8.** Mature spores surrounded by vesicles (HV) of the host ER. The enlargement of the latter is an effect of the retraction of the sporoblasts when they condense into mature spores. ×30,000.

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PKX, the Causative Agent of Proliferative Kidney Disease (PKD) in Pacific Salmonid Fishes and Its Affinities with the Myxozoa[1]

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ABSTRACT. Proliferative kidney disease (PKD), caused by an unclassified protozoan (PKX), is reported from Pacific salmon, *Oncorhynchus tshawytscha* (Walbaum) and *O. kisutch* (Walbaum), and steelhead trout, *Salmo gairdneri* Richardson, held at the Mad River Hatchery in California, USA. The cumulative mortality attributed to the disease was 95, 13, and 18% respectively. The mortalities were greatest at mean water temperatures of 12–14°C during July 1983. The ultrastructure of the PKX organism and its associated pathology during clinical disease in all three species were consistent with those of the parasite in rainbow trout (*Salmo gairdneri*) as described in European outbreaks. Significant mortalities did not occur after August, at which time the parasite could no longer be detected in the salmon species. The steelhead continued to exhibit parasites in the kidney interstitium and epithelium and lumens of the tubules. Myxosporidan trophozoites and developing spores were also observed in the lumens of the kidney tubules of these fish. Although a mixed infection with another parasite may have occurred, evidence suggests that the myxosporidans are later stages of PKX. They were only observed in fish exposed to water with the infective stage and were particularly prominent in recovering fish. The PKX organism is similar to UBO, an unclassified protozoan of carp suspected to be an early stage of *Sphaerospora renicola* Dyková & Lom. Both parasites infect the blood and kidney, divide by endogeny, and are released by disintegration of the primary mother cell. The intraluminal myxosporean forms show similarities to *Sphaerospora* spp. in that they are monosporous and sporoblasts are formed within pseudoplasmodia. It is possible that PKX migrates to the lumen of the kidney tubule and subsequently sporulates. If the myxosporean forms are later stages of PKX, then it would belong to the phlyum Myxozoa.

PROLIFERATIVE kidney disease (PKD), caused by an unclassified protozoan (PKX), has become one of the most important diseases of cultured salmonids in Europe (3, 25). In 1981 the first epizootic of the disease in North America was detected in rainbow trout from the state of Idaho (26). It was subsequently identified in Pacific salmon and steelhead trout (*Salmo gairdneri*) in California (11) and in Vancouver Island, B.C., Canada, by G. Hoskins (pers. commun.). The disease primarily affects underyearling (0+) usually during the summer when water temperatures increase. Mortalities due to PKD may range from 10–95% (3, 11, 25).

The most consistent signs of PKD are exophthalmia, anemia, abdominal swelling, and kidney hypertrophy. The PKX organisms are found primarily in the kidney and may cause tubular atrophy, vasculitis, and a granulomatous nephritis. An increased number of macrophages and lymphocytes in the kidney is characteristic and often these surround the parasites (9). The PKX organism is also frequently observed in the gills and other visceral organs, and apparently these sites become involved via the circulatory system (9).

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