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Blastocystis hominis Revisited

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INTRODUCTION

Despite more than 80 years of debate since the first accepted descriptions of the genus *Blastocystis* (2, 29), not a single issue about this organism has been satisfactorily resolved. Substantial progress has been made in the description of the morphology of *Blastocystis hominis* during recent years, but structures and organelles present in the cell remain of unknown function. The method(s) of division is questioned. The pathogenicity of the organism has not been determined, and the significance of *B. hominis* in immunocompromised patients has not been ascertained. The necessity for treatment and the most appropriate chemotherapeutic strategies have not been defined.

The taxonomy of the organism remains controversial, and it has not been determined if a single species or multiple species exist in human and animal hosts. Hence, in this review, we will refer to *B. hominis* as the parasite isolated from human hosts and to *Blastocystis* spp. as the parasite isolated from other hosts. The life cycle has not been conclusively demonstrated. The mode of transmission of *B. hominis* has not been proven experimentally, and so preventative measures cannot be accurately formulated. *Blastocystis* spp. infections appear to be common in nonhuman hosts, but it is not known if the parasite is zoonotic.

Many of the dogmas currently held are based on minimal factual data, often collated from early reports and uncritical anecdotes. Most of these are based upon superficial light microscopy observations or single case reports. The scarcity of valid experimental data, the large number of conflicting reports, and the perpetuation of unsubstantiated statements have impeded the appraisal of the status of this organism. In this review, we have attempted to provide a balanced overview of the data relating to the identification and clinical relevance of *B. hominis*, concentrating primarily on publications of recent years. A detailed history of *B. hominis* may be found in the review by Zierdt (228), and further information on the morphology and biology of *Blastocystis* spp. from nonhuman hosts may be found in the review by Boreham and Stenzel (23).

History

The first paper to clearly define the genus *Blastocystis* as a distinct organism was presented by Alexeieff (2), who proposed

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the name *B. enterocola*. An extensive morphological description was given, and a life cycle was presented. Brumpt (29) proposed the name *B. hominis* for the organism isolated from human fecal material, and this is the name recognized in the current literature.

After a number of reports of *B. hominis* isolated from human fecal material, particularly in tropical countries, and suggestions of pathogenicity (90, 116, 122, 142, 209), very few data were published until the work of Zierdt et al. (231) renewed interest in the organism. Since that time, a large number of case reports of *B. hominis* has been presented but very few experimental studies have been conducted.

Taxonomy

The taxonomy of *Blastocystis* spp. remains controversial, and the history of the organism reflects the difficulty in defining its taxonomic position. The ultrastructural study by Zierdt et al. (231) provided the first indisputable evidence that the organism was not a yeast or a fungus, as previously suggested by Alexeieff (2), Brumpt (29), O'Connor (142), Beaurepaire-Aragão (11, 12), Knowles and Das Gupta (99), and Lavier (108), or the cyst of another organism, such as a *Trichomonas* spp., as proposed by Bensen (20), Bohne and Prowazek (21), Haughwout (80), and James (90). It is not a degenerating cell, as had been suggested by Swellengrebel (190), and has been shown to be morphologically distinct from *Dientamoeba fragilis* (47). Further ultrastructural studies have supported these conclusions (23, 49, 215, 228).

Morphologically, the *Blastocystis* cell shows protistan features: it contains one or more nuclei, smooth and rough endoplasmic reticulum, Golgi complex, and mitochondrion-like organelles (23, 49, 191, 192, 215, 224, 228, 231). A number of morphologically distinct forms of the organism have been described (22, 23, 49, 179, 192, 228, 231). Forms of *Blastocystis* spp. other than the cyst form (132, 177, 220) are not surrounded by a cell wall but are enclosed by a bilaminar membrane. Physiologically, *Blastocystis* spp. fail to grow on fungal medium, grow optimally at 37°C and at neutral pH, and are not killed by antifungal agents such as amphotericin B (48, 223–226, 231, 236). *Blastocystis* spp. are strict anaerobes and are sensitive to oxygen and to changes in the tonicity of their environment (231). Antibacterial agents such as ampicillin, streptomycin, and gentamicin do not appear to adversely affect the growth of *Blastocystis* spp. (49, 224), whereas a number of antiprotozoal agents appear to inhibit their growth in vitro (48, 233).

Zierdt (224) classified *Blastocystis* in the subphylum Sporozoa on the basis of proposed methods of division, in addition to morphological and culture characteristics. A new class, Blastocystea, and a new order, Blastocystida, were suggested. More recently, Zierdt (227) has reclassified the organism to the subphylum Sarcodina, order Amoebida, in a separate suborder, Blastocystina. However, data to support this reclassification have not been provided.

Molecular sequencing studies on a single axenic human isolate (Netsky stock [232]), performed by small-subunit rRNA sequencing techniques, have shown that *B. hominis* is not monophyletic with the yeasts *Saccharomyces* spp., the fungi *Neurospora* spp., the amebae *Naegleria*, *Acanthamoeba* and *Dictyostelium* spp., or the flagellates *Trypanosoma* and *Euglena* spp. (94). The apicomplexans *Sarcocystis* and *Toxoplasma* spp. were found to be monophyletic but were split from *B. hominis* by the ciliates *Oxytricha*, *Stylonichia*, *Tetrahymena*, *Paramecium*, and *Euplotes* spp. and the dinoflagellate *Prorocentrum* sp. These data suggest that *B. hominis* is not closely related to the yeasts, fungi, sarcodines, or sporozoans.

Jiang and He (93) subsequently proposed a new subphylum Blastocysta, which included the class Blastocystea, the order Blastocystida, the family Blastocystidae, and the genus *Blastocystis*, with *B. hominis* as the type species. While it is apparent that *Blastocystis* does not fit well into the existing protozoan classification, there is insufficient evidence to erect a new classification for the organism (22, 39, 86). Further morphological and molecular data are required, as is clarification of the effect of the physiological state on the ultrastructural appearance of the organism (229). Recent debates on the systematics of the protists (39, 40) have not included the genus *Blastocystis* in any of the suggested classifications.

Questions such as the designation of species of *Blastocystis* and the means by which these species may be differentiated have not been adequately resolved. Four species of *Blastocystis* from nonhuman hosts have been proposed in the recent literature: *B. galli* (18) from chickens, *B. anatis* (13) from domestic ducks, *B. anseri* (15) from domestic geese, and *B. lapemi* (197) from a sea snake. The species isolated from birds were identified by morphological criteria, but the variation seen among individual organisms in isolates from humans (49, 228) suggests that these designations should be regarded cautiously until further confirmatory evidence is obtained. *B. lapemi* was differentiated from *B. hominis* by having different optimal culture requirements (*B. lapemi* grows best at 24°C rather than at 37°C) and a different electrophoretic karyotype from *B. hominis* (197).

Recent data indicate that different groups of *Blastocystis* isolates can be distinguished in human hosts, and this has raised the possibility that more than one species of *Blastocystis* infect humans. However, at this time it is germane to regard these groups as demes in accordance with the nomenclature developed for trypanosomes, where demes are defined as "populations that differ from others of the same species or subspecies in a specified property or set of properties" (211).

Kukoschke and Müller (102) separated four isolates of *B. hominis* from human fecal samples into two distinct demes on the basis of immunological reactions and on polypeptide patterns obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of protein extracts. A more extensive study of 61 isolates identified four serologically distinct demes (138). Analysis of 10 isolates by Boreham et al. (25) also revealed at least two distinct demes. Proteins of the demes were immunologically distinct in immunoblots, and hybridization with random probes generated from the DNA of one stock showed that the DNA content of the two demes also was different (25). At least two zymodemes of *B. hominis* have been distinguished on the basis of isoenzyme patterns of nine isolates (121).

Electrophoretic karyotyping of seven isolates of *B. hominis* indicated that considerable genotypic heterogeneity occurred between isolates (202). However, similarities were noted between one xenic stock isolated in Australia and an axenic stock isolated in the United States more than 10 years previously. In a later study (84), five isolates of *B. hominis* from Singapore showed only minor variations in karyotypic patterns. The number and size of chromosomes identified differed between the two studies (84, 202).

Despite the differences noted, there is insufficient evidence to designate new species of *Blastocystis* from humans without further biochemical and epidemiological data. Therefore, *B. hominis* is the only species of *Blastocystis* which is currently accepted to be present in human hosts.

BIOLOGY

Morphology

Reports of the morphology of *B. hominis* from culture samples commonly have noted three major forms (vacuolar, granular, and ameboid) of the organism (23, 228, 231). A number of reports of *B. hominis* from fecal material and from the host intestine have indicated that additional forms of the organism exist (23, 132, 176, 177, 179, 220, 234), although these forms have not yet been included in diagnostic texts.

Light microscopy provides a rapid method for detecting *Blastocystis* spp. in samples, but almost all of the new information on the cell biology and life cycle of *B. hominis* has resulted from transmission electron microscopy. Recent ultrastructural studies indicate that a continuum of morphological forms of *B. hominis* exists, with the appearance of the organism being dependent upon the environmental conditions (23, 47, 49, 176, 179). Physical factors, such as osmotic changes, the presence of certain drugs, and metabolic status, may influence the morphology of the organism both in vivo and in vitro.

This variation in morphology has important implications for diagnosis, because *B. hominis* in fecal samples usually is identified by the presence of forms approximately 10 to 15 μm in diameter with a large central vacuole. However, recent studies have indicated that this form is not the most predominant form of *B. hominis* present in fresh fecal samples (26, 176, 179). The presence of smaller forms, including multivacuolar and cyst forms (approximately 5 μm in diameter) in fecal samples (23, 132, 176, 177, 179, 220), suggests that many *B. hominis* infections may be missed during laboratory examinations. The lack of information on forms of *B. hominis* other than the vacuolar form, combined with the small size of other forms and the disparate morphology of the organism in some samples, complicates identification, even for experienced laboratory personnel.

Vacuolar and granular forms. The vacuolar form (Fig. 1a and 2a), also referred to as the vacuolated or "central-body" form of *B. hominis* (228), has been considered to be the typical *Blastocystis* cell form. This is the form generally used for diagnosis of *B. hominis* (5, 69, 70). It is the predominant form of the organism in culture (129, 231) and is also found in fecal samples.

The granular form of *B. hominis* (Fig. 1b and 2c) has an ultrastructure similar to that of the vacuolar form, apart from having morphologically and cytochemically different central vacuole contents (49, 192). It does not appear necessary to evoke the concept of a distinct form for the granular cell but, rather, to consider it to be a vacuolar form with granules in the central vacuole. Hence, the morphology of these two forms will be described together.

Vacuolar and granular forms usually are spherical cells, although irregularly shaped cells may be present in culture samples (Fig. 1a and b). Considerable heterogeneity occurs in the ultrastructure of vacuolar and granular forms of *B. hominis* from different isolates, but the ultrastructural appearance is consistent and stable for each isolate, even after prolonged in vitro culture (49). Vacuolar forms vary greatly in size, ranging from 2 μm (204) to more than 200 μm (235) in diameter, with the average diameter of cells usually being between 4 and 15 μm (228). Granular forms often are slightly larger than the average vacuolar forms, and diameters of 10 to 60 μm (231), 15 to 25 μm (223), 3 to 80 μm (236), and 6.5 to 19.5 μm (49) have been reported.

The vacuolar and granular forms display a thin peripheral band of cytoplasm surrounding a large central vacuole (Fig. 2a and c). Long membrane-enclosed cytoplasmic strands, often containing organelles, may project into the central vacuole of vacuolar forms present in fecal material (176, 179, 185). These may be the "endosymbionts" noted by Zierdt and Tan (235).

There is considerable variation in the morphology of the vacuolar contents. In the vacuolar form, the central vacuole usually contains finely granular or flocculent material which is distributed unevenly throughout the vacuole (49). In the granular form, the central vacuole may contain granules of several morphological types (Fig. 2d). Initially, two granule types were proposed on the basis of light microscopy studies (231). It was suggested that one granule type developed into daughter *B. hominis* cells while the other had a role in metabolism (231). Later, a transmission electron microscopy study indicated that three types of granules, described as metabolic, lipid, and reproductive granules, were present in the cells (192). It was reported that the metabolic granules were cytoplasmic, the lipid granules were found in the central vacuole and cytoplasm, and the reproductive granules were present in the central vacuole (223). These observations have not been confirmed, and the study by Dunn et al. (49) described myelin-like inclusions, small vesicles, crystalline granules, and lipid droplets in the central vacuole of the granular form. Lipid droplets also were noted in the cytoplasm (49). Granules that appear similar to those in the central vacuole have been found in small vacuoles and vesicles in the cytoplasm of granular cells (49, 217).

The central vacuole has been reported to function in endodyogeny and schizogony via the development of reproductive granules (227, 228). This has not been supported by further studies. It appears more likely that the granules seen in the central vacuole are the result of metabolism or storage within the cell (49, 182). A suggestion that the granules are mitochondria (228) can be rejected on the basis of morphological evidence.

A surface coat, which also has been called a slime layer or capsule (231), may surround some *B. hominis* cells in culture. The surface coat varies in thickness and morphology (49, 215), and appears to be altered by laboratory culture, becoming thinner or absent after longer culture periods (176, 179). Coated pits are present on the cell membrane (49, 182, 192).

The organelles usually are found in thickened areas of cytoplasm (Fig. 2b), often appearing at opposite poles of the cell. These areas of cytoplasm may protrude into the central vacuole (49, 228) or extend outward to give the cell an irregular outline (49). Most organelles appear to be simple representations of their type. Mitochondrion-like organelles that vary in morphology and number and usually contain small numbers of sacculate cristae are present (49). A Golgi complex is present adjacent to the nucleus and generally appears as a few cisternal stacks with associated vesicles (49, 215).

Multiple nuclei are present in some cultured cells from most isolates, with up to four reported to be common (49, 129, 223). The nuclei are approximately 1 μm in diameter and are surrounded by a nuclear envelope with nuclear pores (176, 215, 217). A crescentic band of electron-opaque material, characteristic of all forms of *B. hominis* (23, 49, 129, 192, 215, 227, 228, 231, 234), is noted in electron micrographs of the nuclei, and occasionally an additional spot of electron-opaque material is seen (23).

A number of culture conditions are known to induce the production of the granular form from the vacuolar form. These include increased serum concentrations in the culture medium (49, 108, 171, 223), transfer of the cells to a different culture medium (47, 179), axenization of the culture (236), and addition of some antibiotics, such as norfloxacin and amphotericin B (47). Occasionally, the granular form has been seen in cultures without increased serum concentration (47, 49), but usu-

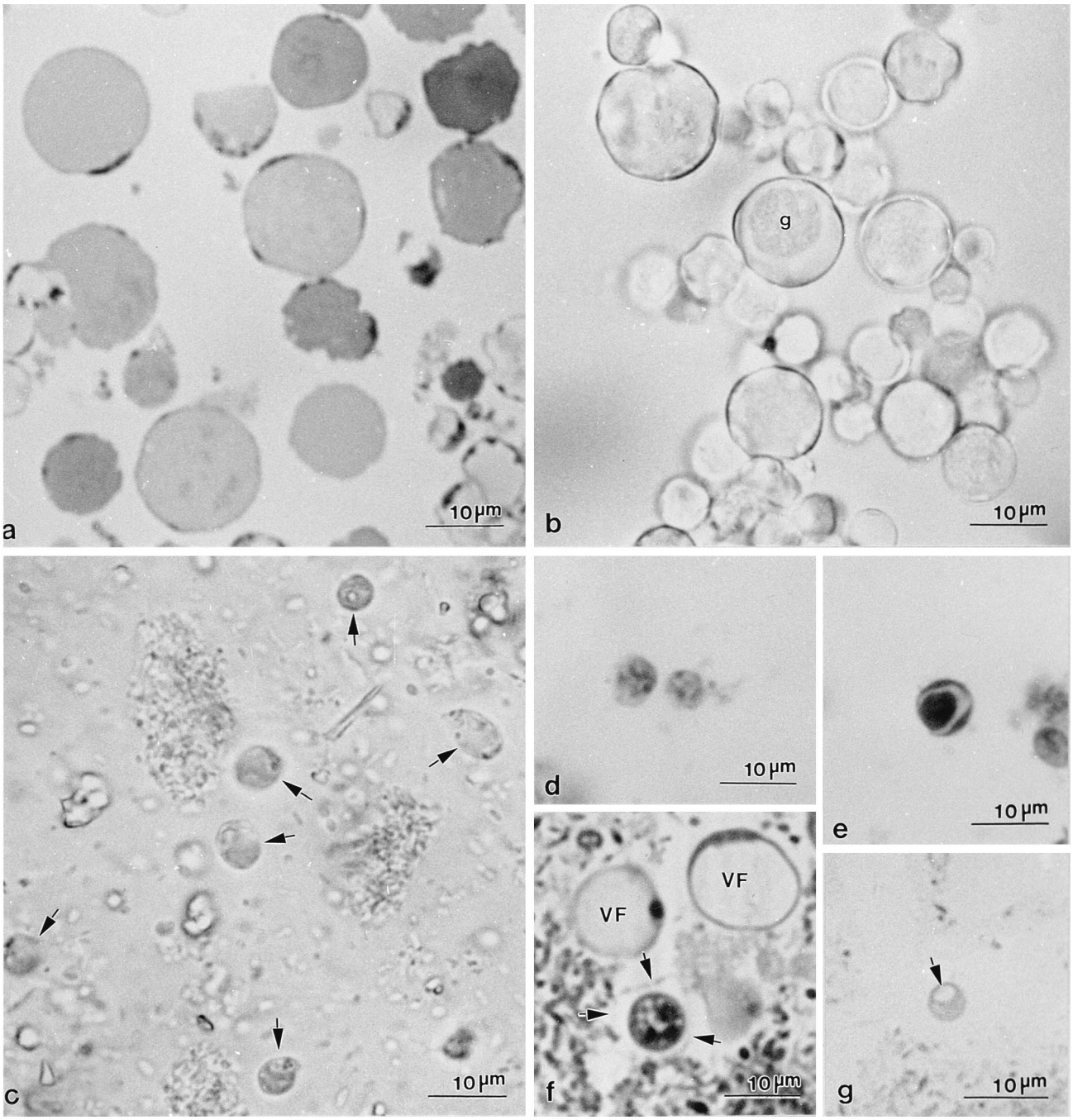

FIG. 1. Light micrographs of *B. hominis*. (a) Vacuolar forms from laboratory culture. Glutaraldehyde fixed, 1-μm epoxy resin section, toluidine blue stain. (b) Granular forms from laboratory culture. Accumulations of granules (g) are noted in the central vacuole of the cells. Glutaraldehyde fixed, wet mount, unstained. (c) Small vacuolar or multivacuolar forms (arrows) in fresh fecal material. Glutaraldehyde fixed, wet mount, unstained. (d) Small multivacuolar forms in fresh fecal material. Polyvinyl alcohol fixed, fecal smear, trichrome stain. (e) Small vacuolar form in fresh fecal material. Polyvinyl alcohol fixed, fecal smear, trichrome stain. (f) Cyst form from laboratory culture, showing remnants of an outer layer surrounding the organism (indicated by arrows). Two vacuolar forms (VF) also are noted in this micrograph. Glutaraldehyde fixed, 1-μm epoxy resin section, toluidine blue stain. (g) Cyst form in fresh fecal material. A large, unstained region (arrow) is noted in the cytoplasm. Glutaraldehyde fixed, 1-μm epoxy resin section, toluidine blue stain.

ally only a few cells exhibit the granular appearance. Conflicting data have been presented suggesting that the granular form may be more numerous in older cultures (231) or in short-term cultures (236).

Although a number of methods for division of the vacuolar and granular forms have been described (23, 93, 228, 229), binary fission is the only demonstrated method of reproduction of these forms of *B. hominis*. The cells have been noted to divide into two approximately equal portions, with distribution of organelles into both portions (49, 129, 131).

Multivacuolar and avacuolar forms. The morphology of *B. hominis* present in fresh human fecal material (Fig. 1c to e and 3) may differ significantly from that of the culture forms (49, 228). Rather than a large single vacuole, as is present in

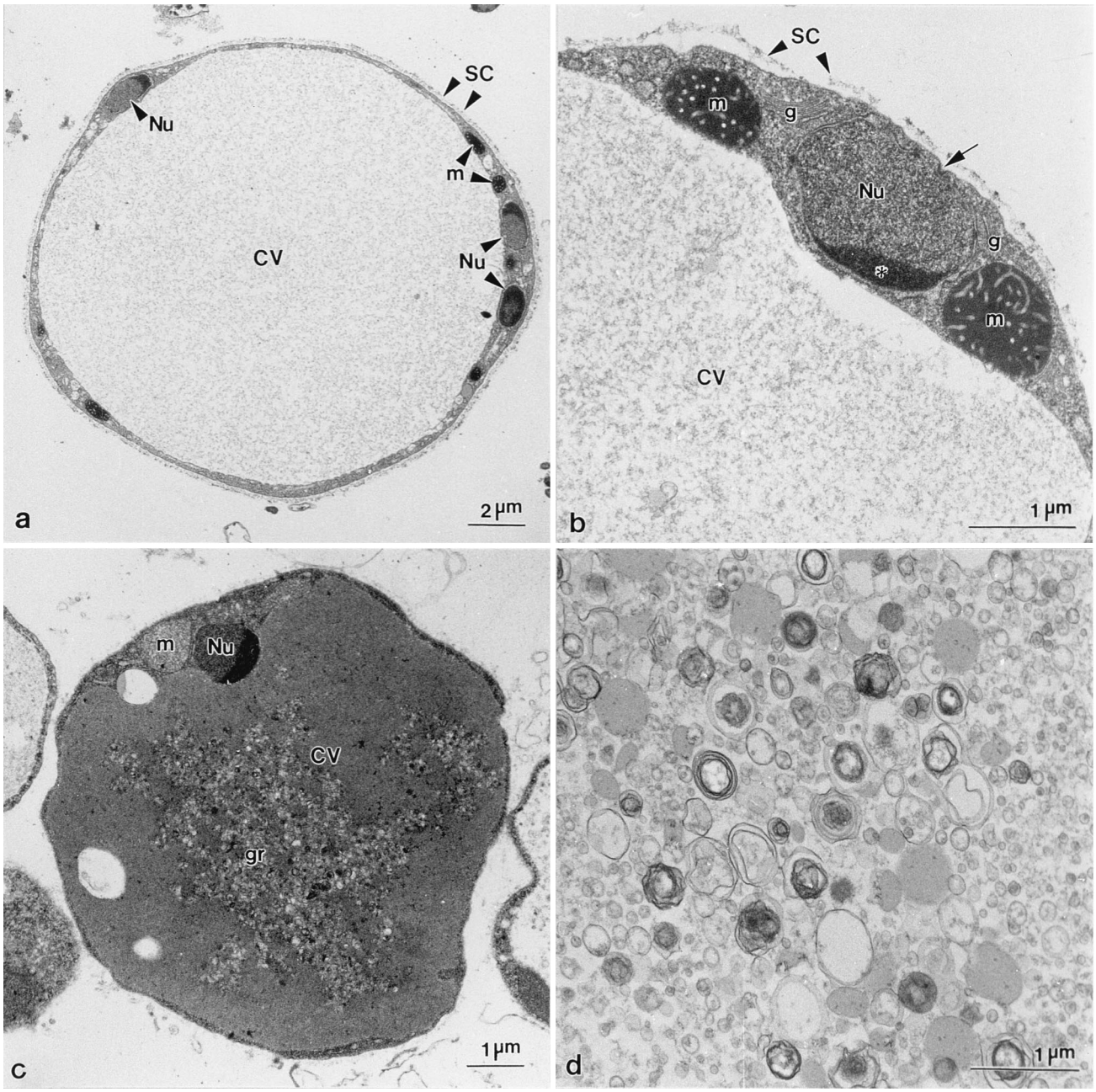

FIG. 2. Transmission electron micrographs of *B. hominis* from laboratory culture. (a) Vacuolar form showing a large central vacuole (CV) surrounded by a thin rim of cytoplasm. Cellular organelles are noted in the cytoplasm. Three nuclei (Nu) are seen in this ultrathin section of the cell. SC, surface coat. (b) High-magnification micrograph, showing details of the nucleus (Nu) and surrounding structures. A crescentic band of electron-opaque material (asterisk) is noted in the nucleus. Mitochondrion-like organelles (m) and the Golgi complex (g) are seen. Coated pits (arrow) are found on the outer cell membrane. (c) Granular form, showing clumps of granules (gr) in the central vacuole (CV). (d) High-magnification details of granules found in the central vacuole of the granular form. Small vesicles, membranous material, myelin-like whorls, and lipid inclusions are noted.

cultured cells, multiple small vacuoles of different sizes and morphologies frequently are noted within *B. hominis* cells from fecal material (26, 176, 179). These multivacuolar forms are smaller (approximately 5 to 8 μm in diameter) than the "typical" vacuolar or granular forms. Information from early reports on the morphology of *B. hominis* suggests that smaller forms were noted in fecal material (11, 12, 117, 130) but were not recognized in the subsequent literature.

It has not been determined whether the small vacuoles present in the multivacuolar forms of *B. hominis* are distinct entities or an interconnected network, although some interconnections do occur (179). Serial ultrathin sectioning of the entire organism would be necessary to demonstrate whether all vacuoles are interconnected, because the vacuoles are too small to be resolved by light or confocal microscopy.

One nucleus (or occasionally two nuclei) has been found in multivacuolar forms of *B. hominis* (179). With few exceptions (176), a crescentic band of electron-opaque material was present at one pole of the nucleus.

A thick surface coat (Fig. 3) surrounded all multivacuolar

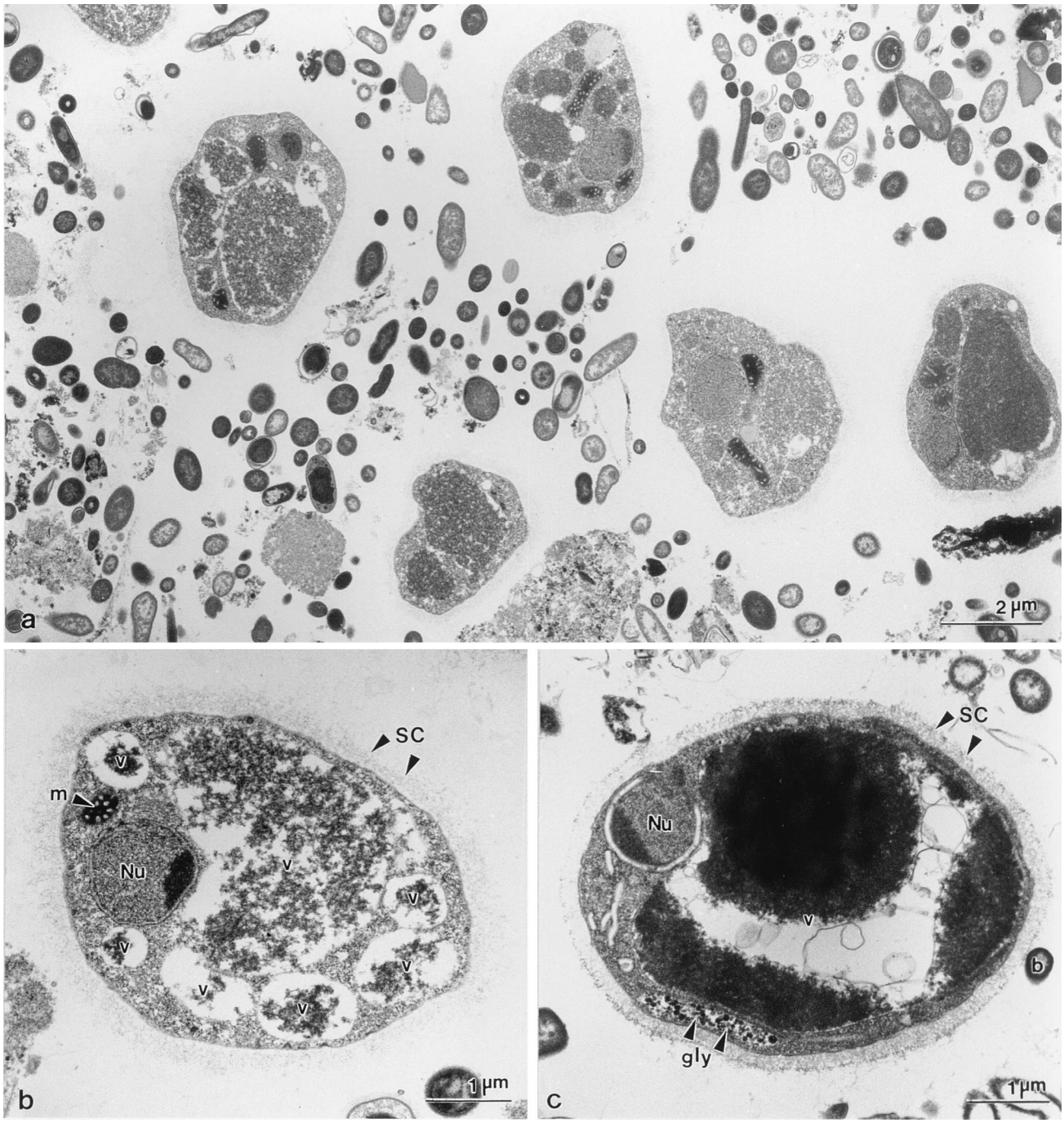

FIG. 3. Transmission electron micrographs of *B. hominis* in fresh fecal material. (a) Multivacuolar forms, demonstrating the variable appearance of the vacuoles and their contents. (b) Multivacuolar form, surrounded by a thick fibrillar surface coat (SC). (c) Small, apparently vacuolar form, showing an accumulation of glycogen (gly) in the cytoplasm. A bacterium (b) is noted in close association with the thick surface coat. Nu, nucleus; m, mitochondrion-like organelle; v, vacuole.

forms of *B. hominis* found in human fecal material (176, 179) and was much thicker than that seen on the cultured forms of *B. hominis* (49, 176, 179). Coated pits were present on the cell membrane (176, 179). Bacteria often were closely associated with the surface coat (33, 176, 179) (Fig. 3c) but were not noted to be internalized by *B. hominis* (176, 179).

Morphological heterogeneity was seen in *B. hominis* isolates from fresh fecal samples (176, 179). This may indicate the presence of different demes or even different species of *Blastocystis* in the host. However, it is more likely to represent a continuum of developmental stages or changes due to differing environmental conditions. After short-term laboratory culture of fecal material which contained multivacuolar forms of *B. hominis*, only vacuolar or granular forms were found (176, 179). After longer periods of culture, only vacuolar forms were found (176, 179).

Two reports in the recent literature have described the form of *B. hominis* thought to be representative of the organism

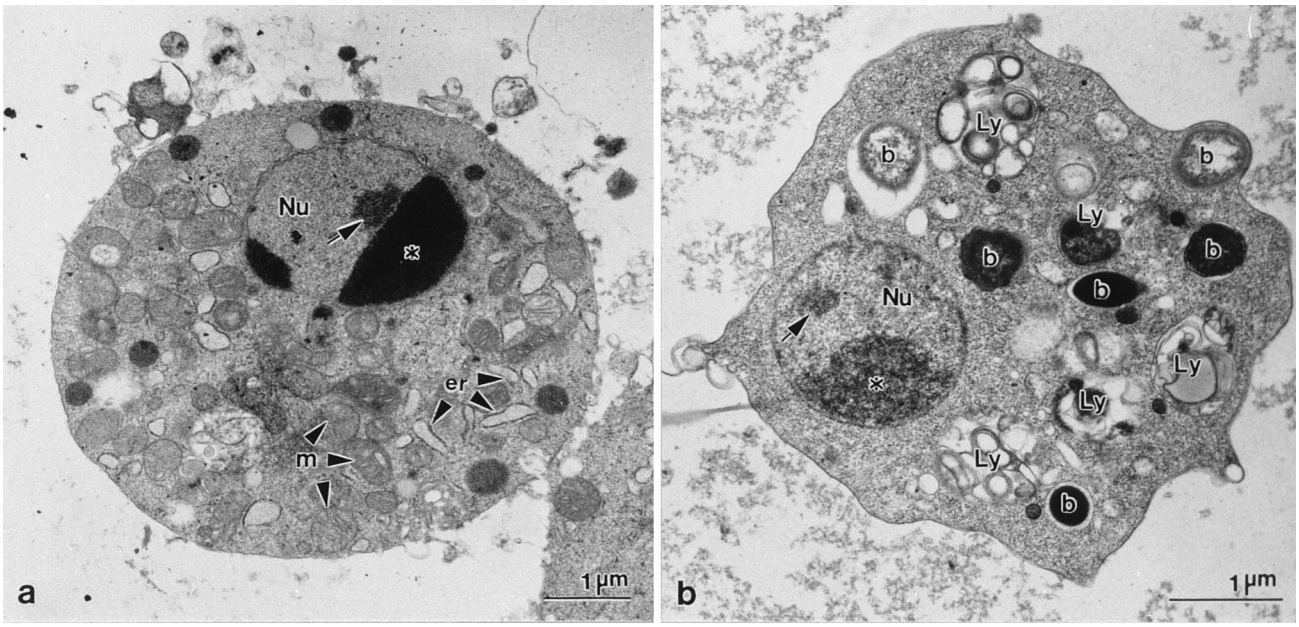

FIG. 4. Transmission electron micrographs of *B. hominis*. (a) Avacuolar form obtained at colonoscopy. In addition to a crescentic band (asterisk), the nucleus (Nu) contains clumps of electron-opaque material (arrow). Mitochondrion-like organelles (m) and rough endoplasmic reticulum (er) are abundant in the cytoplasm. (b) Ameboid form, showing numerous lysosome-like bodies (Ly) in the cytoplasm. Bacteria (b) are seen within these bodies and in other membrane-bounded structures. The nucleus contains a compact band (asterisk) and clumps (as indicated by arrow) of electron-opaque material.

within the intestines of human hosts (Fig. 4a). These reports have noted a similar morphology for *B. hominis* organisms obtained from a patient producing large volumes of diarrheal fluid (234) and from a sample taken at colonoscopy (179). Zierdt and Tan (234) believed that these organisms were the trophozoite form of *B. hominis*, while Boreham and Stenzel (22, 23) have referred to these forms as the avacuolar form. The organisms were smaller (approximately 5 μm in diameter) than *B. hominis* forms from culture (49, 228) and did not contain a central vacuole. The cells contained one or two nuclei, which often were slightly larger than the nuclei reported in the culture forms of *B. hominis*. A crescentic band and often an additional spot of electron-opaque material were present in the nucleus. Zierdt and Tan (234) indicated that this spot was the nucleolus, although this has not been reconciled with a previous report identifying the crescentic band as the nucleolus (231). Differences in the morphology of mitochondrion-like organelles were also noted, with avacuolar forms showing numerous cristae which extended deep into the organelle matrix. In contrast, mitochondrion-like organelles from culture forms have been shown to contain only small numbers of short tubular or sacculate cristae (49, 224, 230). The matrix of mitochondrion-like organelles from the avacuolar cell forms was less electron opaque than in other forms of *B. hominis*. The significance of these differences is not known.

The avacuolar cell form was not surrounded by a surface coat, and this is a feature in common with the ameboid form of *B. hominis* as described by Dunn et al. (49). The study by Stenzel et al. (179) was unable to confirm that culture of the avacuolar form gives rise to the vacuolar form, as reported by Zierdt and Tan (234), since the organisms from the colonoscopy sample failed to grow in culture. Examination of further samples is required to confirm that the morphology is an accurate representation of *B. hominis* in the human intestinal tract and to examine the effects of culture upon its morphology.

Ameboid form. The ameboid form (Fig. 4b) of *B. hominis* has been reported only rarely, and there are a number of conflicting reports on its morphology (49, 131, 192, 223, 228). This form has been termed the ameba-like form (231), the ameba form (192, 228), the amebiform (227), and the ameboid form (49); hence, standardization of the nomenclature is required. Dunn et al. (49) provided a detailed ultrastructural description of the ameboid form of *B. hominis* from culture material. The cells were 2.6 to 7.8 μm in diameter, were irregular in shape, and often had extended pseudopodia. Engulfed bacteria were seen in lysosome-like bodies within the cytoplasm and appeared to be digested by the cell. A large central vacuole was not seen. The characteristic nuclear morphology, with a crescentic band of electron-opaque material, was present. The cells showed few additional morphological features seen in the other forms of *B. hominis*. A Golgi complex, surface coat, coated pits, and mitochondria were not noted.

This morphology correlated with the ameboid form previously reported to be present in small numbers in older cultures, cultures treated with antibiotics, and fecal samples (223). The organism was described as irregular and lobed in outline, with pseudopodia extending from the cell. The cells were reported to feed on bacteria. A central vacuole was not seen, but one or two nuclei were present, located at the center of the cell.

However, in another report, Tan and Zierdt (192) described a quite different morphology for the ameboid form. The organisms were oval in outline, with one or two large pseudopods. The cells were reported to contain a large central vacuole, and it was noted that electron-lucent granules were present in the central vacuole of organisms from cultures with a higher serum content. Membranes were not seen surrounding the cell, the central vacuole, or the nucleus (192), and this seemed to be the primary criterion for defining the organisms as the ameboid form. It is unlikely that the organism could remain viable without a surrounding membrane or cell wall,

and the lack of such structures is insufficient to define a separate form.

The mode of division of ameboid cells is unproven. Budding (223, 231), sporulation (183), and plasmotomy (228) have been suggested as methods of reproduction. However, there is no conclusive evidence to support any of these reproductive modes. Suresh et al. (186) and Singh et al. (174) have suggested that the ameboid form is an intermediate between the vacuolar and cyst forms of *B. hominis*, but this has not been proven. These reports also indicated that bacteria were ingested by the ameboid form to provide nutrition for the process of encystment (174, 186).

Cyst form. Several early studies reported a small, resistant form of *B. hominis* (11, 12, 100, 108, 117), but the presence of a cyst form remained unconfirmed until recently. The current definition of the species (227, 228) still indicates that a cyst form of *B. hominis* does not exist, and this needs to be amended.

Cyst-like forms of *B. hominis* were first found in the fresh feces of a patient with AIDS by Mehlhorn (132), but it was not until the study by Stenzel and Boreham (177) that a detailed morphological description of the cyst form was provided. The cyst forms were found in fecal material, particularly material that had been stored for several days before being fixed, and occasionally in laboratory cultures (177). Zaman et al. (220) also have provided an ultrastructural description of the cyst form and have reported a method for concentrating this form from fecal material by repeated washing in distilled water and centrifugation on Ficoll-Paque.

The studies by Suresh et al. (186, 189) purport to identify the cyst form, but the micrographs in these reports show organisms morphologically identical to the granular form described by other authors (23, 49, 192). Similarly, the thick-walled and thin-walled cysts described by Singh et al. (174) are illustrated by electron micrographs of granular forms and remnants of the central vacuole, respectively, and these cannot be accepted as cyst forms of *B. hominis.*

The cyst forms of *B. hominis* (Fig. 1f and g and 5) are smaller than vacuolar and granular forms found in cultures and generally are smaller than the multivacuolar forms found in fresh fecal material (176, 177, 220). They have been reported to be 5 to 10 μm (132), 3.7 to 5 μm (177), and 3 to 6 μm (220) in diameter. A thick, multilayered cyst wall surrounds the organism (Fig. 5d). The cyst wall appears to form beneath the surface coat, with the surface coat and cell debris subsequently separating from the cyst form (176, 177).

A condensed cytoplasm containing many small vacuoles, similar to that of other cysts (133), is noted in the cyst forms of *B. hominis*. Cytoplasmic reserves of glycogen and lipid inclusions frequently are present (23, 176, 177, 220). However, these inclusions may be extracted by some methods of sample preparation for transmission electron microscopy. Multiple mitochondrion-like organelles, usually containing poorly developed cristae, have been noted (176, 220). Mehlhorn (132) described up to four nuclei per cell, but Stenzel and Boreham (177) found only one nucleus per cell in *B. hominis* cyst forms from human fecal material and from culture. Subsequent data have indicated that two nuclei often are present in the cyst forms (176). Evidence of nuclear division in the cyst forms has not been presented. The formation of vacuolar forms within the cyst forms of *B. hominis*, by a "multiple fission-like" mode of asexual reproduction, has been described previously (187), but this has not been confirmed and the "cysts" described by these authors (174, 186, 187) are inconsistent with the descriptions of the cyst form of *B. hominis* given by other researchers (23, 132, 176, 177).

The cyst form of *B. hominis* probably confers resistance in the external environment, as is the case for many other protozoa (133), but this has not been confirmed experimentally. Cyst forms of *B. hominis* did not lyse when placed into distilled water, suggesting that they possess at least some resistance to environmental conditions (189, 220). The presence of cyst forms in culture medium containing antiprotozoal drugs (172) also supports this thesis.

Other forms. The existence of other forms of *B. hominis* cannot be discounted but remains unproven. Although the review by Zierdt (228) indicated that a number of other cell forms occur in culture, adequate descriptions were not given and the existence of these forms has not been verified by other studies. The effects of culture conditions on the morphology of *B. hominis* have only recently been addressed (47, 176, 179), but the influences of osmolarity, pH, temperature, and chemical composition of the culture media have not been fully assessed. It has been suggested that the metabolic state of the cells also influences the morphology of *B. hominis* (229). Hence, before describing new forms of *B. hominis*, it is essential to discriminate between morphological differences induced by the microenvironment and physiology of the cells and distinct life cycle stages.

Life Cycle

A number of life cycles have been proposed for *B. hominis* (2, 23, 31, 174, 223), although controversy about the modes of division of the organism has always existed (11, 12, 37, 93, 99, 108, 117, 228, 229). The life cycle presented in most recent texts is that proposed by Zierdt (223) on the basis of his light microscopy observations. This life cycle indicates that the vacuolar form differentiates either into the granular form, subsequently producing daughter vacuolar cells within the central vacuole, or into the ameboid form, subsequently producing vacuolar cells by budding. Although some aspects are consistent with current data, this life cycle must be reconsidered in the light of recent ultrastructural studies.

The life cycle recently postulated by Singh et al. (174) must be rejected, because it contradicts morphological data from numerous other studies (23, 47, 49, 129, 132, 172, 173, 176, 177, 179–181, 192, 215, 223, 234). The forms shown, particularly the cyst forms, are not in accordance with other descriptions (23, 132, 176, 177, 220), and despite assertions of the importance of the granular form given in the text, this form is not shown on the proposed life cycle (174). These authors (174) propose a cycle of external transmission, with the vacuolar form differentiating into the amoeboid form and subsequently into a precyst form. Progeny are indicated to arise by schizogony within the precyst form, resulting in a thick-walled cyst, which ruptures to release daughter vacuolar forms. A cycle of "autoinfection" also is proposed (174), although this is not consistent with the definition of autoinfection associated with other parasites, such as *Enterobius* and *Strongyloides* spp. (69, 70). In this cycle, the vacuolar form is indicated to differentiate into the thin-walled cyst form via the multivacuolar and precyst forms. Schizogony is suggested to occur within the thin-walled cyst, which then ruptures to release daughter vacuolar forms.

In our view, binary fission is the only plausible method of reproduction for *B. hominis* that has been demonstrated. Plasmotomy, endodyogeny, schizogony, and sporulation are not supported by recent morphological data. It is probable that the presence of granules or inclusions in the cells or the presence of membrane-bound projections and cytoplasmic bridges that appear to divide the central vacuole (47, 176, 185) was interpreted as these supposed means of division during observation

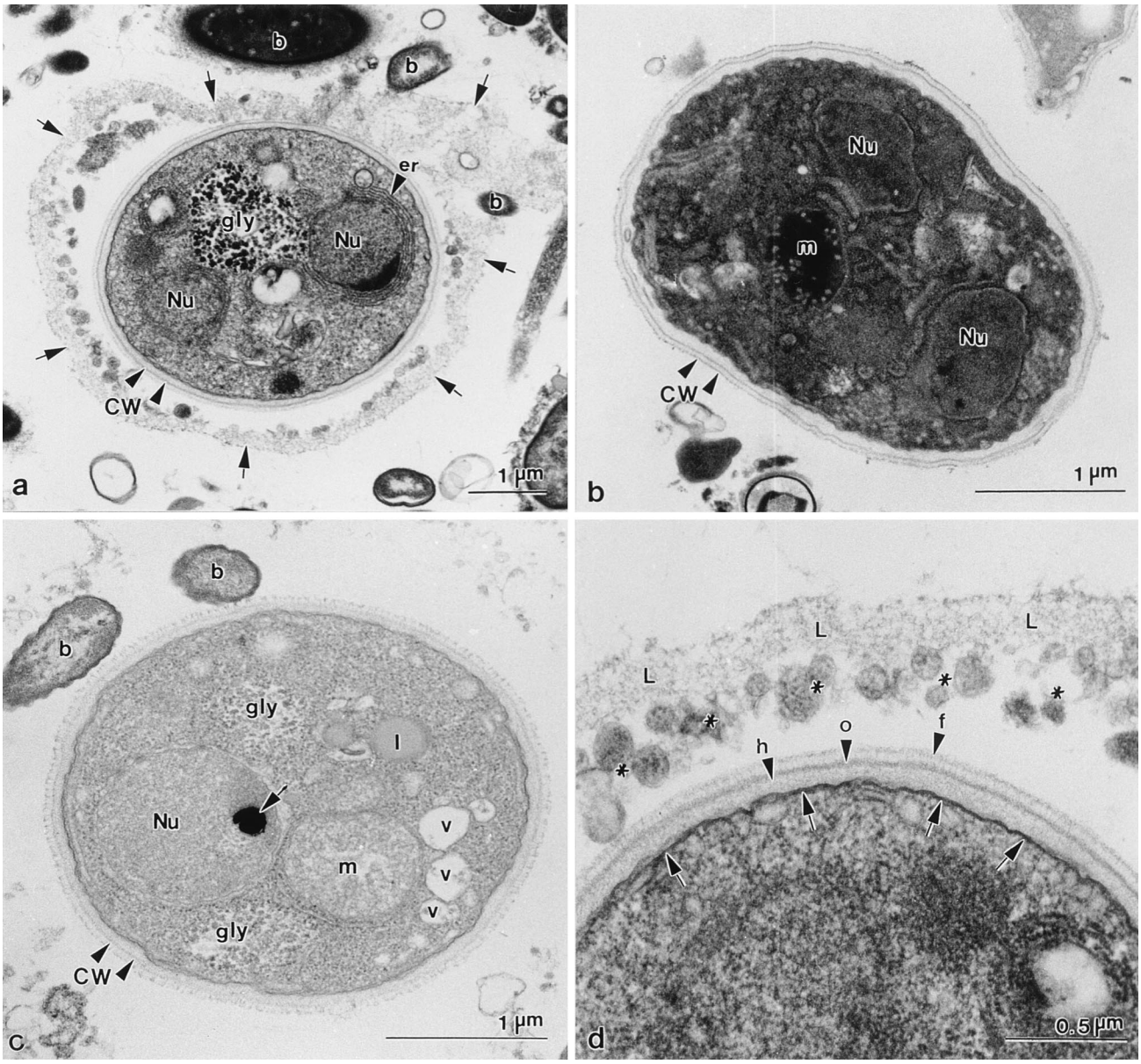

FIG. 5. Transmission electron micrographs of the cyst form of *B. hominis*. (a) Cyst form showing the multilayered cyst wall (CW) and an outer fibrillar layer (arrows), which appears to be separating from the cyst. Two nuclei (Nu) are noted in this ultrathin section of the organism. A large accumulation of glycogen (gly) is seen in the cytoplasm. (b) Cyst form, showing the multilayered cyst wall (CW) without additional surrounding fibrillar material. (c) Cyst form showing atypical nuclear morphology. An electron-opaque spot (arrow) is seen within the elliptical band of electron-opaque material in the nucleus. Large accumulations of glycogen (gly) are present in the cytoplasm. Small vesicles (v) and lipid inclusions (I) also are noted. (d) High-magnification micrograph, showing details of the surface structures of the cyst form. The cyst wall shows an inner homogeneous layer (h), an electron-opaque layer (o), and an outer fibrillar layer (f). Some cyst forms show an additional layer of fibrillar material (L) and vesicular or membranous material (asterisks) between this layer and the cyst wall. A bilaminar membrane (arrows) is noted beneath the cyst wall. m, mitochondrion-like organelle; er, rough endoplasmic reticulum; b, bacterium.

of *B. hominis* by light microscopy. A cyst form of *B. hominis* (132, 177, 220) also must be included in the life cycle.

The life cycle shown in Fig. 6 has been proposed for *B. hominis* on the basis of current data (23). The form present in the intestines of humans appears to be a small avacuolar cell without a surface coat. As the avacuolar form passes through the intestine, the small vesicles present in the cytoplasm probably coalesce, and subsequently the cell appears as the multivacuolar form. The multivacuolar form, found as the predominant form in fecal material, is surrounded by a thick surface coat. The cyst wall appears to form beneath the surface coat, which subsequently appears to slough off. The resultant cyst form is likely to be the infective form of *B. hominis*. Preliminary support for this hypothesis has been provided by a study indicating that the cyst form is infective to rats (189), although further experimental verification of this and of the ability of the cyst form to survive external environmental conditions is required. Ingestion by a new host and excystation of the cell would complete the cycle. Excystation may occur as a result of exposure of the cyst form to gastric acid and intestinal enzymes, as has been described for *Giardia* spp. (162). The general cytoplasmic organization of the cyst form appears similar

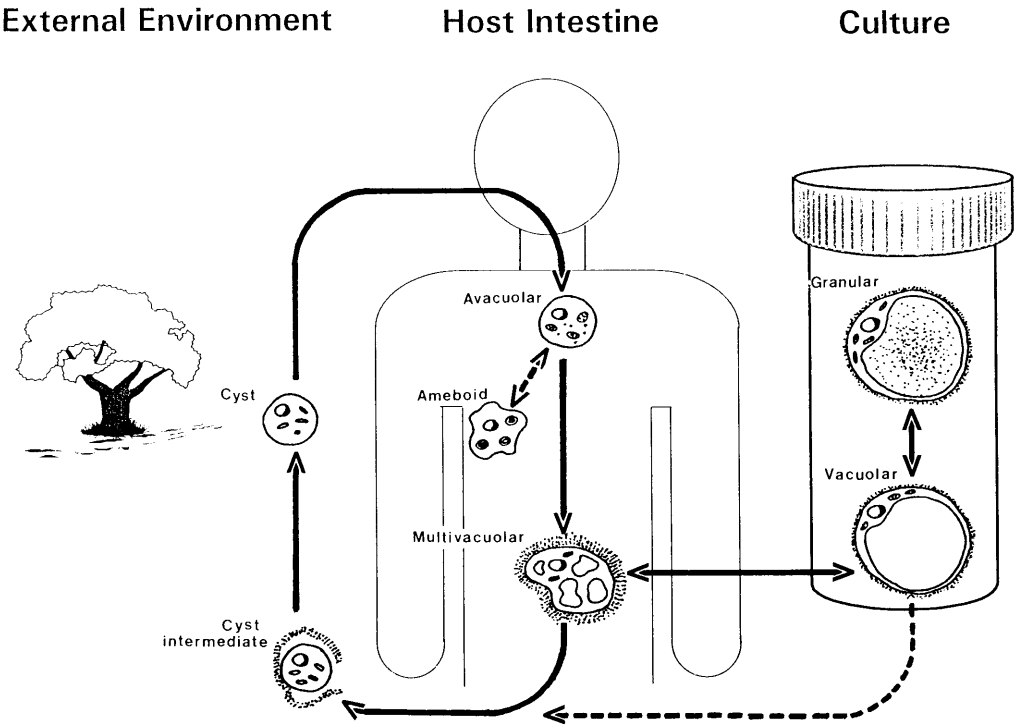

FIG. 6. Proposed life cycle for *B. hominis*. See the text for details.

to that of the avacuolar form of *B. hominis* found in the intestine. The cyst form was noted to be more common in stored fecal material than in fresh stools (177), suggesting that this form may develop in response to passage from the host or to external environmental factors.

Very little information is available on the differentiation to the ameboid form, because few ameboid cells have been found in any samples. It is possible that the ameboid form, as described by Dunn et al. (49), arises from the avacuolar form, since there are some morphological similarities. A precedent for this type of differentiation is the ameba-flagellate transformation described in some free-living amebae, such as *Naegleria* spp. (163), in which the mechanism of transformation appears to be dependent upon physiological conditions. Like the transformation of *Naegleria* spp., the transformation of *B. hominis* from the avacuolar form to the ameboid form probably is reversible, but experimental data for this have not been provided.

The vacuolar form has been demonstrated to form after culture of the multivacuolar form, apparently by the coalescence and enlargement of the smaller, multiple vacuoles to make up the large central vacuole (176, 179). It appears likely that this morphological alteration also occurs when *B. hominis* is placed in various preservative, diluent, and staining solutions, such as are used in clinical laboratories (176). Thus, the vacuolar form may not play a major role in the natural life cycle of *B. hominis*. It remains to be determined whether the vacuolar form is infective to humans. Experimental evidence suggests that it has low, if any, infective potential when inoculated orally into animals (147, 189). Cyst forms occasionally have been found in long-term laboratory cultures, indicating that encystation may occur from the vacuolar form, the only other form present in the cultures (176, 177). This also provides supporting evidence that the cyst form is *B. hominis* and not a disparate organism (176, 177).

Altered culture conditions have been demonstrated to give rise to the granular cell (49, 108, 171, 179, 223, 236). It is assumed that the granular form differentiates from the vacuolar form by the accumulation of granules in the central vacuole, and this is supported by light microscopic and transmission electron microscopic evidence (49, 192, 223). The alternative explanation of the granular form arising from selective reproduction of the extremely small numbers of granular cells seen in vacuolar cell cultures seems unlikely, given the rapidity of transition of the culture from the vacuolar to granular cells (179).

The life cycle and modes of reproduction of *B. hominis* have not been conclusively demonstrated. Further studies may elucidate differentiation pathways that are not indicated on the proposed life cycle (Fig. 6), and the existence of additional forms of the organism cannot yet be confuted.

Biochemistry and Cytochemistry

B. hominis is a strictly anaerobic organism (47, 49, 223, 224, 226, 231, 232, 236), although it contains organelles that morphologically resemble mitochondria. It may be cultured on prereduced Boeck and Drbohlav's inspissated egg medium (236), on which growth is optimal at neutral or slightly alkaline pH. Successful culturing of *B. hominis* also has been reported to occur on Dobell and Laidlaw medium covered with Ringer solution supplemented with 20% human serum and streptomycin sulfate (171), Loeffler medium covered with Ringer solution containing 20% human serum (173), Diamond's Trypticase panmede serum (TP-S-1) monophasic medium (136), minimal essential medium containing 10% horse serum (48, 202), and Iscove's modified Dulbecco's medium supplemented with 10% horse serum (83). The organism isolated from human fecal material grows in culture when incubated at 37°C but not at 30°C or ambient temperature (236). *Blastocystis* spp.

from other hosts may have different culture requirements (14, 16, 17, 197), but these requirements have not been extensively investigated.

Comprehensive studies of the basic biochemical cycles and the cytochemistry of *B. hominis* have not been performed. There are a number of reports of limited cytochemical examinations at the light microscopic level (47, 49, 108, 128, 131, 192, 214, 223, 226, 230, 231, 234), but because of the small size of *B. hominis* and the correspondingly small size of the cellular components, light microscopic resolution is insufficient to obtain precise localization of molecules in this organism. Only three studies (176, 182, 216), two of which were limited to the investigation of *B. hominis* from laboratory culture, have reported ultrastructural cytochemistry. The few biochemical studies of *B. hominis* have examined organisms from culture (97, 98, 226, 230), because there currently is no known method of isolating viable *B. hominis* cells directly from fecal material.

Morphological and cytochemical studies indicate that *B. hominis* cells may accumulate lipid, both in the cytoplasm and in the central vacuole of the cells (47, 49, 176, 179, 227). Biochemical analysis of six axenic strains of *B. hominis* has shown a lipid profile typical of animal cells, with a spectrum of phospholipids, neutral lipids, and polar lipids detected (97). A metabolic study of three axenic strains suggested that *B. hominis* was able to synthesize triacylglycerols, diacylglycerols, and phospholipids (98). Cholesterol and cholesterol esters were obtained from the culture medium in this study (98), so it is unknown whether the organism is capable of de novo synthesis of these compounds.

Isoenzyme electrophoresis had indicated that the enzymes glucose phosphate isomerase, phosphoglucomutase, and L-malate:$NADP^+$ oxidoreductase (oxalacetate decarboxylating) were not present in *B. hominis*, although all of these enzymes were found in a number of intestinal amebae examined in the same study (160). However, a more recent study (121) has identified and provided an isoenzyme analysis of glucose phosphate isomerase, phosphoglucomutase, malic enzyme, 6-phosphogluconate dehydrogenase, and hexokinase in isolates of *B. hominis.*

The function and metabolic capabilities of mitochondrion-like organelles in *B. hominis* have not been ascertained. These organelles have been shown to stain selectively with stains used to identify mitochondria at the light microscopic level in other cell types. Positive staining has been achieved with the vital stain Janus green (47, 108, 224, 230, 234) and with rhodamine 123 (226, 230). The mitochondrion-like organelles of *B. hominis* appear to be able to generate energy and to maintain the transmembrane potential required for the accumulation of rhodamine 123, indicating that they are functional and not vestigial remnants of nonfunctional organelles. Mitochondrial function in *B. hominis* also has been supported by the finding that high concentrations of rhodamine 123 (100 μg/ml) inhibited or blocked division of the cells (230). Hollebeke and Mayberry (85) reported that the respiratory chain in the mitochondrion-like organelles of *B. hominis* was insensitive to cyanide, but other metabolic inhibitors and uncouplers have not yet been applied to determine functional pathways within the organelles. It has been suggested that these organelles may play a role in lipid synthesis (230). This has not been tested experimentally.

A number of enzymes associated with energy metabolism and which are considered to be marker enzymes for mitochondria appear to be absent in *B. hominis*. Biochemical analysis has indicated that cytochrome oxidase, catalase, peroxidase, the pyruvate dehydrogenase complex, ketoglutarate dehydrogenase complex, isocitrate dehydrogenase, glutamate dehydrogenase, and cytochrome *c* oxidase are not present (226, 230). Conflicting data on the presence in *B. hominis* of the enzymes diaphorase, lactate dehydrogenase, and aldolase have been reported (227, 230). These results must be regarded circumspectly until substantiated by further analysis, including appropriate control experiments.

The analysis of the lipid composition of *B. hominis* strains from culture has indicated that cardiolipin is present (97). This lipid plays a functional role in the cytochrome oxidase complex in mammalian cells and is thought to be a characteristic lipid of mitochondria (8). Further investigation is required to determine the subcellular location of this lipid in *B. hominis* and to reconcile its presence with the reported absence of cytochrome oxidase (226, 230).

The fluorescent dye 4′,6-diamidino-2-phenylindole (DAPI), which complexes specifically with DNA, showed some staining of the mitochondrion-like organelles, suggesting that DNA is present (129). This has been supported by selective staining for nucleic acids in these organelles at the ultrastructural level (176). It has been suggested that the mitochondrion-like organelles present in *B. hominis* may be hydrogenosomes with a morphological resemblance to mitochondria (23). The presence of hydrogenosomes would be more easily explained, considering the anaerobic environment required for the survival and growth of *B. hominis.*

Cytochemical stains have been used to examine the central vacuole in an attempt to determine its function, but the results have been contradictory. The results suggest that at least in some circumstances, the central vacuole may have a function in the metabolism or storage of molecules, but further studies are required. The absence of a central vacuole in the cyst form and in the forms found in the gastrointestinal tract indicates that the large central vacuole is not essential for the survival of at least some of the forms of the organism; therefore, its role remains an enigma.

Cytochemical staining has indicated that lipids, including phospholipids and fatty acids, are present in the vacuolar form (131, 192, 223), the granular form (47, 49, 108, 192), and older cells in culture (231). Iodine stains for starch usually have given negative results (131, 223), although a low proportion of cells have shown positive staining in one study of *Blastocystis* spp. from chickens and monkeys (214). It has been suggested that starch accumulates in the cells under adverse culture conditions (100, 171). Carbohydrates have been detected in the central vacuole by using a number of selective stains and lectins, and variability in the staining reactions and distribution of carbohydrates has been noted (176, 216).

The presence of glycogen in *Blastocystis* spp. has been examined cytochemically at the light microscope level, although the results have been conflicting. Several early reports (100, 108) had indicated that *Blastocystis* spp. from fecal material and from culture did not stain for glycogen. However, both reports described the presence of smaller "spore" forms of *Blastocystis* spp. which appeared to contain abundant glycogen. These forms (100, 108) probably represent the cyst forms of *Blastocystis* spp. described in recent studies (132, 177), and several ultrastructural studies now have indicated that large accumulations of glycogen may be present in the cyst form (23, 176, 220). Periodic acid-Schiff staining methods have given negative results for the vacuolar and granular forms of *B. hominis* from culture (192, 223, 231) and from fecal material (231), but Silard (171) suggested that glycogen was present in the central vacuole of vacuolar forms differentiating to the granular form. McClure et al. (131) did not detect glycogen in *Blastocystis* spp. from simian fecal material by light microscopic staining, whereas Yamada et al. (214) found variable staining,

with some cells showing intense staining within the central vacuole. In ultrastructural studies, glycogen was detected in the cytoplasm and vacuoles of small numbers of multivacuolar forms of *B. hominis* from human fecal samples but was not apparent in *B. hominis* from culture (176). Clusters of glycogen particles have been noted during electron microscopic examination of the cytoplasm of *Blastocystis* spp. from simian fecal material (131). Further studies are required to determine if glycogen is utilized when *Blastocystis* cells are grown in culture, or if glycogen is formed in the cells only during transformation to the cyst form.

Cytochemical analysis of the central vacuole has indicated that acid phosphatase, a hydrolytic enzyme, is not present (182). Alkaline phosphatase was detected in the central vacuole and some Golgi cisternae of the vacuolar form when cytochemical staining methods were used at the ultrastructural level (176), although a previous biochemical study had not identified this enzyme in *B. hominis* (230).

Coated pits and vesicles have been found on the cell membrane of *B. hominis* cells from culture (49, 182) and from fecal material (170). Clathrin, a major structural protein of coated pits of many eukaryotic cells (145), was detected in the coated pits and vesicles of *B. hominis* from culture by immunoelectron microscopy (182). Small vesicles associated with the Golgi complex also were seen to label with the anti-clathrin antibody. It has not been determined if the labelling noted in *B. hominis* cells is of clathrin as previously characterized in mammalian cells or of a cross-reacting protein, since isolation and purification of coated vesicles and their constituent molecules have not been performed for *B. hominis*. Since there are no other reports of clathrin in protozoan organisms, it is important to determine the nature of the protein associated with the coated pits and vesicles of *B. hominis.*

An ultrastructural study in which cationized ferritin was used as a tracer for endocytosis indicated that the endocytic pathway in the vacuolar and granular forms of *B. hominis* is similar to that described for other eukaryotic cells, with cationized ferritin internalized via coated pits and vesicles (182). Uncoated vesicles, larger vacuoles, and tubules contained cationized ferritin after short incubation periods. Ultimately, cationized ferritin particles accumulated in the central vacuole of the vacuolar and granular forms of *B. hominis*. Cationized ferritin particles were not found within the granules of the granular forms but were interspersed among them. Endocytosis has not been studied in forms of *B. hominis* other than the vacuolar and granular forms.

A role for the central vacuole in reproduction, suggested by Zierdt et al. (231), has not been supported by cytochemical data. Methyl green-pyronin staining indicated that RNA was not present in the central vacuole, although staining was seen in the peripheral cytoplasm (192). Fluorescence was not seen in the central vacuole when DAPI staining for DNA was performed, although the nucleus showed strong fluorescence (129).

The presence of DNA within the nucleus of *B. hominis* has been ascertained at the light microscopic level by the use of specific stains, including Hoechst 33258, acridine orange, methyl green, and brilliant cresyl blue (47, 188, 192), as well as DAPI (129). At the ultrastructural level, a number of cytochemical methods have indicated that the crescentic band in the nucleus of *B. hominis* is not a nucleolus (176), as has been previously suggested (230, 231). The structure contains basic proteins, indicated to be histones, and appears to be condensed chromatin (176).

Karyotypic analysis of *B. hominis* by field inversion gel electrophoresis has indicated that 10 to 12 chromosomes, ranging in size from approximately 200 kbp to more than 1 Mbp, are present (202). Variability in karyotype was noted among seven stocks (202). Another study, using pulsed-field gradient electrophoresis, discerned 14 to 16 chromosomes ranging in size from approximately 600 kbp to 2 Mbp, with similar karyotypic patterns seen in the five isolates examined (84). Further biochemical methods should be used to determine the structure and properties of nucleic acids and associated molecules in *B. hominis.*

Ultrastructural studies have demonstrated a thick, fibrillar surface coat on *B. hominis* in fecal material (23, 176, 179), suggesting that this structure plays a role in parasite survival in vivo. The composition and role of the surface coat remain to be elucidated, and it has not been determined if humans infected with *B. hominis* produce antibodies against the surface coat. Selective staining methods to detect carbohydrates by light and electron microscopy have indicated that carbohydrates are present in the surface coat of *B. hominis* (216). However, lectin localization at the ultrastructural level, performed with a range of lectins, has suggested that carbohydrates are not a major component of the structure (176). The highest degree of labelling was achieved with lectins which recognize *N*-acetyl-β-D-galactosamine residues (176). As the interface between the parasite and the host or external environment, the surface coat warrants further study to elucidate its composition and function and to determine the extent of antigenic variation among isolates.

Chitin appears to be present in the cyst wall of *B. hominis*, as indicated by wheat germ agglutinin binding to the inner band of the cyst wall (176). This chitin may not be exposed at the surface of the cyst, and, hence, intact cysts may not stain with light microscopic stains for chitin, such as calcofluor white. The composition of the other layers of the cyst wall is unknown.

Although limited in scope, the cytochemical and biochemical studies which have been performed have given some indications of the composition and function of structures and organelles of *B. hominis*. However, most studies have been performed on cultured forms, and the applicability of the data to other forms of the organism has yet to be determined. Additional studies are required to confirm the previous results and to provide data for the structures and organelles of the in vivo forms. Alteration of cellular physiology and biochemistry as a result of laboratory culture and other physical conditions needs to be investigated more thoroughly and must be clarified before conclusions about the validity of information obtained from *B. hominis* in vitro can be reached. The presence of endosymbionts (176, 178, 235) and virus-like particles (176, 195) in *Blastocystis* spp. may result in significantly altered cellular characteristics, indicating that further caution is warranted when performing biochemical, molecular, or cytochemical analyses.

CLINICAL ASPECTS

Clinical Presentation

Symptoms commonly attributed to infection with *B. hominis* are nonspecific and include diarrhea; abdominal pain, cramps, or discomfort; and nausea (9, 10, 45, 46, 51, 67, 77, 78, 89, 96, 140, 141, 154, 167, 184, 194, 210, 219, 228, 238, 239). Profuse, watery diarrhea has been reported in acute cases (115, 192, 219), although this may be less pronounced in chronic cases (219). Fatigue, anorexia, flatulence, and other nonspecific gastrointestinal effects also may be associated with *B. hominis* infection (10, 51, 67, 140, 141). Fever has been reported, particularly in acute cases (55, 219), but has not been noted in

other studies (9, 10, 109, 194, 238). Other signs and symptoms sometimes reported include fecal leukocytes (38, 45, 77, 157, 203), rectal bleeding (3, 28), eosinophilia (60, 67, 106, 167, 219), hepatomegaly and splenomegaly (60), cutaneous rashes (60), and itching (67). One report has indicated that joint pains and swelling may result from infection of the synovial fluid by *B. hominis* (110).

A number of case reports have suggested that *B. hominis* may be the causative agent of a variety of diseases including enteritis (55, 73), colitis (157, 169), terminal ileitis (200), and arthritis (101, 105, 110) and may complicate ulcerative colitis (92). *B. hominis* has been associated with diabetes (41, 51, 162, 168) and leukemia (65). None of these associations have been substantiated and often are based on single case studies. In a report of a patient with tropical pulmonary eosinophilia (52), the *B. hominis* infection appears unrelated to the underlying disease.

Although detailed epidemiological studies have not been performed to ascertain the true prevalence, symptomless infections appear to be common (51, 63, 76, 78, 88, 89, 107, 155, 170, 184, 201). Large numbers of *B. hominis* cells may be present in fecal material from patients who do not show symptoms (26, 201, 219). The numbers of symptomless infections probably have been grossly underestimated, because not all forms of *B. hominis* have been used in diagnosis and many studies indicate the presence of *B. hominis* in fecal samples only if five or more organisms per "high magnification field" or "oil immersion field" are detected (36, 46, 75, 115, 154, 184, 219, 238).

The presence of *B. hominis* in stool samples from patients showing gastrointestinal symptoms does not necessarily imply that symptoms are due to this organism, and other infective and noninfective causes should be investigated (9, 23, 123, 124, 135, 184, 201). Similarly, large numbers of *B. hominis* cells may be an incidental finding in stools of patients being investigated for other infections or disease conditions (52, 124, 184). The possibility of asymptomatic shedding of *B. hominis* during convalescence from a symptomatic episode of infection has not been investigated (95).

It is possible that only certain life cycle stages or demes of *B. hominis* are responsible for symptoms in humans. Some authors have suggested that the patient's overall health may influence the development of symptoms and that symptoms are unlikely in healthy hosts (9, 32). The possibility that host immunosuppression influences the presence or severity of symptoms has been raised (45, 62, 110), but the occurrence of *B. hominis* infections in immunocompromised hosts has not been fully assessed. Certainly, *B. hominis* infections, both with and without severe symptoms, have been reported in patients with a number of immunocompromising disorders such as AIDS (34, 36, 57, 61, 62, 67, 82, 107, 112, 114, 137, 139, 154, 238), poorly controlled diabetes (41, 57, 62, 67, 161), and leukemia (57, 65, 154) and in patients who have undertaken immunosuppressive therapy (110).

In the absence of other identified causes of symptoms, patients presenting with diarrhea or other gastrointestinal symptoms should be assessed for the presence of *B. hominis*. The symptoms may be tentatively ascribed to *B. hominis*. However, the possibility of other unidentified etiological agents, especially viruses, toxins, and noninfectious causes, should not be discounted, even if large numbers of *B. hominis* cells are found in fecal samples. The recent designation of Zierdt-Garavelli disease for infection with *B. hominis* (56, 66) is not acceptable until it is conclusively shown that the organism causes disease.

Pathogenicity

It is unknown whether *B. hominis* is a truly pathogenic organism or a commensal or perhaps is capable of being a pathogen in specific circumstances. There have been many reports suggesting that *B. hominis* causes disease (3, 9, 10, 41, 45, 46, 51, 55, 58, 62, 67, 71, 73, 77, 89, 96, 106, 109, 110, 113, 120, 139, 140, 149, 152–154, 157, 166, 167, 193, 194, 203, 218, 219). However, there also have been a number of reports to the contrary (6, 35, 74, 103, 104, 124–126, 135, 155, 156, 165, 184, 201). The difficulty of eliminating all other infectious and noninfectious causes of symptoms is a major problem, especially when the pathogenicity of many other concomitant organisms also is uncertain (46, 123). A number of studies examining the pathogenicity of *B. hominis* have not eliminated all known viral, bacterial, or other protozoal infections as a possible cause of symptoms (9, 10, 46, 71, 96, 219, 238), while others have not considered noninfectious causes (126, 135, 210). Case-controlled studies have not been performed.

Endoscopy and biopsy results usually have indicated that *B. hominis* does not invade the colonic mucosa in human patients (41, 43, 45, 46, 62, 66, 96, 200, 238), although edema and inflammation of the intestinal mucosa may be present (62, 66, 96, 110, 157, 239). In contrast to these results, a recent case report (3) describes colonic ulceration determined by colonoscopy and biopsy, with *B. hominis* found in superficial ulcers and infiltrating to the superficial lamina propria and gland spaces. Inflammation, with chronic inflammatory cells and eosinophils, was present in the cecum, transverse colon, and rectum of the patient. Endoscopy of the upper gastrointestinal tract showed normal epithelia. This study had eliminated the possibility of other known bacterial, viral, and parasitic pathogens, as well as *Clostridium difficile* toxin. Noninfectious causes of the symptoms were not found. *B. hominis* was the only parasite found in the feces and also was identified in biopsy material, associated with inflamed areas of the gastrointestinal tract (3).

B. hominis has been suggested to cause toxic-allergic reactions, leading to a nonspecific inflammation of the colonic mucosa (62, 63, 66), but this has not been verified. The review by Zierdt (228) gives previously unpublished information from studies involving isolated segments of rabbit ileum, which suggests the presence of toxins in fractions taken from *B. hominis* culture medium. The possibility of contamination of these fractions with bacterial or other toxins was not considered. Detailed studies of *B. hominis* to determine the presence of toxins or other substances likely to be harmful to the gastrointestinal tract have not been published and do not appear to have been performed.

Zuckerman et al. (239), using an excreted radioactive marker, found impaired intestinal permeability in patients with *B. hominis* infection. This is in contrast to enteric infections with other organisms or intestinal diseases which cause mucosal injury or significant inflammation, in which increased permeability of the mucosa is demonstrated (239).

There appears to be no evidence of acquisition of protective immunity to *B. hominis* at a community level (6). Ashford and Atkinson (6) suggested that if the organism is pathogenic, only a small proportion of the community is susceptible to infection, so that any individual immunity has no effect on the overall community pattern. Other authors suggest that the self-limiting nature and spontaneous elimination of some *B. hominis* infections are due to protective immunity (140, 184). It has been noted in several studies that older children and adults have lower infection rates and fewer symptoms than younger children, and this may reflect immunity induced by previous infections (140, 141). However, this remains controversial, be-

cause a number of other studies have found higher rates of infection in adults than in children (6, 46, 76, 89, 115, 158). Serum immunoglobulin G antibody responses to *B. hominis* have not been detected (35), but the possibility of a secretory immunoglobulin A antibody response to the organism has not been investigated.

It has been suggested that *B. hominis* may be an opportunistic pathogen in immunosuppressed patients (41, 57, 58, 114, 139, 154), although convincing evidence has not been presented. However, since few clinicians or pathology laboratories have regarded *B. hominis* as more than a harmless commensal, infections may have been overlooked and diarrhea and other symptoms may have been attributed to other or unknown causes. In the most detailed study (36), *B. hominis* was found to be the most common parasitic infection in homosexual men, both with and without human immunodeficiency virus (HIV) infection, but was not considered to be a pathogen.

Very little information is available on the pathogenicity of *B. hominis* in patients with immunodeficiencies other than AIDS, because most publications consist of single case reports. Symptoms due to *B. hominis* infections have been reported to be more severe in patients with immunodeficiencies due to alcoholic cirrhosis, hepatitis, diabetes, carcinoma, and systemic lupus erythematosus than in immunocompetent persons (62). Similarly, the single case report of systemic dissemination of *B. hominis* described a patient undergoing immunosuppressive treatment for arthritis (110).

Some authors have concluded that *B. hominis* is pathogenic, since chemotherapy has resulted in a reduction of symptoms and elimination of organisms (106, 110). However, the drugs used, primarily 5-nitroimidazoles, act on a range of protozoa, as well as gram-positive and gram-negative bacteria present in the gastrointestinal tract, and may remove other pathogens. Conversely, the spontaneous subsidence of symptoms should not be taken as evidence that *B. hominis* is not the causative agent of disease, because many other causes of infectious diarrhea are known to be self-limiting (46, 184).

Several reports, including some very early data, indicate that *B. hominis* may cause symptoms when present in large numbers (71, 109, 166, 167, 193, 203, 209, 225, 228). The severity of symptoms has been reported to correlate with the numbers of *B. hominis* present in the feces (51, 140, 141, 157). Other reports have found no such correlation between numbers of *B. hominis* in fecal material and the presence or severity of symptoms (46, 74, 78, 95, 96, 128, 139, 165, 170, 201), and a study by Kukoschke and Müller (103) found an increased incidence of *B. hominis* in healthy, asymptomatic persons compared with symptomatic patients.

It has been suggested that the numbers of *B. hominis* present in the stools may vary during the course of infection (95), and, like *Entamoeba histolytica* and *Giardia lamblia*, the number of organisms may be an inadequate marker for pathogenicity (96). The possibility of an asymptomatic carrier state also needs to be considered (46, 62).

Although these aspects of *B. hominis* infection have not been resolved, a number of recent research papers and diagnostic texts suggest that numbers of *B. hominis* in fecal samples correlate with signs and symptoms of infection and that an indication of the numbers of organisms present should be recorded in pathological reports (69, 70, 111, 149, 157, 167, 218, 228, 229). This seems unnecessary until there is sound evidence that *B. hominis* can be a pathogen and that the number of organisms is related to pathogenicity. Small numbers of *B. hominis* cells should be recorded as positive and not disregarded as insignificant, as has been the case in a number of studies (36, 46, 115, 154, 184, 219, 238). It also is necessary to define the magnification used for diagnosis by light microscopy, rather than use equivocal terms such as "high-magnification field" and "oil immersion field."

Currently, the inability to fulfill Koch's postulates (7, 205), primarily because of the lack of experimental animal models, and the difficulty in excluding all other causes of symptoms mean that the role of *B. hominis* as a causative agent of human disease remains undefined (81, 95, 155). Protozoa, such as *Cryptosporidium* spp. and the microsporidia, which were previously considered to be nonpathogenic or to have low pathogenicity are now recognized to initiate disease, especially in immunocompromised patients (7, 79, 81, 208). It is possible that even if *B. hominis* is shown to be primarily a commensal organism, it may be a pathogen under specific host conditions, such as immunosuppression, poor nutrition, or concomitant infections (4). The existence of different demes of *B. hominis* with differing pathogenicities has not been clarified (25, 45, 63, 88, 95, 103, 104), and at present it is prudent to consider *B. hominis* a potential pathogen (4, 23, 71, 72, 120, 203).

Epidemiology and Prevalence

The epidemiology of *B. hominis* remains almost totally unknown, because studies have been hampered by misinformation and confusion over the status of the organism (6, 23, 70, 81). *B. hominis* is often the most frequent protozoan reported in human fecal samples, both from symptomatic patients (53, 149, 205, 237) and from healthy individuals (6, 53, 72, 148, 207). However, attempts have not been made to determine the true prevalence of *B. hominis* infections in humans.

B. hominis was originally reported as being associated with diarrhea in the tropics and subtropics (159, 212); however, more recent reports have indicated that *B. hominis* infections are common in residents of tropical, subtropical, and developing countries (6, 10, 34, 44, 51, 53, 54, 76, 89, 134, 140, 141, 150, 152, 198, 218, 219). Immigrants, refugees, and adopted children from developing countries appear to have higher incidences of *B. hominis* infection than do adults and children raised from birth in their new community (74, 75, 111, 139). The occurrence of *B. hominis* infections has been related to weather conditions, with the suggestion that infections are more common during hot weather (51, 99) or during the premonsoonal months (10). Conversely, other studies (34, 59, 165) have not found variations in the number of infections at different times throughout the year. Hahn and Fleischer (78) suggested that *B. hominis* frequently is acquired during travel to tropical countries, and a number of other studies have provided limited evidence to support this hypothesis (9, 38, 46, 96, 106, 167, 205, 213).

The prevalence of infections in various communities has been determined, although the results must be regarded circumspectly, because most studies were based on samples submitted to parasitology laboratories and are largely associated with symptomatic patients (23). It is difficult to compare studies, because most provide insufficient details of the patients chosen for study, and, while some studies report the number of patients, others report the number of infected fecal samples. Additionally, these infections were diagnosed by the recognition of the vacuolar form of *B. hominis* in fecal material, and the presence of other forms of the organism may have been missed. However, a number of reports may be regarded to give an indication of community infection rates. In general, reports from developing countries give higher prevalences of *B. hominis* (approximately 30 to 50% [6, 76, 134, 150, 198]) than those from developed countries (approximately 1.5 to 10% [46, 74, 115, 120, 165, 184, 205, 213, 238]), although exceptions do

occur (54, 72, 152, 207). Within communities, lower socioeconomic groups or those with lower standards of hygiene may show higher prevalences than the remainder of the community (54, 113, 141, 198, 213).

Higher prevalences in adults than in children have been noted in a number of studies (6, 46, 76, 89, 115, 158), and young adults appear to have the highest rates of infection (128, 152). Infection rates may decrease in older adult groups, possibly with another increase in old age (6). *Endolimax nana* and *Escherichia coli* show age prevalences similar to those of *B. hominis* (6), indicating that transmission methods may be similar. It is possible that multiple infections or other concomitant factors are required to establish *B. hominis* infection. Several studies (115, 238) found that *B. hominis* was more common in older persons as the sole infecting agent, although no significant differences were noted in the numbers of children and adults infected. However, a recent study by Nimri and Batchoun (141) found statistically significant declining rates of infection in older children when comparing children in the three age groups of 6 to 7, 8 to 9, and 10 to 12 years.

There does not appear to be any major difference in the prevalence of *B. hominis* between genders (71, 77, 89, 96, 184, 198), although some studies have reported slightly increased female to male incidences (46, 96, 158, 165). Two studies of *B. hominis* infections in children have reported male to female ratios of 2:1 (140, 141), but the significance of this has not been determined.

Infection with *B. hominis* appears to be common in immunocompromised patients (34, 36, 57, 58, 71, 82, 107, 114, 137, 154), and one study of children with chronic diarrhea found that only those with human immunodeficiency virus infection were infected with *B. hominis* (34). However, only limited data are available on the prevalence and significance of *B. hominis* infections in immunocompromised patients, both with and without human immunodeficiency virus infection, and comprehensive comparative studies with immunocompetent persons have not been performed.

Summarizing the current data, *B. hominis* infections do not appear to be restricted by climatic conditions or socioeconomic group, nor to any geographical area, with published data indicating a worldwide distribution. Infection probably is not gender related but may be influenced by the ages of the patients, their immunological status, and factors relating to hygiene.

Transmission

It generally is assumed that *B. hominis* is transmitted by the fecal-oral route, in a manner similar to other gastrointestinal protozoa (5, 23, 32, 69, 70, 96, 106, 135, 141, 174, 228), but this has not been confirmed experimentally. Although the vacuolar form of *B. hominis* is the form most commonly found in cultures and is the form normally used for diagnosis in fecal material (70, 228, 231), this form appears to have low infective potential when orally inoculated into gnotobiotic guinea pigs (147) and rats (189). Infection rates of only approximately 20% were achieved in guinea pigs, despite repeated oral inoculation with large numbers (8×10^5 to 10×10^5 organisms) of *B. hominis* vacuolar forms (147). A slightly higher rate of infection (approximately 35%) was achieved with intracecal inoculation of 4×10^5 to 5×10^5 organisms (147). These low infection rates probably were due to the destruction of the vacuolar or granular forms by the gastric secretions. The vacuolar form also appears extremely sensitive to environmental conditions, such as exposure to air, ambient or lower temperatures, and desiccation (223, 224, 227, 228, 231); therefore, it appears unlikely to be the major natural infective stage.

The cyst form was reported to be infective to rats when inoculated orally (189), because large numbers of *B. hominis* cells were detected by light microscopic examination of the cecal contents at autopsy 7 days postinfection. Smaller numbers of *B. hominis* also were detected in the large intestine (189). Details of the morphology of the organisms found in the intestine of the rat were not given. The cyst wall of *B. hominis* may provide protection against the environment encountered in the stomach (23, 176). Hence, it appears likely that the cyst form, rather than the vacuolar form, is the principal infective form of *B. hominis.*

Waterborne transmission of *B. hominis*, via untreated water or poor sanitary conditions, has been indicated (96, 140, 141), and food-borne transmission has been suggested (32, 59). These modes of transmission have not been proven but are consistent with reports of *B. hominis* infection associated with travel, particularly to developing countries and wilderness areas (9, 10, 46, 78, 99, 106, 167, 193, 210, 213).

Transmission has been reported among family members (73, 158, 198), among mentally deficient persons in institutions (87, 113, 213), and in communities without adequate sanitary facilities (54, 141, 198). *B. hominis* infections appear to be common in children in day care centres (76, 150), although controlled studies have not been performed. These reports all are consistent with a fecal-oral mode of transmission for *B. hominis.*

It has been suggested that sexual transmission of *B. hominis* may occur among homosexuals (137), although evidence for this has not been obtained. Studies of *B. hominis* in homosexual populations report widely varying rates of infection (36, 58, 107, 114, 137) and neither support nor refute the possibility of sexual transmission of the organism.

Garavelli and Scaglione (59) suggested that *B. hominis* is a zoonosis, and this was supported by the study of Doyle et al. (46) which indicated that patients with histories of exposure to pets or farm animals were more likely to be infected. However, another study did not find a correlation between *B. hominis* infection and the presence of animals (141). A recent report suggested that cockroaches may act as vectors for infection (222).

Blastocystis spp. are common in a range of mammalian, avian, and reptilian hosts, including domestic animals, pets, and other animals in close contact with humans (13, 15, 16, 18, 23, 24, 30, 131, 143, 144, 151, 176, 180, 181, 196, 214). In studies to date, the only group of animals in which *Blastocystis* spp. has not been found is the Osteichthyes (19). It is not known whether the organisms isolated from nonhuman hosts are *B. hominis* or other species of *Blastocystis*, although several other species have been proposed (13, 15, 18, 197). It also has not been determined if *Blastocystis* spp. have a low host specificity and to what extent the organisms can be transmitted among host species. Experimental cross-transmission has been achieved in guinea pigs (147), rats (185, 189), and mice (118) with *B. hominis* from humans and in domestic ducks with *Blastocystis* spp. from mallards (144) but has not been successful with *Blastocystis* spp. from chickens to geese and from pigs to chickens (19). If *Blastocystis* species do have low host specificity or if the organisms from animals are capable of infecting humans, the number and range of animals found to be infected with *Blastocystis* spp. indicate a vast potential reservoir for infection of humans.

DIAGNOSIS

The authors of the older diagnostic texts had not recognized *B. hominis* as a protozoan organism, and considered it a yeast

TABLE 1. Summary of the reported diagnostic features of different forms of *B. hominis*

Form	Size (μm)	Source	Central vacuole	Surface coat	No. of nuclei	Other remarks
Vacuolar	2–>200	Culture, feces	Present	Present (thin) or absent	1–4	Central vacuole occupies most of cell volume
Granular	6.5–80	Culture, feces	Present	Present (thin) or absent	1–4	Granules in central vacuole; morphology similar to vacuolar form
Multivacuolar	5–8	Feces, culture	Absent	Present (thick)	1 or 2	Multiple small vacuoles (may be too small to resolve by light microscopy)
Avacuolar	~5	Intestine, feces	Absent	Absent	1 or 2	Rarely reported
Amoeboid	2.6–7.8	Feces, culture	Absent	Absent	1 or 2	Rarely reported; conflicting information on morphology
Cyst	3–10	Feces, culture	Absent	Present or absent	1–4	Cyst wall present (beneath surface coat)

(68, 127, 175) or a fungus (146). However, the more recent texts report *B. hominis* as a potentially pathogenic protozoan organism and give a description of the diagnosis (5, 69, 70, 183). *B. hominis* usually is identified microscopically by the presence of the vacuolar form (69, 70), which is easily recognized by its "large size and characteristic appearance" (104). The organism must be distinguished from leukocytes (225, 228), *Cryptosporidium* spp. (55), and the cysts of other protozoa, particularly those of *Entamoeba histolytica*, *Entamoeba hartmanii*, and *Endolimax nana* (43, 55, 183).

Concentration methods, as used for other protozoa and fecal parasites, generally appear unsuitable for *B. hominis*, because they cause disruption of the vacuolar, multivacuolar, and granular forms of the organism (26, 135). In a detailed study, Miller and Minshew (135) often were able to detect *B. hominis* in stained smears of fecal material but not in concentrated specimens from the same fecal sample. This has been confirmed by other laboratories (26). However, other authors have reported that concentration methods are effective for *B. hominis* (1, 71, 76, 89, 109, 115, 228). Spontaneous sedimentation has been suggested as a suitable method for separating *B. hominis* from fecal material (76), and, although time-consuming, this may be the most appropriate method until further comparative studies are performed.

The addition of distilled water to the fecal material results in lysis of vacuolar forms of *B. hominis*, and saline has been recommended if dilutions or washes are performed during the preparation of samples for diagnosis of this organism (5, 69, 70, 220). Examination of multiple stool specimens is recommended to enhance the detection of low levels of *B. hominis* and other intestinal protozoa (70, 76).

Microscopy

B. hominis most frequently has been diagnosed by light microscopic examination of fecal material (228). Table 1 indicates morphological features which may aid in the identification of the various forms of *B. hominis*. Wet mounts, either unstained or stained with iodine, may be used (89, 104, 194). Aqueous nigrosin has been used as a counterstain in wet mounts (206), and India ink staining highlights the surface coat or "slime capsule" of *B. hominis* (153, 184).

Trichrome staining of permanent smears has been recommended for routine use in the diagnosis of *B. hominis* (69–71, 81, 109, 111, 126), although many other stains may be used successfully. These include iron hematoxylin (76, 119, 165), Giemsa (41, 153, 184), Gram (135, 228), and Wright's (203) stains. Phase-contrast microscopy also has been used for diagnosis (111, 213).

Permanent smears appear to be the procedure of choice for light microscopic diagnosis of *B. hominis*, because the organism may be difficult to identify in wet mounts (70, 126, 167, 219). However, variability in staining, particularly of the central vacuole, has been reported to occur with trichrome (70) and iron hematoxylin (119) stains. Variability in the size and shape of the organism also has been noted, and a size range of 6 to 40 μm has been reported from smears of fecal material (69, 70). Recent studies indicate that the forms found in very fresh fecal material may be at the lower end of this size range (46, 119) and may be multivacuolar forms rather than the large vacuolar form (179). The appearance of the organism may be altered by environmental conditions or treatment of the fecal specimen prior to examination (176), although the effects of preservative, diluent, and staining solutions commonly used in clinical laboratories have not been adequately assessed. There is evidence of alteration of morphology as a result of osmotic changes and of the time of storage of unfixed fecal material prior to microscopy (176).

The cyst forms of *B. hominis* also may be present in fecal material (177, 220), and unpublished data indicate that in some instances, the cyst forms may be the predominant or only forms of *B. hominis* seen in some human fecal samples (27). Because of the presence of large lipid inclusions and glycogen deposits, staining patterns of the cyst forms may vary significantly (176). Additionally, the cyst forms are small, commonly noted to be in the range of 3 to 5 μm in diameter (176, 177, 220). Hence, these forms can present a diagnostic challenge.

Transmission electron microscopy may assist in confirming the diagnosis if atypical morphological forms of *B. hominis*, such as small cyst forms with large lipid or glycogen inclusions, are noted. However, electron microscopy generally is not required for routine diagnosis of the organism and is of greater significance in research. Fecal material to be processed for transmission electron microscopy should be fixed with glutaraldehyde fixative as soon as possible after passage of the stool, to minimize morphological changes and cellular degeneration (176). Fixatives used for light microscopy generally are not adequate and are not recommended if electron microscopy is to be performed.

Immunological Diagnosis

Serologic testing has been used in an attempt to identify patients with *B. hominis* infections, with very limited success. A

lack of humoral immune response was found by Chen et al. (35), using immunoblotting techniques with antigens from cultured *B. hominis* isolated from four patients. Only immunoglobulin G was examined in the study, and further investigations may show a response with other immunoglobulin subclasses. Garavelli et al. (64) reported the success of the immunofluorescence assay and enzyme-linked immunosorbent assay (ELISA) in detecting serum antibodies to *B. hominis* in four patients. An ELISA has been used to detect immunoglobulin G antibodies in the sera of 28 patients, although the threshold dilution for a positive result was reported as 1/50 dilution (237).

The review by Zierdt (228) reported previously unpublished data on the use of the immunofluorescence assay for the detection of *B. hominis*. Rabbit antisera to unheated whole-cell *B. hominis* antigens reacted with the vacuolar, granular, and ameboid forms of *B. hominis*. The antisera did not react with bacterial, fungal, or mammalian cells (228).

The availability of specific antibodies for application to fecal material would ease the diagnosis of *B. hominis*, especially in patients with small numbers or small forms of the organism or those with forms with atypical morphology. At present, appropriate antibodies are not available for clinical diagnostic applications.

Other Diagnostic Procedures

Invasive techniques occasionally have detected *B. hominis* in the intestine but have not been evaluated and are not recommended as routine methods for diagnosis of the organism. Fluid aspirated during endoscopy has been used to detect *B. hominis* organisms in the lumen and cecum (129), and duodenal secretions from the enteral string test were found to contain *B. hominis* (149, 203). *B. hominis* has been reported in colonic scrapings obtained during colonoscopy (43). Touch cytologic testing, performed by rolling endoscopic biopsy specimens onto glass slides and then using a rapid staining method similar to May-Grunwald-Giemsa stain, has been used to diagnose infections (42). Endoscopy and sigmoidoscopy also have been used to investigate the intestinal damage attributed to *B. hominis* infections (45, 46, 96, 106, 110, 157, 239).

Culture from fecal material appears to have no advantages over light microscopy of fresh fecal material for the detection of *B. hominis* and requires increased time, costs, and personnel (103, 104). It also has been reported that culture was successful only if large numbers of *B. hominis* were present in the fecal material (225, 228); therefore, cases with small numbers of organisms may not be detected. Any increase in the detection efficiency appears to be due to the increase in size and to the "typical" vacuolar appearance of *B. hominis* cells from culture and, hence, to the ease of detection of the organisms by light microscopy (103, 104, 225). However, it must be recognized that forms other than the vacuolar form of *B. hominis* may be present in the human intestinal tract and from human fecal material, and the detection of these forms from culture of fecal material has not been assessed. Additionally, the numbers of organisms present in the original sample cannot be determined from cultured material.

TREATMENT AND CONTROL

The requirement for treatment of *B. hominis* infections remains controversial. In the absence of conclusive evidence of pathogenicity of the organism, treatment with potentially dangerous drugs and the failure to investigate the true cause of symptoms in patients are of concern to many physicians (124). However, other physicians believe that treatment of *B. hominis* infections is warranted when debilitating symptoms are present and no other cause of disease is obvious (106, 109, 203). It also should be considered that the infection may be self-limiting and so intervention may not be required (9, 46, 123, 184, 206, 219, 228).

Chemotherapy

There are very few experimental data to verify the efficacy of the chemotherapy of *B. hominis*. Very few studies have considered the treatment of large numbers of patients, and case-controlled studies have not been performed. Hence, the choice and dosage of drugs used for the treatment of *B. hominis* infections remain largely empirical.

The efficacy of chemotherapy of *B. hominis* has been assessed as the total absence of parasites in subsequent stool samples, a reduction in parasite numbers, or the alleviation or elimination of symptoms. This assessment needs to be standardized, and double-blind clinical trials need to be conducted. Until the pathogenicity of the organism is determined, it is not known whether total elimination of parasites is required for the cessation of the symptoms of *B. hominis* infections or whether a low level of parasites can be tolerated.

Antiprotozoal drugs, particularly metronidazole (3, 9, 28, 45, 46, 52, 55, 58, 67, 73, 77, 89, 92, 96, 106, 109, 110, 112, 153, 154, 157, 194, 203, 206, 218, 219, 238) or iodoquinol (10, 46, 91, 124, 218, 238), commonly are recommended for treatment of *B. hominis* infections. Metronidazole and iodoquinol have been administered together in one study (154). It should be noted, however, that iodoquinol is no longer available in many parts of the world, including Australia, because of its toxicity.

Other drugs may be highly or moderately efficacious in treating *B. hominis* infections but have been reported only in individual case reports or in studies with very limited numbers of patients. These drugs include furazolidone (139), quinacrine (10, 184), ornidazole (78), tinidazole (10, 50), trimethoprim-sulfamethoxazole (154, 228), co-trimoxazole (164, 219), ketoconazole (38, 219) and Entero-vioform (228). Emetine and the arsenicals Stovarsol (acetarsone), Carboarsone, and Narsonal (228) have not been used in recent studies because of toxicity. Tetracycline apparently decreased the recovery rate of *B. hominis* from the stools of infected persons in one study (164), but this has not been thoroughly assessed.

Recommended doses for metronidazole treatment of *B. hominis* infections include 250 to 750 mg three times per day for 5 to 10 days (89, 96, 194), 200 mg four times per day for up to 7 days (77), or 2 g/day for 5 days (57, 62, 67). Iodoquinol has been administered at 300 mg three times per day for 10 days (218) and at 650 mg three times per day for 20 days (238).

There have been reports that metronidazole was ineffective in the treatment of *B. hominis* infections (38, 114, 124, 164, 165, 234) or was effective only in a small number of the treated cases (57, 62, 184). Similarly, there are reports of the ineffectiveness of iodoquinol (124, 165), furazolidone (165), quinacrine (124), and paromomycin (124). It is not known whether these treatment failures are due to drug resistance, as seen in other protozoal infections (199), or to other factors, such as patient noncompliance, different pharmacokinetic properties in different patients, different drug sensitivities of different demes of *B. hominis*, inaccessibility of the organisms to drugs, or inactivation of drugs by concomitant organisms.

Few studies have reported the results of chemotherapy for *B. hominis* infections in immunocompromised patients. Metronidazole, given at a dose of 1 to 2 g/day, has been reported to be successful in eliminating the organism (57, 62, 67, 112,

154), as have trimethoprim-sulfamethoxazole and a combination of metronidazole plus iodoquinol (154). Furazolidone was reported to be effective in one study in which metronidazole was not tolerated by the patient (139) and has been suggested as an alternative compound for treating AIDS patients who might not tolerate trimethoprim-sulfamethoxazole therapy (139).

To date, only two studies have examined the effects of drugs on *B. hominis* in vitro. Zierdt et al. (233) investigated the effects of 10 antiprotozoal drugs on the growth of four isolates of *B. hominis*. The study was not quantitative but classified the drugs into inhibitory (emetine dihydrochloride, metronidazole, furazolidone, trimethoprim-sulfamethoxazole, Entero-vioform, and pentamidine isethionate), moderately inhibitory (iodoquinol and chloroquine), and noninhibitory (diloxanide furoate and paromomycin sulfate) compounds. Some variability in the response to most drugs was noted among the four isolates of *B. hominis.*

A quantitative study by Dunn and Boreham (48), based on the incorporation of [^{3}H]hypoxanthine, reported the comparative efficacy of 42 drugs, including 12 5-nitroimidazoles, to inhibit the growth of a single isolate of *B. hominis*. The drugs found to be most effective in vitro were emetine dihydrochloride, furazolidone, satranidazole, and S75 0400 A [1-methyl-2-(4-dimethylamino-methylenimino-phenoxymethyl)-5-nitroimidazole hydrochloride]. Flunidazole, ronidazole, quinacrine dihydrochloride, niridazole, and metronidazole also were effective, as were, to a lesser extent, ornidazole, chlorimipramine hydrochloride, tinidazole, trimethoprim, and secnidazole. However, Entero-vioform, chloroquine diphosphate, desipramine hydrochloride, ketoconazole, nimorazole, amodiaquine base, fexinidazole, panidazole, amphotericin B, and iodoquinol showed poor inhibition of the growth of *B. hominis*. Suramin, albendazole, mebendazole, diloxanide furoate, penicillin, streptomycin sulfate, miconazole, azithromycin, amitriptyline hydrochloride, gentamicin, erythromycin estolate, griseofulvin, sulfadiazine, sulfamethoxazole, sulfanilamide, paromomycin sulfate, and imidocarb dipropionate were ineffective, even at concentrations greater than 8.0×10^{-5} mol/liter.

Antibacterial compounds, such as ampicillin, penicillin, streptomycin, gentamicin, colistin, ceftizoxime, and vancomycin, and the antifungal agent amphotericin B are not considered to inhibit the growth of *B. hominis* in vitro and commonly are used to prevent bacterial and fungal overgrowth during culture (48, 49, 228, 236). Addition of chloramphenicol to the culture medium has been reported to kill *B. hominis* after several passages (233).

The drugs commonly used therapeutically, primarily metronidazole and iodoquinol, showed in vitro activity against *B. hominis* (48, 233), although iodoquinol, commonly recommended as an alternative to metronidazole, had only low or moderate activity in vitro (48). Further in vitro testing with a wider range of *B. hominis* isolates is required to determine the extent to which variability occurs in the response to drugs among different isolates. However, in vitro responses cannot necessarily be extrapolated to drug efficacies in humans or other animals, and the current lack of an appropriate experimental animal model has prevented in vivo testing of the effectiveness of potential chemotherapeutic compounds and regimens.

From the results of clinical reports and limited in vitro studies, the drug of choice for chemotherapy of *B. hominis* appears to be metronidazole or other nitroimidazoles, or iodoquinol in the countries where it is available (23, 48). Failures may occur, and then the decision on further treatment is largely empirical. Co-trimoxazole, trimethoprim-sulfamethoxazole, or the aminoglycoside paromomycin, which is not absorbed from the intestine, is possibly the most appropriate second-choice drug. Chemotherapy does not appear to be required in asymptomatic individuals and may not be warranted in patients with mild or transient symptoms. Until the pathogenicity of *B. hominis* is properly defined, the existence of carrier states is determined, and the spontaneous elimination of parasites is assessed, the chemotherapy of infections with this organism will remain controversial. Treatment should be used, with caution, only after a thorough clinical review of other possible causes of symptoms.

Other Management Strategies

Dietary management in patients with *B. hominis* infection was found to be effective in reducing symptoms or parasite numbers in several studies (96, 190). The study by Kain et al. (96) noted that all of their six patients with small numbers of *B. hominis* showed clinical improvement with lactose-free and/or high-fiber diets. This was a higher proportion of patients than those responding to metronidazole treatment in the study (96). However, it is possible that *B. hominis* was not the cause of symptoms in the patients showing clinical improvement, and the total number of patients in the study was very small.

Conclusive evaluations of dietary management of *B. hominis* infections have not been performed. Some studies have suggested that altered intestinal conditions, such as changed nutrient levels, reduced redox potential, or change in bacterial flora, may enhance the growth of *B. hominis* (96, 135), and in such cases, dietary changes that redress these alterations may be of benefit to the patient.

Prevention

Considering the present data, it is reasonable to assume the fecal-oral route as the most likely for transmission of *B. hominis*. Thus, control measures would include good personal hygiene, improvement in community sanitary facilities, and education to prevent fecal contamination of the environment and ingestion of contaminated material. It has not yet been determined if *Blastocystis* infection is a zoonosis and if animals and their excreta represent a risk factor for human infection.

It is not known which, if any, sterilization procedures are effective against *B. hominis*. It has been reported that the vacuolar and granular forms of the organism are sensitive to oxygen, air, and desiccation (223, 227, 228, 231). Hence, the vacuolar and granular forms would not be expected to pose significant contamination problems in the environment. The resistance of other forms of *B. hominis* has not been determined, although the cyst form appears able to survive in some environmental conditions (189) and the organism has been isolated from sewage (221).

CONCLUSIONS

Our current knowledge of *B. hominis* and the putative disease it causes is insufficient to determine the significance of the parasite in humans. A major problem with the research to date is the small number of investigators engaged in the study of *Blastocystis* infections and the potential for bias that may result. Greater collaboration among the research groups is required. The current literature must still be considered with a great deal of caution, because ambiguous and unsubstantiated data still are presented.

Over the past few years, a considerable amount of data relating to the morphology of the parasite has been collected, so that now a working hypothesis of the life cycle, based on known facts, has been postulated (23). The proposed life cycle

can be tested, but this does require the development of a suitable animal model. Significant advances are being made in this field, with the successful experimental infection of rats (185, 189), mice (118), and chickens (14).

A detailed knowledge of the biology of the organism is essential to define effective methods for diagnosis, treatment, and control. At present, chemotherapy of *B. hominis* infections is empirical. Because our knowledge of the biochemistry of the organism is rudimentary, we cannot target specific metabolic sites with drugs. It is imperative to determine which is the infective stage of the organism, what are the stages susceptible to chemotherapy in the host, and to what extent are there variations in susceptibility to commonly used drugs between different demes of *B. hominis*. It is important also to determine the role and function of unique organelles and structures, such as the mitochondrion-like organelles, the central vacuole, and the nuclear substructures, which might give indications of appropriate control targets.

As far as the clinical aspects of the putative disease are concerned, much more data are required before *Blastocystis* spp. can be conclusively shown to cause disease. Koch's postulates should be fulfilled, and a prerequisite of this is the identification of an appropriate animal model. Case studies are of limited value, because it is almost impossible to rule out all other infectious and noninfectious causes of the nonspecific symptoms that have been associated with *B. hominis* infection. Our knowledge about the epidemiology of *Blastocystis* infection and the risk factors associated with the acquisition of the parasite is extremely limited. We need to determine the true prevalence in different populations and whether there are different demes, strains, or species of the organism that cause the patient to be symptomatic or not and to verify the exact mode of transmission.

Because of our current lack of information about this organism, it is difficult to predict the status of *Blastocystis* spp. in 5 to 10 years time. It is certain, however, that this unusual organism still has a number of surprises to reveal to the careful researcher.

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