CLINICAL MICROBIOLOGY REVIEWS, Jan. 1991, p. 61–79
0893-8512/91/010061-19$02.00/0

Vol. 4, No. 1

Blastocystis hominis—Past and Future

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HISTORY AND INTRODUCTION

A review of *Blastocystis hominis* requires a thorough historical description to appreciate events leading to its reclassification and consideration as a respected human pathogen. The history of this complex parasite is a panorama of early advance followed by decades of retreat into obscurity and, then, reintroduction into the modern era. The problems preventing the orderly development of knowledge of *B. hominis* and its deleterious effects were largely the fault of the organism itself.

On first discovery, the unique mimicry of form of *B. hominis* led to a state of confusion. The predominant hollow spherical form was, and still is, confused with unrelated cells from many animals. It has falsely been reported as occurring in almost every insect, reptile, bird, fish, and mammal examined. The earliest descriptions, with the required drawings that purportedly were of the organism later called *B. hominis*, were those of Brittan (6) and Swayne (70), who studied and wrote voluminously of the London cholera epidemic of 1849. They worked separately but shared their findings. The cells that Swayne called "cholera bodies" and Brittan called "annular cells" were regarded by them as the cause of cholera, and they convinced other London physicians who were studying the epidemic, including William Budd, who had accurately described the epidemiology of typhoid fever in 1840. Critical analysis of their works, however, does not support the contention that they discovered *B. hominis*.

Yakimoff (82, 83) has stated that the work of Brittan and Swayne included descriptions of *B. hominis* but Swayne's drawings of cholera bodies, in their clearest form, are *Ascaris lumbricoides* ova. Brittan provided drawings of "condensed air" of cholera patients (apparently a suspension of room dust in water condensed from the air) as well as feces from cholera patients and from healthy persons. He observed his annular bodies in all specimens except those from healthy persons. While his report of annular bodies in condensed air and specimens from cholera patients seems incredible, his drawings of diarrheic excretions show a few cells that might be *B. hominis*. The same drawings unmistakably depict *A. lumbricoides* and *Trichuris trichiura* ova. Thus, the credit given to Brittan and Swayne for the discovery of *B. hominis* is misplaced.

Kean et al. (33) credited Fedor Aleksandrovich Lösch, the discoverer of *Entamoeba histolytica*, with the discovery of *B. hominis* in 1849, but Lösch's reports and illustrations do not corroborate this discovery (37, 38). Because written descriptions may not be specific enough, more reliance must be placed on drawings. Lösch's drawings are too indefinite to be identified as any specific ameba. Coupled with his

extensive descriptions, they are of *E. histolytica*, but not *B. hominis*. From manuscripts that we have examined, Perroncito is more likely to have discovered *B. hominis* in 1899 (56), but again there is insufficient documentation. Perroncito provided an adequate written description of *B. hominis*, but no drawings. He stated that the organism was probably a member of the coccidia. In the same year, Borini (5), a worker in Perroncito's laboratory, referred to it as "a new Perroncito parasite," and called it a protozoan as well as "a corpuscle." No illustrations were provided. Borini, Perroncito's assistant, made no mention of a binomial in his publication later in 1899. Micheletti referred to Borini as "holding like opinions," but as we have seen, Borini provided no name, other than protozoan and corpuscle. In 1932, Micheletti (49) used the genus name provided by Alexieff (1), *Blastocystis*, added *jalinus* (origin unknown), and called the protozoan *Blastocystis jalinus* (Perroncito). He stated that, by priority, the organism was *B. jalinus* Perroncito 1901 and not *Blastocystis hominis* Brumpt 1912. This report is surrounded by mystery because Micheletti did not cite any 1901 publication by Perroncito, and no such publication has been uncovered to date. Perroncito apparently did not name the organism *Coccidium jalinum* in 1899. Part of the answer to this quandary may lie in verbal communication, personal correspondence, or published material unavailable to us.

The first description fulfilling requirements of nomenclatural priority goes to Alexieff (1) for his 1911 description, naming the organism *Blastocystis enterocola*, a yeast. He applied the same binomial to cells that he observed in rats, guinea pigs, chickens, reptiles, and leeches. His appelation is prior to that of Brumpt, who in 1912 coined the name *Blastocystis hominis* (7), changing the specific epithet from *enterocola* to *hominis*. The belief that Brumpt's classification had priority because he worked only with human material had an early influence, and the name *B. hominis* is now firmly entrenched and recognized worldwide. Because of the possibility that the organism will be further reassigned within the protozoa, this is not a good time to change the name back to *B. enterocola*.

Some early workers observed *Blastocystis* cells in many animals, including flies, snakes, rodents, and cockroaches (47), but these observations have been largely ignored. In the early days of microscopy, the tendency to be fooled by artifacts, degraded cells, and normal cells of all description was widespread. Many degenerate tissue cells, vegetable cells, and yeast cells, as well as many artifacts, resemble *B. hominis*. Degenerated hepatocytes in liver have been mistaken for *B. hominis*, which is then cited as an invasive liver parasite. Rosenbusch (61) mistakenly attributed the disease blackhead in turkeys to *B. hominis* through such an error in diagnosis, and in the same way human hepatic pathology has been attributed mistakenly to *B. hominis*.

Isolation of *B. hominis* has been confirmed only in humans, monkeys, apes, pigs, and, perhaps, guinea pigs. It is likely that more than one *Blastocystis* species is involved. Alexieff was accused of creating his new genus by confusing pollen grains and helminth ova, such as *A. lumbricoides*, with a new organism. The accusation was also aimed at Brittan and Swayne, against whom it was more accurately made.

Eminent early workers, including Swellingrebel (71), Alexieff (1), Wenyon (77), and Cicchitto (12), thought that *B. hominis* was a degenerate or cyst form of the flagellates *Trichomonas intestinalis* and *Chilomastix mesnili* or of *Endolimax nana*. Their opinion was accepted by many other workers in the field, and peripherally in other disciplines, and continued in force into the middle of the 20th century. Brumpt's publication (7) has been most cited. His classification and a brief description of *B. hominis* as a harmless intestinal yeast, important only because it might be confused with *E. histolytica*, became the standard entry in succeeding editions of parasitology and medical texts. The published descriptions of epidemic disease in humans remained buried in the Russian, Italian, French, German, Argentine, Portuguese, Rumanian, English, and other literature, without entrance into the parasitology and infectious disease texts of any country. These old papers in translation are credible descriptions of infections, written by physician-parasitologists expert in field work and enteric parasitology, many of whom saw thousands of cases of *B. hominis* disease (4, 9, 10, 39, 47, 54, 55, 63, 64, 65, 82).

Eventually, reports of *B. hominis* as an agent of intestinal disease in individuals as well as in group settings, e.g., during military campaigns and in institutions, began to appear. A strange paradox was the inclusion of *B. hominis* in surveys of intestinal parasites (amebae, flagellates, and ciliates), often without reference to its classification as a yeast. Efforts to further characterize this "enigma," as Bach and Keifer (3) called it, did not result in significant change in its status. Succeeding publications affirmed the yeast classification and assigned the organism to different yeast genera, for example, *Schizosaccharomyces* and *Saccharomyces* (7). Ciferri and Redaelli placed it with the achlorophyllic algae, in the genus *Prototheca* (13). As late as 1972, Wolynska and Soroczan classified *B. hominis* as a phycomycete (80).

There were good reasons for classifying *B. hominis* as a yeast. It had a yeastlike glistening appearance in fresh wet fecal mounts, no pseudopodia in the usual unheated wet preparations, and no evidence of locomotion. The size range was more extreme than expected in a protozoan, as was the diversity of forms seen in a single specimen. Division by binary fission was easily misconstrued as budding. No cyst form was found and the ameba forms of the organisms were not recognized as *B. hominis*. The nuclei were not visible by bright-field optics in unstained wet mounts. No life cycle in humans was evident; no secondary or alternate hosts were known, and its mode of transmission was not demonstrated. *B. hominis* was not recognized as possibly tissue invasive, a characteristic providing acceptance of a parasite as pathogenic.

TAXONOMY

Zierdt et al. (91) first described the protozoan characteristics of this intestinal yeast. While no single character strongly allied *B. hominis* with the yeasts, many allied it with the protozoa (Table 1). Continued research on culture, physiology, and ultrastructure (84, 87) strengthened the need for this change in classification.

B. hominis is difficult to classify and may eventually be placed in an individual niche within the protozoa, based on the unique diversity of its shapes and sizes, its unique physiology, and some of its reproductive modes. Shared RNA sequences with other eucaryotic cells (31) confirm that *B. hominis* is a protozoan and perhaps belongs in a group by itself. Since its reclassification in 1976, it has been placed with the family Sporozoa (94). However, the findings of motility by pseudopodia as well as feeding pseudopodia, extracellular location in the host, lack of an apical complex,

TABLE 1. Characteristics used to reclassify *B. hominis* from the fungi to the protozoa

Characteristic	Yeast	*B. hominis*
Strict anaerobe	No	Yes
No growth on fungal or bacteriological media	No	Yes
Growth on media developed for intestinal protozoa	No	Yes
No growth on surface of solid media	No	Yes
Requires presence of bacteria for growth; axenic growth achieved slowly under carefully controlled conditions	No	Yes
Capable of ingesting bacteria and other particulate matter	No	Yes
Cultures die in 2–3 days at 22°C or overnight at 4°C	No	Yes
No growth below 33°C, but cell death at 30°C	No	Yes
Optimal growth at 37°C	No	Yes
Optimal growth at neutral pH	No	Yes
No growth at pH 5.5	No	Yes
Resistant to 400 μg of amphotericin per ml	No	Yes
Sensitive to drugs effective against intestinal protozoa	No	Yes
No cell wall	No	Yes
Reproduction by endodyogony, schizogony, binary fission, and plasmotomy (cutting off) individual *B. hominis* from the ameba form	No	Yes
No budding	No	Yes
Supports stable bacterial endosymbiont (an obligate mutualism)	No	Yes
Slow-feeding pseudopods	No	Yes
Rapid locomotion pseudopods	No	Yes
Limiting membrane with micropinocytotic vesicles	No	Yes
Membrane-bound CB, a reproductive organelle, formerly called vacuole	No	Yes
Mitochondria in all cells; mitochondria show a general morphology similar to that in other protozoa, with short saccate cristae when in the resting mode	No	Yes

and other minor characteristics indicate a better assignment is to the Sarcodina. The initial (mistaken) classification was in phylum Protozoa Goldfuss 1818 emend. von Liebold, 1845, subphylum Sporozoa Leuckart, 1879 emend. Levine 1963. A new class, Blastocystea, and order, Blastocystida, were required for the genus *Blastocystis*, species *hominis*.

According to the classification schema of Levine et al. (36), the 1985 classification and description of *B. hominis* are as follows: kingdom Protista, subkingdom Protozoa, phylum Sarcomastigophora, subphylum Sarcodina, superclass Rhizopoda, class Lobosea, subclass Gymnamoeba, order Amoebida, new suborder Blastocystina, genus *Blastocystis*, species *hominis*—"cells spherical and widely variable in size, can be amebiform in disease, limiting membrane only, no cyst form, may have glycocalyx (capsule), large membrane-bound central body for schizogony occupying large volume of cell but absent in amebiform cell, delineating a peripheral band of cytoplasm including most of the cell's organelles, multinucleate, cytochrome-free mitochondria always present, division usually by binary fission, also plasmotomy, endodyogony, and schizogony, slow feeding pseudopodia, rapid locomotion pseudopodia, pinocytotic feeding, mesomitotic, no flagella, intestinal parasite of higher primates" (89). This classification seems to suffice for the present.

MORPHOLOGY

Light Microscopy

For diagnostic work, bright-field optics are preferred, because bright field is more universally available. For research, examination by differential interference contrast optics (DIC) of wet mount live preparations is required (Fig. 1). Built-in photographic capability of proven quality should be included. By Nomarski optics, *B. hominis* nuclei are visible and normally associated with the brightest organelles, the mitochondria, which form rosettes about the nuclei (Fig. 2C and D). The mitochondria are normally spherical, but may elongate rapidly.

In clinical samples, *B. hominis* is brightly refractile and of widely variable diameter (4 to 15 μm) and contains visible mitochondria. The organelles are gathered as one, two, or four thickened opposed pods in the thin band of peripheral cytoplasm. These pods bulge the central body membrane inward. The outer membrane remains entire and glistening, with no protuberances. A distinct band of capsular material, or slime, may surround the cell (Fig. 1C) and is apparent because it forms a transparent circle of variable thickness delineated on the inside by the cell membrane and on the outside by bacteria, cells, and food debris in the fecal suspension. In wet mounts, *B. hominis* cells at the edge of the cover glass quickly deform from oxygen intrusion, collapse, and release cytoplasmic contents, finally leaving empty membranes, looking like burst balloons (Fig. 2A). The *B. hominis* cell is extremely plastic, passing through constricted passages in its environment much like an erythrocyte. The mitochondria are stained selectively with Janus green and even better with the fluorescent dye rhodamine 123 (90).

In cells from most clinical samples, the central body (CB), once called the vacuole, appears completely empty. It occupies a variable volume of the cell, from 50 to 95%, with average displacement of about 80%. It is always concentric with the outer membrane, never eccentric. The CB contents appear dense and stain as amorphous material or particles with Gram, Feulgen, trichrome, and hematoxylin-eosin stains, but by transmission electron microscopy (TEM) are seen to be membrane spheres of widely variant diameters. Trichrome stain may remain inside the CB, apparently trapped there, so that the CB appears uniformly dark.

In some infections the ameba form may be the only form seen, and diagnosis is difficult because it resembles leukocytes. The numbers of amebae, however, often exceed those

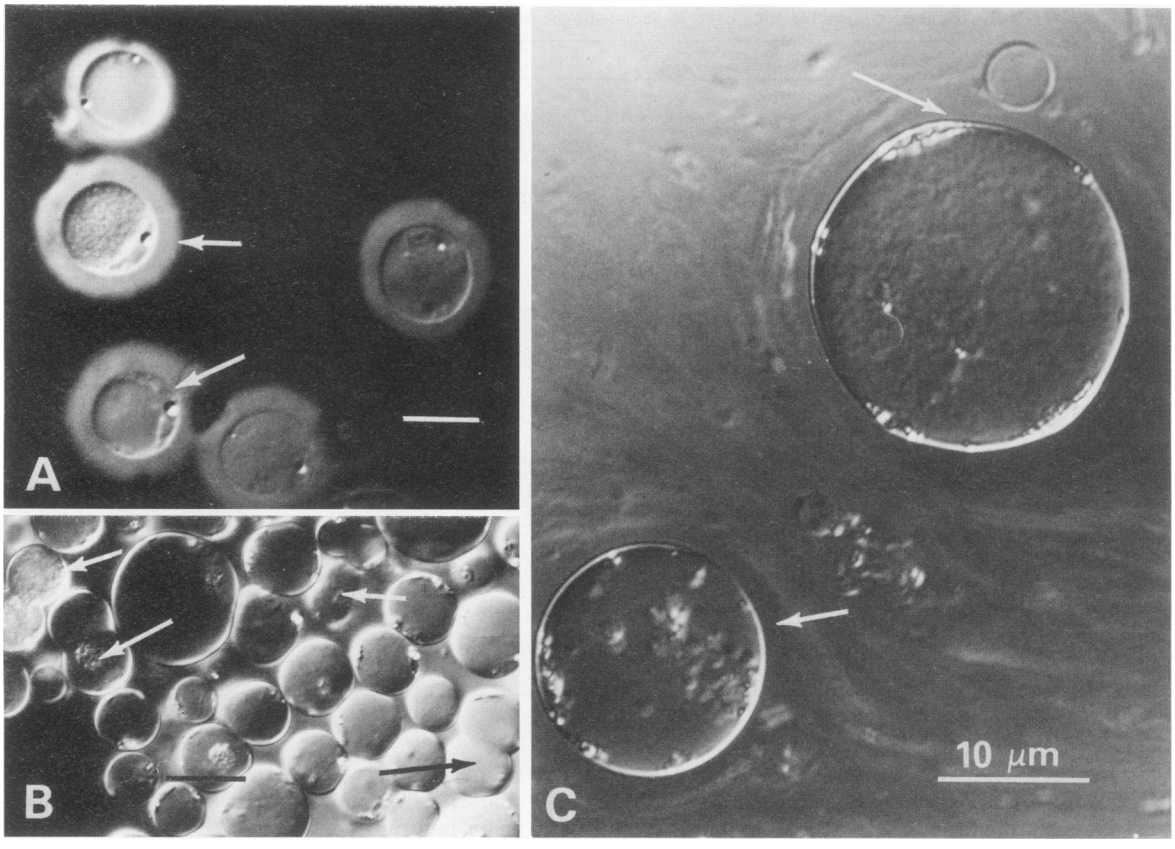

FIG. 1. *B. hominis* seen by DIC optics. All wet mounts. (A) Slime coat or capsule (arrows). India ink mounts. (B) Cultured cells showing binary fission (arrows). (C) Slime coat as clear zone around cell (arrows).

of accompanying leukocytes. The cell outline is distinguishable from that of leukocytes. It is more diverse in morphology and appears stretched out along mucus strands because of its plasticity. Giemsa stain reveals well-defined and much larger nuclei in leukocytes, while the nuclei of the amebae are perfect spheres of 1-μm diameter, staining faintly. The ameba form is often seen in culture.

TEM

Fixation for electron microscopy is done anaerobically by adding 8% (vol/vol) glutaraldehyde with a pipette tip submerged directly in the growth sediment at the base of the egg slant culture (see section, "Culture"). This step initiates fixation and kills the *B. hominis* cells so that the compact growth sediment can be transferred to another tube of 4% glutaraldehyde in cacodylate buffer (72, 73). After 1 h, the suspension is centrifuged at 500 × *g* for 10 min, decanted, resuspended in 1% glutaraldehyde buffer, and stored at 4°C. Dehydration and embedding are best done within 24 h of specimen collection but can be delayed when necessary.

The major forms of *B. hominis* are the CB form, the granular (mitochondrion) cell form, and the ameba form. A minor form is the uncommon schizont. In the gastrointestinal tract, the CB form predominates except in rampant infections, when the ameba form may be the only one seen or the two are seen together. Figure 3 illustrates some *B. hominis* forms seen by TEM.

CB form. As indicated, the granular form is so designated because of the high concentration of mitochondria in the cytoplasmic sphere, obscuring the CB. The CB form can be considered a *B. hominis* cell having a minimal number of mitochondria. Although the CB is commonly so large as to restrict the cytoplasm to a spherical band only 30 to 80 nm wide, in clinical material and cultures it is also seen as a diminished body remaining in the center of the cell. The cytoplasm then exceeds the CB in volume, and when the CB disappears, the total volume of the cell consists of cytoplasm plus organelles. The CB is directly involved with schizogony and becomes filled with progeny during this asexual reproductive mode. In developing to a schizont, the CB cell eventually loses the CB membrane, and its contents intermingle with the cytoplasm. There is evidence of passage of mitochondria and nuclei from cytoplasm to CB through the CB membrane or commingling after dissolution of the CB membrane. The complete role of the CB in schizogony and perhaps in other cell functions is complex and remains to be elucidated.

Ameba form. The ameba form of *B. hominis* provides the greatest challenge for diagnosis. In cultures, the ameba form reverts to the CB form whose morphologic characteristics and ability to undergo binary fission make it readily identifiable as *B. hominis*. In diarrheal fluid, however, the ameba form mimics leukocytes. An immediate differential test is to perform a Gram stain of air-dried but not heat- or alcohol-fixed smears. The ameba form lyses, while neutrophils and macrophages stain and are easily recognized. There is no cyst form of *B. hominis* and, so far as is known, no resistance to air exposure, water, or desiccation.

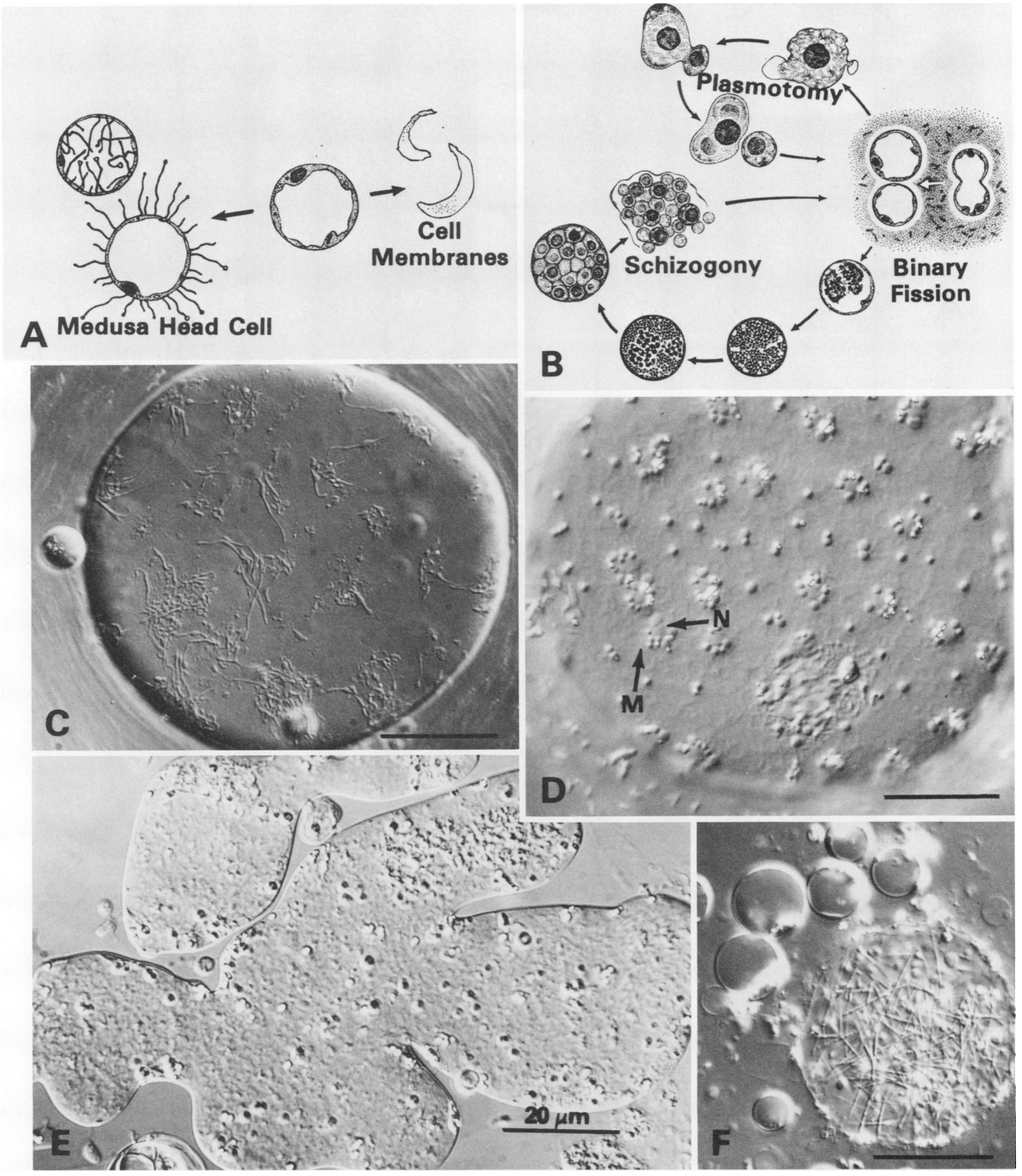

FIG. 2. (A) Schematic diagram of structural change caused by exposure to air. Collapsed cells and membrane extrusions shown. The extrusions fall back on and adhere to the cell. (B) Division modes of *B. hominis* showing three modes. Endodyogony is not shown. (C) Giant cell showing mitochondria in the filamentous condition; many include a bulbous swelling. The mitochondria are growing radially from their rosette arrangement. Panels C to F are DIC optics on wet mounts. (D) Rosettes of brightly refractile mitochondria around single nuclei or clusters of nuclei. (E) Giant amebiform cells from culture. (F) Classic CB form cells and a giant cell with filamentous mitochondria arranged like jackstraws. Mitochondria are also interspersed at regular intervals between rosettes.

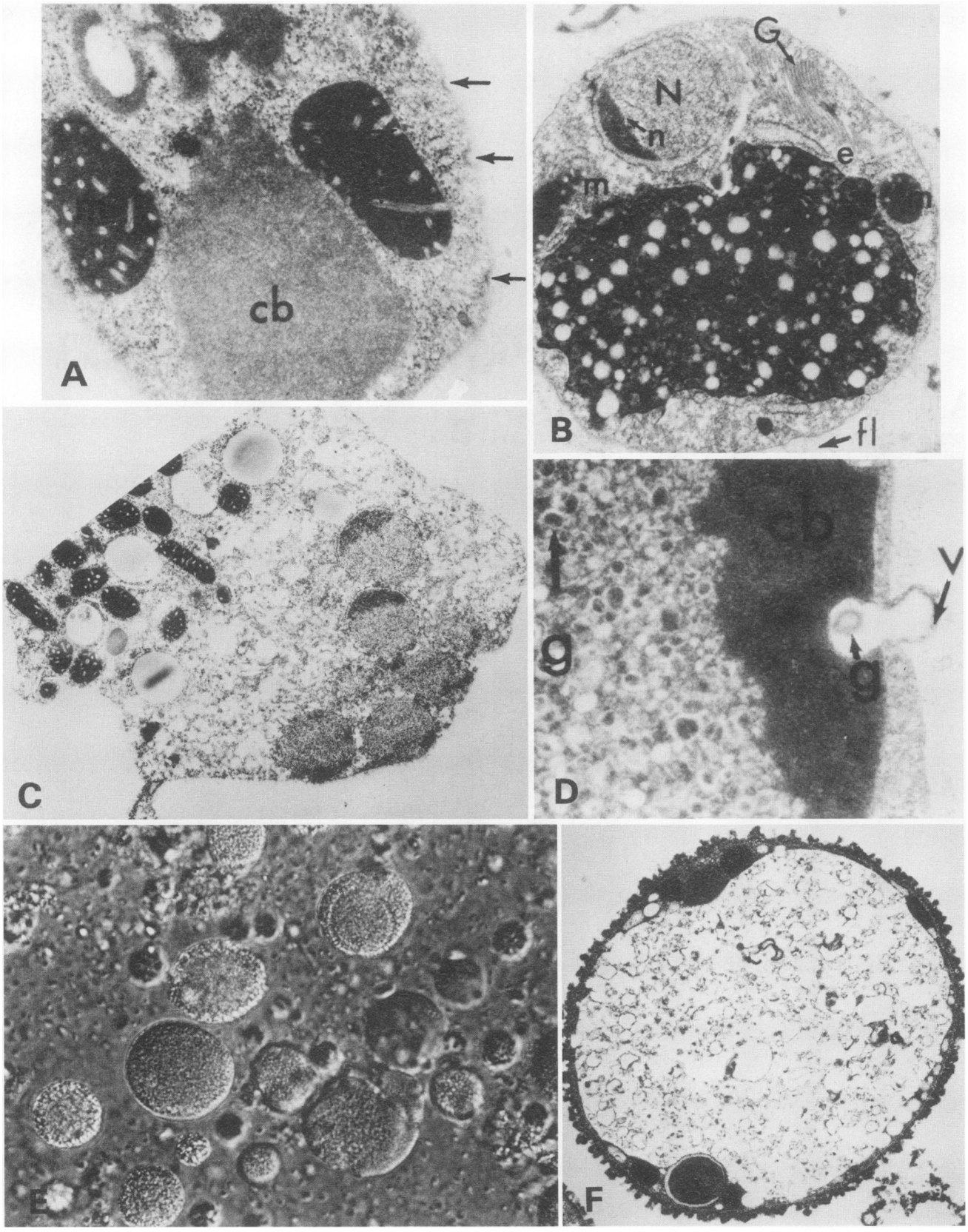

FIG. 3. *B. hominis*. Panels A to D and F are TEM; panel E is DIC. (A) Pockets (pores) in the outer membrane (arrows). (B) Much granular differentiation in CB. (C) Multiple nuclei and massed mitochondria in a granular cell. (D) Vesicle containing a granule in outer membrane. (E) Granule (mitochondrial) cells. Wet mount from culture. (F) Entire center of cell (CB) is reproductive organelle. Beyond is thin band of cytoplasm containing two nuclei bracketed by mitochondria. Outside of the cell membrane is the fixation-altered slime coat, fibrillar in nature. The coat is lost in continuous culture. cb, Central body; N, nucleus; n, nucleolus; G, Golgi apparatus; e, endoplasmic reticulum; fl, fibrillar layer; m, mitochondrion; g, granules; V, vesicle. Magnification, ×7,000, except (D) ×14,000 and (E) ×1,200.

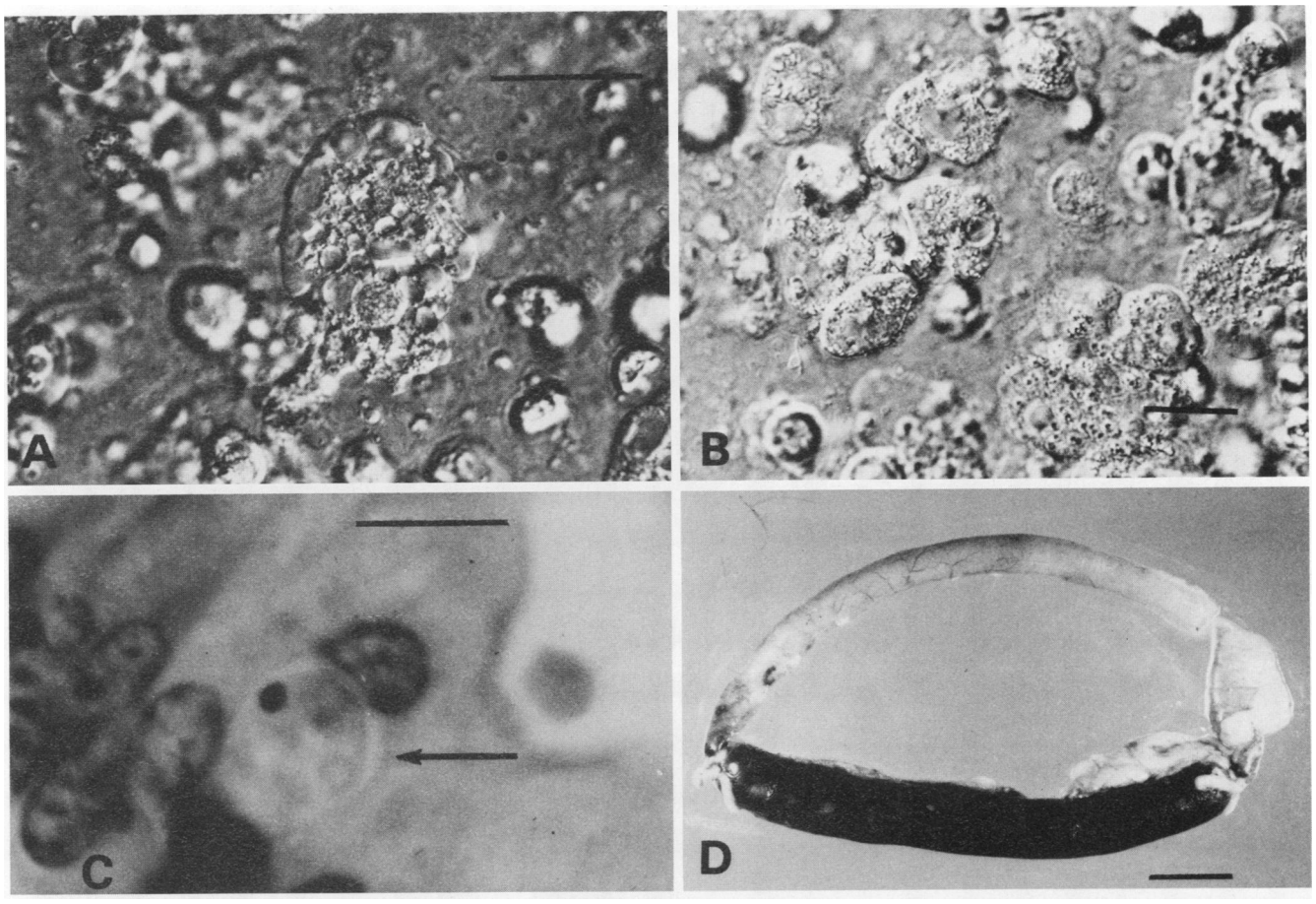

FIG. 4. *B. hominis*. (A, B) DIC optics. Ameba cells in diarrheal fluid, which contained 10^6 ameba cells per ml. Bar, 10 μm. (C) *B. hominis* in ileal mucosa of gnotobiotic guinea pig (arrow). Bar, 10 μm. (D) Ligated segments of rabbit ileum. Upper segment is control. Lower segment was injected with unheated column-purified toxin from *B. hominis* culture filtrate. Bar, 1 cm.

TEM is useful for examining the ameba form because the crescentic chromatin, or nucleolus (Fig. 3B), characteristic for *B. hominis* is well visualized. With experience, the ameba form is identifiable in wet mounts viewed by light or Nomarski optics. The ameba form, seen in diarrheal fluid of a patient who died of his disease (94), was trapped in and between parallel mucus strains (Fig. 4A and B). Mucus exudate was an outstanding feature of this case; the patient had choleralike diarrhea of 3-month standing. Many *B. hominis* cells were elongated by mechanical stretching along the mucus strands. The large cells were ovoid and irregularly convoluted. One or two large pseudopods were present in some cells.

An ameba form cell from culture is seen in Fig. 2E. TEM shows that the cell surface is covered by a finely filamentous layer, 0.2 to 0.25 μm thick. Pockets 90 nm in diameter are distributed at 0.5- to 1.0-μm intervals in the cell membrane. The cytoplasm lacks well-developed smooth or rough endoplasmic reticulum, in contrast to the other forms. All cytoplasmic areas show moderately dense concentrations of ribosomal particles.

Granular form. The granular form (Fig. 3 and 5) can be seen in clinical samples or in cultures in which these cells may predominate as the culture matures. The granules are mitochondria, which are so numerous in the peripheral sphere of cytoplasm that, by DIC optics, the cell appears to be a solid ball of granules. Sections examined by electron microscopy, however, show that the large, relatively empty CB is still there, occupying the greater volume of the cell. Granule cells are of average diameter (10 μm). Giant cells have many mitochondria that are widely dispersed as rosettes surrounding single nuclei or clusters of nuclei. In contrast to the ameba form, in the granule form a distinct cell membrane is present. Other types of CB granules, unseen by light microscopy, are membrane bound and range from finely granular material to electron-lucent empty granules and moderately electron-dense oval to spherical granules. The CB is a reproductive organelle (85) and the granules described probably have a role in schizogony.

Rough and smooth endoplasmic reticulum (Fig. 3B) is present only in the peripheral sphere of cytoplasm, in lengths up to 0.75 μm. Most tubules are 50 to 60 nm wide, with dilation evident even in short segments. Finely granular electron-dense material forming filamentous rings and clumps is present in some segments of endoplasmic reticulum. Mitochondria are smaller than those in the ameba form but appear identical otherwise. Multiple nuclei are common. Single nuclei are spherical and finely granular, with electron density equal to that of the cytoplasm. A marked double nuclear membrane is present, delineating a perinuclear space filled with small amounts of evenly distributed granular material. The Golgi apparatus appears as numerous parallel

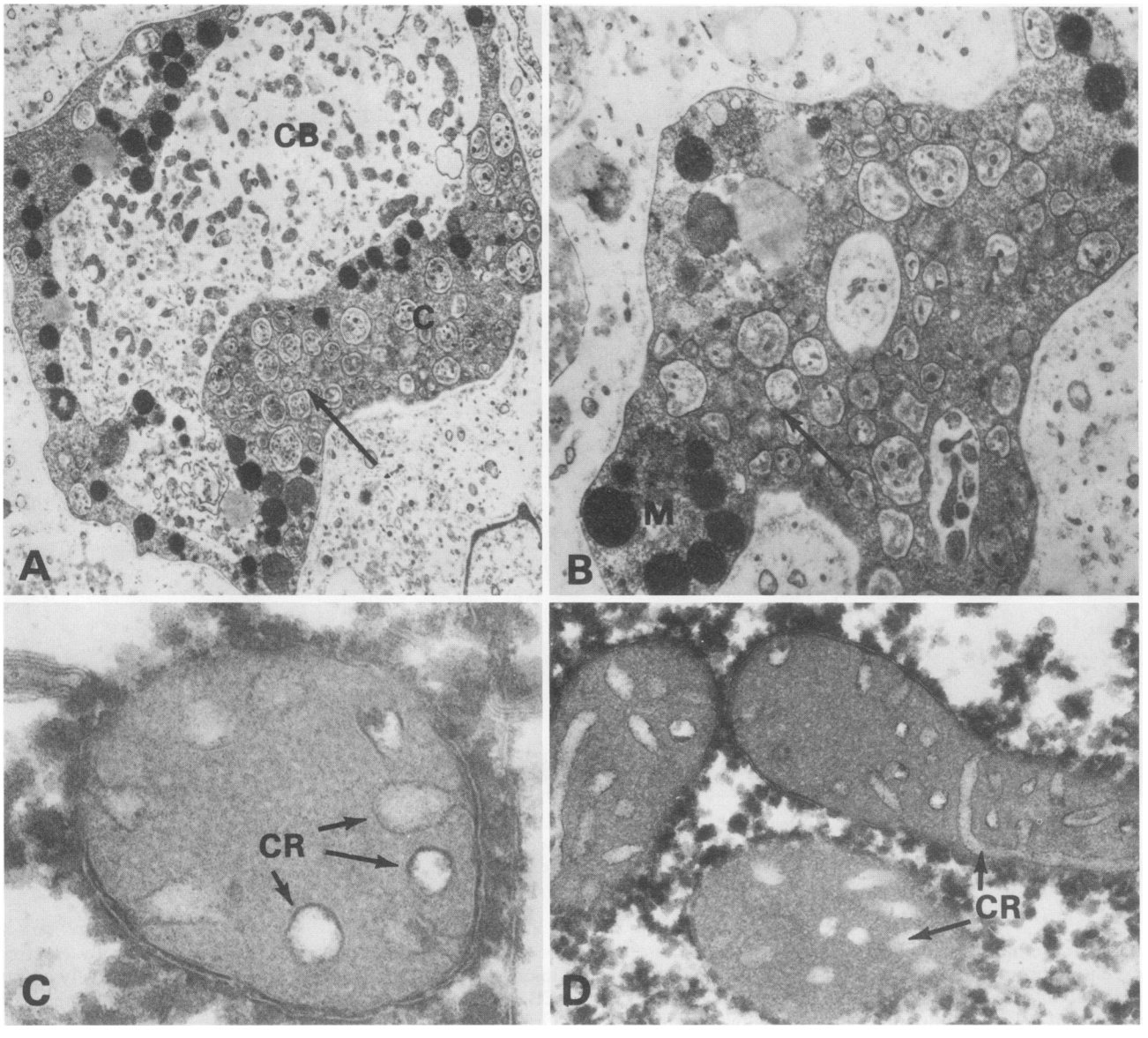

FIG. 5. Ultrastructure of *B. hominis*. TEM. (A, B). The unusual intracellular bodies (arrows) are unexplained, but may be spent or degenerate mitochondria. They are seen here adjacent to typical electron-dense mitochondria. (C, D) High-magnification micrographs of mitochondria. (C) Saccate cristae open into the intracristal space (arrow), demonstrating that this anaerobic organelle is morphologically an archetypical mitochondrion. Magnification, ×100,000. (D) Three mitochondria showing elongate, branched, and hooked cristae. Magnification, ×46,500. C, Cytoplasm; CR, cristae; M, mitochondria.

membranes lying either as a rectangular or semicircular array (Fig. 3B). Vesicular structures along the cell surface extend from the CB and appear to cause an evagination of a thinner segment of the cytoplasmic membrane toward the outside (Fig. 3D).

FEM

Tan et al. (72), after a freeze-etch electron microscopic (FEM) study, reported marked structural differences between the CB membrane and the outer or cytoplasmic membrane. Although *B. hominis* is a difficult organism to fix properly, remarkable agreement exists between FEM and TEM findings concerning cell shape, the nature of the CB, and the character of cell nucleus and organelles. The only additional information contributed by FEM to previously reported observations is the general completeness of membranes covering the CB granules and the difference in structure between inner and outer nuclear membranes. In addition, the three-dimensional views of all membrane surfaces permit a partial characterization of the tubular endosymbiont forms present in the cytoplasm and CB, observed initially by DIC microscopy (93).

The freeze fracture technique cleaves lipid cell membranes down the middle presumably through the hydrophobic portions. Thus, any surfaces revealed by the fracture process are covered by lipid membranes. FEM evidence indicates that, except for size, no external morphologic difference exists between the granules of the granular and CB forms. Moreover, the CB, exclusive of granules, is

devoid of characteristics that distinguish it from the more electron-lucent (by TEM) "vacuole." Therefore, the term "central body" is appropriate to use for all structures of this type. Occasional, fortuitous, freeze fractures of *B. hominis* yield cross-sectional views through apparent pores, demonstrating continuity of membranes from inner to outer cytoplasmic surfaces. All connections revealed in this manner, however, are 5 to 10 times greater in diameter than those of the usual cell membrane pore. It is possible that the former represents dilation of channels to allow passage of material between extracellular and intracellular environments. Membranous particles are present throughout the inner membrane but are often absent from oval areas which, by three-dimensional analysis, are either above or below the plane of the remaining membrane surface. It is not known whether the indentations, smooth oval areas, and holes represent stages in the opening and closing of the inner membrane, although biologic membranes are capable of relatively rapid structural changes in local surface areas. Movement of membranous particles can also occur.

The outer membrane has pores, approximately 50 nm in diameter, which are evenly distributed throughout the membrane surface. The inner or CB membrane has intramembranous particles and indentations. The indentations are distributed in a pattern similar to outer membrane pores. Outer and inner membranes may communicate directly by means of the pore indentation system.

The nucleus is delimited by two membranes. The outer nuclear membrane contains intramembranous particles that are twice as numerous as those of the inner nuclear membrane. The individual features of the CB, cytoplasmic organelles, and general shape of *B. hominis* seen by TEM (73, 91, 94) are confirmed by FEM.

An ultrastructural comparison of 10 stock cultures by Dunn et al. (19) shows wide size range in the CB form and in the thickness and density of surface coats. The cell membrane beneath the surface coats has electron-dense pits. The mitochondria display a wide size range reflecting changes concomitant with division and metabolism. "Budding" or apparent phagocytosis of mitochondria into the CB via pockets in the CB membrane is reported, but passage into the CB has not yet been seen.

Unusual *B. hominis* Cells

A number of unidentified structures have been seen intermittently in a few strains of cultured *B. hominis* cells, e.g., a rod-shaped structure seen both by TEM and light microscopy (83a). By TEM, the rods are crystalline and have the electron lucency characteristic of protein. They are found intracellularly but are often not contained within a single cell (Fig. 6A to C); they may extend through up to three or four adjacent cells. The rods are up to 10 μm wide and of highly variable length.

A "chestnut burr" cell may predominate in degenerating cultures (Fig. 6D). The refractile spikes originate from the cell membrane and give the cell a brightly refractile golden appearance. The culture does not recover from this condition.

Old cultures synthesize excess lipid, which collects inside the cell as tiny globules. These coalesce and form larger globules until the cell is full of lipid (Fig. 6E). The lipid globules apparently are not membrane enclosed and may stem from synthetic activity of the special anaerobic mitochondria.

Another cell type (Fig. 6F) appears to have no cytoplasm, only the outer membrane enclosing one to four granular bodies of irregular size and contour. Possibly, these are abnormal progeny arising from schizogony. At present, the origin and function of this cell type is unknown.

The intracellular bodies seen by TEM (Fig. 5A and B) are not identified with certainty but may be of mitochondrial origin. They are similar in size to mitochondria (dark bodies in Fig. 5A and B) but of ghostlike appearance, with irregular inclusions of varying electron density. The inclusions in some of the bodies appear to be degenerate cristae.

MITOCHONDRIA

Considerable research has been done on the *B. hominis* mitochondria because of the paradox of a cell with strict anaerobic metabolism containing hundreds of mitochondria as the most numerous organelle. They make up more of the cell's bulk than the sum of the other organelles. Purification by density gradient centrifugation has failed because of massive and tenacious mitochondrial aggregation on release from the cell, a common problem in mitochondrial research. Purification by differential centrifugation is adequate for determination of enzymes unique to mitochondria (88). The *B. hominis* mitochondria are devoid of cytochromes. There is no activity of the mitochondrial enzymes pyruvate dehydrogenase complex, ketoglutarate dehydrogenase complex, isocitrate dehydrogenase, glutamate dehydrogenase, and cytochrome *c* oxidase (90). Thus, the function of the anaerobic mitochondria in *B. hominis* remains unknown. Other enzymes absent from *B. hominis* are gammaglutamyl transpeptidase, alkaline phosphatase (a lysosomal marker), and creatine kinase isoenzymes (90).

The basic ultrastructure of the *B. hominis* mitochondrion was shown by TEM to match that of an archetypical mitochondrion (90). *B. hominis* mitochondrial cristae are short, saccate or globular structures in the evidently resting organelle (Fig. 5C), but within a few hours can elongate to long tubules as the mitochondrion itself elongates radially from the rosettes (Fig. 2D). The cristae also elongate (Fig. 5D), branch, and make abrupt right-angle bends. Evidence that the mitochondria are functional includes the following: (i) it is unlikely that a vestigial, useless organelle would be retained by the cell; (ii) they normally surround the nucleus as small spheres (0.2 to 0.5 μm in diameter) and then elongate as tubular, sometimes branched, organelles up to 5 μm in length and migrate from the cell nucleus as the cell ages; (iii) they stain brightly and specifically with Janus green or rhodamine 123, indicating that they have a typical, physiologically active outer membrane; (iv) their numbers per cell are not fixed but vary from two to four in *B. hominis* cells in the intestine and in rapidly dividing cells in vitro in late log growth phase (other than granule cells which have hundreds of them); (v) *B. hominis* synthesizes and stores lipid in quantities sufficient to displace most of the volume of the cytoplasm; there is a possibility that the mitochondria synthesize the lipid. This hypothesis is based partially on observations of cells in heated-stage culture chambers showing massive aggregations of thousands of mitochondria that escaped from ruptured cells. Rivulets of lipid stream from inside the mass and form droplets at the periphery which coalesce to form globules (89).

DIVISION IN *B. HOMINIS*

There are four known modes of division, all of them asexual: binary fission, plasmotomy, schizogony, and en-

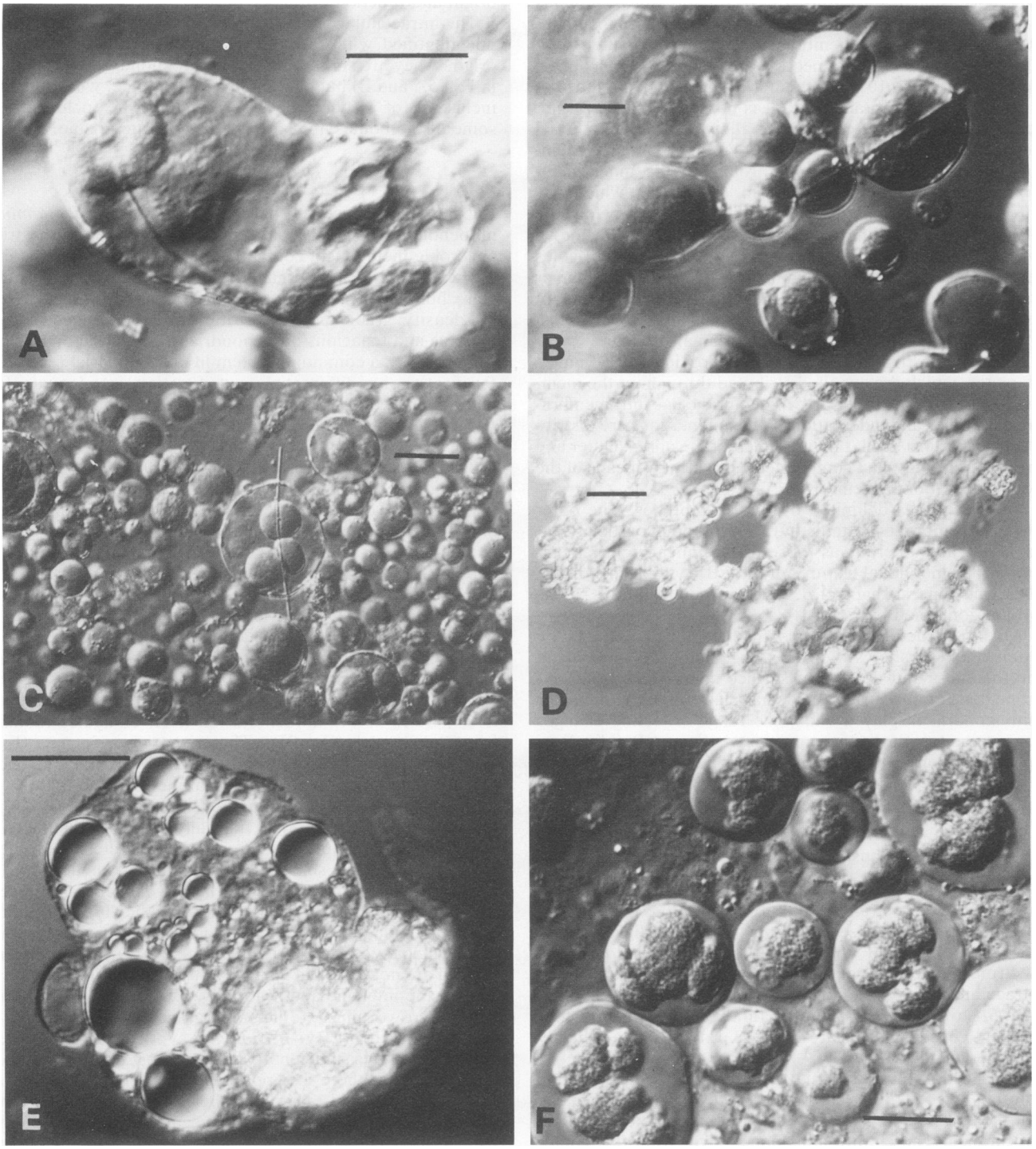

FIG. 6. *B. hominis*. DIC optics. (A) Large cell enclosing a proteinaceous rod of unknown function. (B) The same phenomenon, but in this photo a large rod has grown directly through four *B. hominis* cells. (C) A rod passes through this schizont, protruding from the cell on each side. (D) Some strains undergo an irreversible dying out process. These bright golden "chestnut burr" cells, formed by membranous extrusions, are seen during this "suicide" phenomenon. (E) A fat cell of *B. hominis*, common in aging cultures. (F) Unusual cells with irregular granular bodies included. These may be malformed schizonts. Bar, 10 μm.

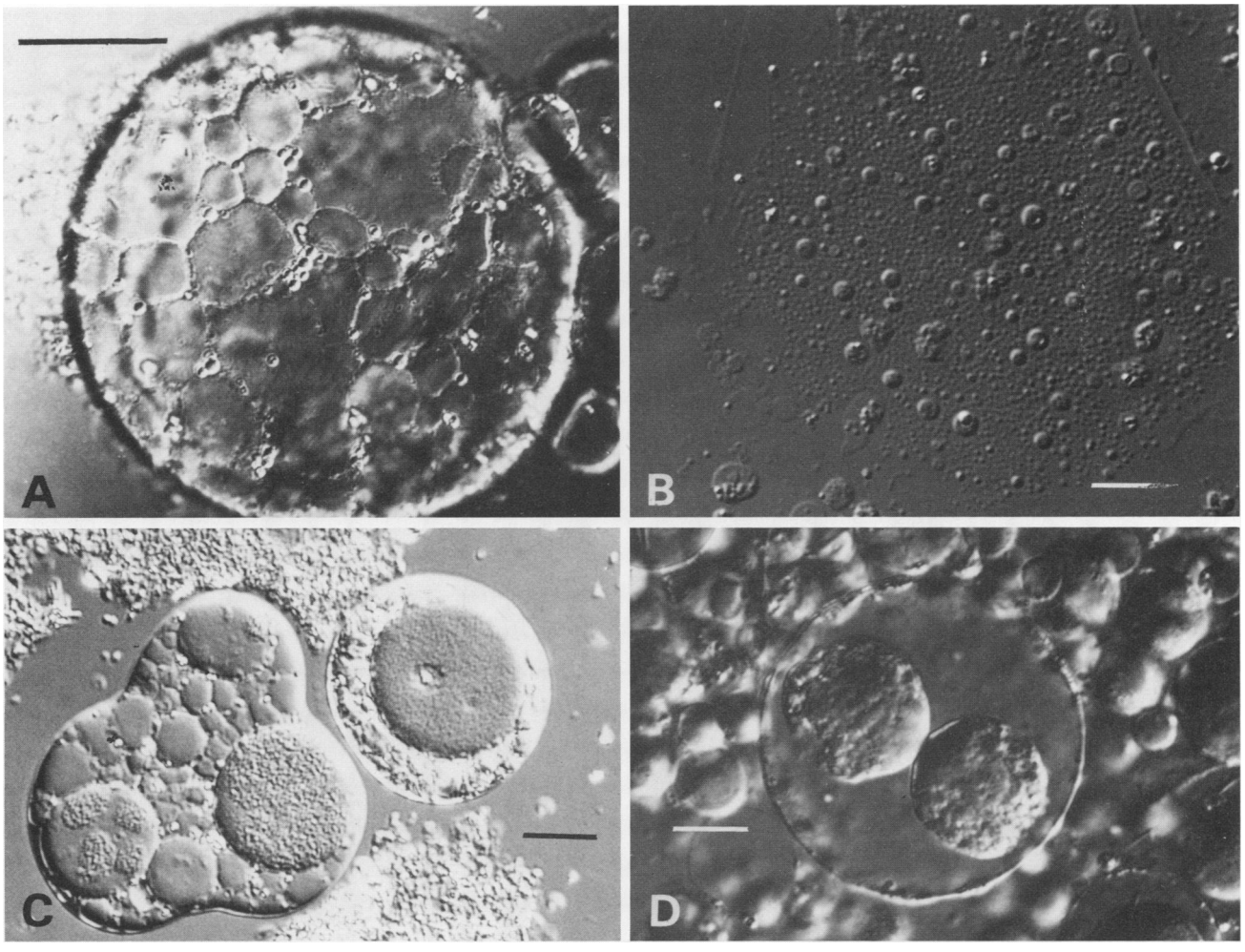

FIG. 7. *B. hominis*. DIC optics. Schizogony. (A) Schizont filled with progeny, or daughter cells. (B) Ruptured schizont has released small, condensed, brownish progency characteristic of only rare strains wherein a higher proportion of cells undergo this asexual division, resulting in smaller progeny, perhaps due to competitive nutrition. Bar, 10 μm. (C) Schizont with progeny in varying stages of maturity. Bar, 5 μm. (D) Cell in endodyogony, the creation of two progeny within the parent cell. Bar, 5 μm.

dodyogony. In the host, division is usually by binary fission (Fig. 2B). The ameba form may reproduce by plasmotomy, i.e., the cutting off of one or more progeny from roughly circular extensions of the cell. These progeny contain one or more nuclei, but are without a CB.

The CB is the organelle in which schizogony occurs. Progeny may fill the parent cell, or schizont (Fig. 7A), until the cell bursts, releasing them to the environment (Fig. 7B). Numbers of progeny range from one to hundreds, the organisms diminishing inversely in size as numbers increase. Progeny of widely varying size and development may occupy the same schizont (Fig. 7C). Endodyogony is less common and produces two large progeny with the CB (Fig. 7D).

Viable progeny from schizonts appear as tiny *B. hominis* cells, somewhat dark, condensed, and nonrefractile (Fig. 7B). A possibility remains that these viable granules, or progeny, are more resistant to air exposure, drying, and less than optimal temperature, but this has not been tested. These brown condensed *B. hominis* cells have not been studied by TEM. Sexual reproduction has not been described in *B. hominis*.

Generation time for the CB form in culture is 8.5 to 19.4 h, depending on the strain studied, the average being 11.7 h (92). Only about 5% of the cells in log-phase culture are in division, but the generation time formula considers every cell to be in division. Division times of individually observed cells just initiating division are much less, 40 to 60 min (92).

DIAGNOSIS

Slide Preparation

Wet mounts and trichrome-stained smears are recommended for stool examination. This laboratory has successfully used the FPC ova and parasite concentration device (Evergreen Scientific, Los Angeles, Calif.) for many years. Trophozoites of *B. hominis* remain intact after concentration for examination by wet mount or stained slide preparations. Representative morphology of trichrome-stained cells is seen in Fig. 8.

Gram-stained smears of clinical material are not usually successful because of *B. hominis* cell lysis, but the cells may be recognizable even though swollen and empty appearing.

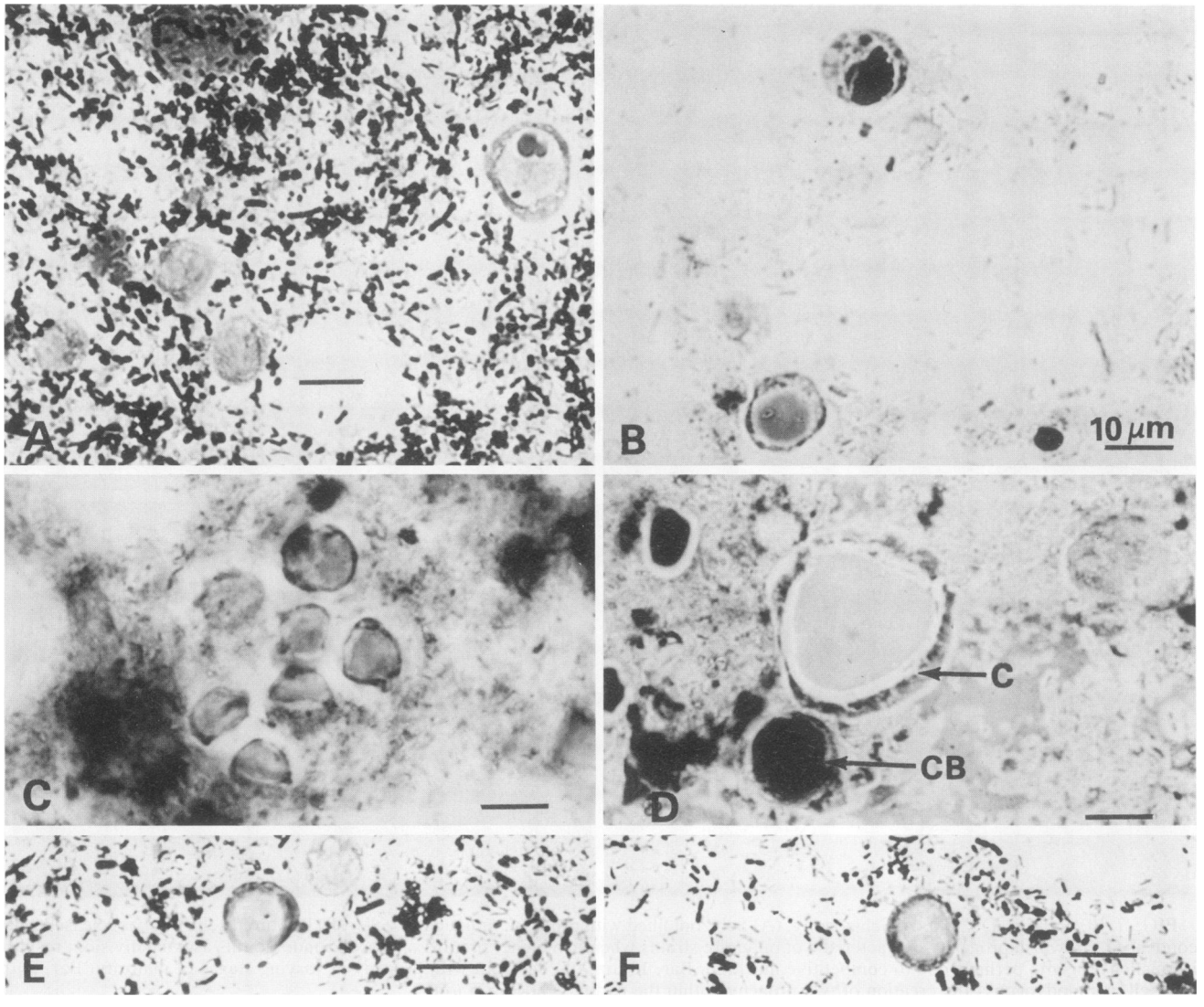

FIG. 8. Trichrome-stained fecal smears from patients. CB may not be clear-cut in all *B. hominis* cells. The diversity of form and size is well illustrated in this series. Inclusions are often artifactural. The stained CB in panel D (arrow) may represent trapped stain within the CB. The cytoplasmic band is clearly visible in most cells. Bar, 10 μm. C, Cytoplasm.

Cells from culture are particularly lysis susceptible during Gram stain, but glutaraldehyde-fixed cells may show nuclei well. If the cells are protected in a thick smear, they are more likely to survive Gram stain. The entire cell is gram negative. The Feulgen stain delineates nuclei better than other stains.

Quantitation

There are many reports about numbers of *B. hominis* seen per high-power field (×400 magnification) and their correlation with symptomatic disease. An empirical figure, five or more per high-power field, correlates with the presence of symptoms associated with blastocystosis. Kain et al. (32), however, presented convincing data that patients with ≤5 *B. hominis* per oil immersion field (OIF; ×1,000) expressed symptoms as often as those with ≥5 *B. hominis* per OIF. This classic well-controlled work is the definitive modern study of *B. hominis* infections.

Immunologic Diagnosis

Immunofluorescent staining. Rabbit antisera to unheated whole-cell *B. hominis* antigen (with Freund complete adjuvant) have been prepared in my laboratory and used successfully for immunofluorescence staining of the CB, ameba, and granule forms from both culture and feces (unpublished data). The reactions were clear-cut at a 1:200 dilution of antiserum. No nonspecific staining of bacterial, fungal, and mammalian cells was encountered in fecal smears. Antisera to *B. hominis* are not yet available from commercial sources, but are being developed. Availability of specific antiserum would greatly ease problems in diagnosis.

Serum antibodies. Chen et al. (11) studied four blastocystosis patients for the presence of humoral antibody. Immunoblots on nitrocellulose paper were probed with ^{125}I-labeled protein A. No serum antibody response to *B. hominis* proteins was detected; however, other pathogenic intestinal protozoa invoke only weak serum responses. Protein A probes detect only immunoglobulins G1 and G3 and not

other antibody classes. The use of other probes and the search for other antibody classes are indicated.

CULTURE

Culture of clinical specimens is not recommended as a routine procedure but is beneficial when microscopic diagnosis is uncertain. The medium of choice is modified whole-egg slant medium with Locke solution overlay (89), to which 30% horse serum has been added. Medium in screw-capped tubes is reduced by incubation under anaerobic conditions for 3 days or longer. The caps are loosened to permit gas exchange and tightened when removed from the anaerobic atmosphere. Inoculated tubes are incubated under the same atmospheric conditions. When sufficient numbers of *B. hominis* are present in a fecal sample so they are detected on direct examination, culture is usually successful. Cultures become positive quickly, and examination after 24 h is feasible. Specimens that have been refrigerated or even allowed to sit overnight at room temperature should not be cultured, because the organism dies rapidly under these conditions.

Stock cultures should be transferred every 3 to 4 days. To ensure a debris-free culture, as small a portion as possible of the growth sediment at the base of the slant is transferred. An inoculum of approximately 10^6 *B. hominis* cells is required. Cultures with few organisms require that the entire sediment be transferred. Tubes with heavy growth can be split to as many as five tubes. In our experience, and by unknown mechanisms, *B. hominis* cultures die out after an indefinite number of transfers, up to about 1,000, and none of our early strains survive today. One unusual strain survived for 10 years, lasting for 1,131 transfers.

Giant cells (Fig. 2C) are fairly common in egg slant medium and achieve diameters up to 400 μm. If cells of this size (0.4 mm) were opaque rather than transparent, they would be visible to the unaided eye. Giant cells do not divide by any mode; they are almost totally composed of an empty CB. The cytoplasm may contain upward of 100 nuclei with mitochondrial rosettes. Other mitochondria are evenly spaced around the cell (Fig. 2D). Giant cells may be defined arbitrarily as cells of >20-μm diameter, with a mean diameter of 30 to 40 μm.

Axenization

B. hominis grows to greater numbers and more consistently on egg slant medium with horse serum than on Diamond TPY medium (17). The bacterial component of *B. hominis* cultures may be eliminated by adding ampicillin, colistin, and streptomycin to the cultures (95). Ceftizoxime and vancomycin may be added to eliminate resistant bacteria. Axenization is successful with some cultures but fails in others because some *B. hominis* strains seem to depend on bacterial support. If the *B. hominis* population becomes critically low during axenization, recovery can be achieved by deleting antimicrobial agents. By alternating the addition and deletion of antimicrobial agents, *B. hominis* may gradually adapt to axenic growth. Typically, axenization over a period of weeks or even months reduces bacterial numbers and species until one species, usually a *Bacteroides* sp., remains. At this point, an antibiogram of the surviving strain can indicate antimicrobial candidates for inclusion in the antimicrobial agent mixture. Elimination of the last bacterial species does not ensure success, because the *B. hominis* strain may be unable to survive without its support. To save the strain, the culture may be continued as a monoxenic culture. The surviving bacterial species is usually the anaerobe, *Bacteroides fragilis*. After axenization, culture may be attempted on a synthetic medium to which has been added bovine serum albumin and other adjuvants such as linolenic, linoleic, and arachidonic acids and cholesterol (89a).

Preservation by Freezing

Preservation by lyophilization has not been successful in our laboratory trials; however, *B. hominis* culture sediments may be frozen successfully. The sediment from 3-day-old cultures is treated by submerging a 1-ml pipette tip through the overlay to the sediment at the base of the slant and releasing 0.1 ml of glycerol. In the same manner, 0.1 ml of dimethyl sulfoxide is added. The overlay is covered with 3 ml of sterile mineral oil and the culture is immediately frozen slowly to −70°C. To slow the rate of freezing, the tubes are first wrapped in a few layers of tissue paper and enclosed in a cardboard mailing tube. In this state, the cells are viable for at least 2 years. *B. hominis* ATCC 50177 is available as a frozen culture from the American Type Culture Collection, Rockville, Md.

SURVEYS OF INTESTINAL PARASITES INCLUDING *B. HOMINIS*

Although the question has been raised as to whether *B. hominis* is an intestinal pathogen, during the early 20th century, most articles that described diarrheal disease caused by protozoans included *B. hominis* without distinction. In 1916, Fantham (20) examined 3,800 stools from 1,305 British soldiers who had returned to England from Gallipoli and Flanders for convalescence. He reported a general increase in numbers of intestinal parasites, including *B. hominis*, in returning servicemen. In 1917, Lynch (41) reported on a survey of intestinal parasites in South Carolina residents. He found that *B. hominis* was prevalent in persons with pellagra. In 1920, Haughwout and Horrilleno (28) examined 100 Filipino children for parasites and reported the presence of *B. hominis*. Kofoid and Swezy (34), in 1921, surveyed intestinal parasites, including *B. hominis*, in soldiers returning from overseas. They noted a marked increase in occurrence of this organism in soldiers as compared with civilians. In 1936, Byrd (8) studied 537 people on the relief rolls in Athens, Ga., and included *B. hominis* in the survey. Stabler (69), in 1941, surveyed intestinal protozoa, including *B. hominis*, in 106 parasitology students.

As recently as 1988, Reinthaler et al. included *B. hominis* with other intestinal parasites in a survey conducted in Ogun state of southwest Nigeria (59). Also in 1988, Taylor et al. (74), in a survey of bacterial and protozoan enteropathogens in Nepal, reported *B. hominis* in 33% of 328 expatriate patients, confirming the high incidence of this parasite reported previously in Kathmandu (2). By comparison, enterotoxigenic *Escherichia coli* was found in 24% of patients; *Shigella* sp., in 14%; *G. lamblia*, in 12%; *Campylobacter* sp., in 9%, and rotavirus, in 8% (74). These values demonstrate a high rate of pathogen acquisition among travelers to Nepal. Nguyen and Krech surveyed 1,460 Swiss gastoenteritis patients in 1989 (53). *B. hominis* was identified in 69 (4.7%). In 45 (65%) of the 69 patients, *B. hominis* was found alone, but 24 (35%) also had other protozoan or helminthic parasites.

CLINICAL BLASTOCYSTOSIS

Beginning with Perroncito in 1899 (56), occasional independent reports of *B. hominis* intestinal disease were published, but recently the number of reports has increased markedly. In 1916, Low (39), who referred to *B. hominis* infection as "an infection that is difficult to get rid of," treated patients with emetine. In 1917, Lynch provided two reports of *B. hominis* infections. One of these (42) related *B. hominis* to ulcers lining the "pellagrous intestine." He noted that *B. hominis* morphology within the ulcers was strikingly different from that of organisms in the lumen of the intestine. This observation correlates with recent studies showing that the ameba form is more common than the CB form in the intestinal mucosa. Lynch's second paper was the previously cited report of *B. hominis* infections in pellagra patients in South Carolina (41). In a third paper (43), he described a study with human serum as culture medium either undiluted or diluted in saline. He asserted that Alexieff (1) had published the first and, by priority, the correct name for *B. hominis*, *B. enterocola*. Lynch declared himself neutral on the question of the pathogenic nature of *B. hominis*, but in 1923 he described intestinal inflammation accompanying *B. hominis* infection (44). In 1922, Mazza (47) reported on 180 infections in Argentina: 80 patients had *B. hominis* mixed with other protozoa and 100 had *B. hominis* alone. He treated most patients successfully with the arsenicals Stovarsol and Narsenol.

In 1922, Castex and Greenway (10), also in Argentina, reported 88 infections by *B. hominis* and other protozoa. They used Stovarsol successfully for treatment. In 1923, Yakimoff, a Petrograd Russian, studied intestinal infections and concluded that *B. hominis* was pathogenic (82). In 1925, Yakimoff and Wassilewsky (83) reported on a Petrograd epidemic of 250 cases in which blastocystosis was studied and treated. Barilari (4), in 1924, reported eight cases from Buenos Aires successfully treated with Narsenol. Another case of blastocystosis, from France, was reported by Dargein et al. (15). Parodi and Nino (55) reported from Buenos Aires in 1926 that *B. hominis* was a significant intestinal pathogen. Panayotatou (54) in 1928 reported on three cases of blastocystosis treated with Stovarsol and Yatren. In 1929, Silberstern (68) described enteritis in humans caused by *B. hominis*. Sangiorgi (64) described the "critical" picture of *B. hominis* infection as the presence of numerous "blastocysts" in the "most florid period of their life," with abnormal numbers of leukocytes, erythrocytes, and epithelial cells and mucus. The "postcritical" period saw regression of these elements and change in *B. hominis* morphology to "constrained elements, primarily distributed in dense groups. Are these elements equivalent to spores?" These elements were probably progeny resulting from schizogony. Sangiorgi studied 2,000 Italian soldiers in Albania in 1918 (63) and in 1933 (65) reported on an outbreak among 100 Italian soldiers in Albania who were infected with *B. hominis* alone. From Italy, Milella, in 1936, reported on 116 cases of diarrhea caused by *B. hominis* (50). In 1937, Calderin (9) affirmed the entity of blastocystosis in Italy.

In 1972, Wolynska and Soroczan (80) examined 312 Polish peasant women for vaginal parasites. They reported *B. hominis* with "various grades of invasion" in 47 women (11.5%), *Trichomonas vaginalis* in 19 (6.0%), *Enterobius vermicularis* in 27 (8.6%), and "*Monilia*" in 11 (3.4%). In 16 cases, *B. hominis* was found in the rectal area, in 22 cases in the vagina only, and in 9 cases in both rectal and vaginal areas. The women had colpitis and cervical erosion. The authors attributed the disease to poor personal hygiene.

One well-studied case of blastocystosis reported in 1976 (94) involved a 45-year-old alcoholic male who developed a fulminant refractory diarrhea. He produced from 5 to 20 liters of diarrheal fluid daily and required continuous administration of large volumes of intravenous fluids. *B. hominis* counts (performed in Neubauer hemacytometer chambers) rose to and remained at an average of 8.3×10^6/ml in the diarrheal fluid. The patient died of aspiration pneumonia after 3 months. Before his death, treatment for 7 days with metronidazole (250 mg, three times daily) reduced *B. hominis* numbers by one-third and caused the appearance of injured protozoan cells. In retrospect, considering the elimination time and dilution in the fluid volume, the dose and treatment span were inadequate. Intensive search by medical personnel disclosed no underlying disease or other etiologic agent of this fatal case. During infection, the mixed presence of CB and ameba forms gave way to the ameba form only. Identification of the agent as *B. hominis* was done by wet mount morphology under DIC optics, by TEM, and by indirect fluorescent-antibody staining. TEM verification depended particularly on finding typical crescentic nuclear chromatin and mitochondria. No CB were present.

An epizootic with animal deaths was reported in 1980 (48) in a primate colony. Cases have been described in human and nonhuman primates in a zoo. In 1983, May et al. (46) reported that 52% of 180 male homosexuals in south Florida had *B. hominis*, whereas 16% of 45 heterosexual men had *B. hominis*. In 1984, reports of blastocystosis began to increase. In that year, Garcia et al. (25) reported positively on the clinical significance of *B. hominis* and Ricci et al. (60) referred to *B. hominis* as a "neglected cause of diarrhea." Babcock et al. (2), in 1985, reported cases of blastocystosis in Kathmandu, Nepal, and Vannatta et al. (76) reported cases in Kansas City. LeBar et al. (35) and Gallagher and Venglarcik (21) contributed more cases in 1985. In 1986, Sheehan et al. (66) reported an "association of *Blastocystis hominis* with signs and symptoms of human disease" and Jarecki-Black et al. (30) reported a case of blastocystosis in a 2-year-old child.

In 1987, Kain et al. (32) reported a definitive retrospective study of 1,496 patients over a 3-year period, of whom 190 (12.7%) carried *B. hominis*. Ten characteristics were used to characterize the patients and their disease: (i) duration of symptoms either acute (<2 weeks) or chronic (>2 weeks) before seeking medical attention; (ii) history of underlying gastrointestinal disease or immune deficiency that might produce gastrointestinal symptoms; (iii) recent medications that might affect gastrointestinal function; (iv) travel history to the tropics or consumption of untreated water in rural or wilderness areas of North America within 6 months of initial stool examination; (v) symptom complex including diarrhea, abdominal pain, nausea and vomiting, arthralgia, or arthritis; (vi) concomitant infection with other potentially pathogenic bowel parasite or bacteria; (vii) leukocytosis (>11,000 leukocytes per μl) and eosinophilia (>450 eosinophils per μl); (viii) endoscopic, histologic, and radiologic observations; (ix) clinical response by return to an asymptomatic state or distinct improvement in gastrointestinal signs or symptoms on follow-up examination; and (x) microbiologic response by clearance or decrease of *B. hominis* numbers.

Of 100 patients infected with *B. hominis*, 70 had <5 *B. hominis* per OIF; 15 had one or more stools with >5 *B. hominis* per OIF and one or more with <5 *B. hominis* per OIF; and 15 had >5 *B. hominis* per OIF in all stools. The

study included 50 *B. hominis*-negative control subjects who had other intestinal parasites or pathogenic bacteria, chosen by sex and age to match the *B. hominis*-positive group. Travel or consumption of untreated water was reported by 57.5% of the 100 *B. hominis*-infected patients in contrast to only 12.2% of the 50 control subjects. Travel to Southeast Asia, Central or South America, and Africa was noted or hiking and camping in North America. The infections tended to be self-limiting. There was no statistically significant difference in loss of symptoms or parasite among patients treated with metronidazole, diet, or not treated. The authors concluded that *B. hominis* infection was (i) related to travel, (ii) symptomatic even in the presence of <5 *B. hominis* per OIF, (iii) more severe when *B. hominis* numbers were >5 per OIF, (iv) related to symptoms in the absence of another identified pathogen in 55 (94.6%) of 57 patients, and (v) related to symptoms in the absence of another pathogen or underlying disease in 35 (94.6%) of 37 patients.

In a 1987 report of 103 patients with pure *B. hominis* infection, Guirges and Al-Waili (27) noted that excessive flatulence was the chief gastrointestinal symptom. All of their patients showed good response with metronidazole treatment. At 1 and 2 months after therapy, 74 patients examined were negative for *B. hominis*. In 1987, Diaczok and Rival reported on a patient with *B. hominis* diarrhea in Detroit, Mich. (16). In 1988, Russo et al. (62) provided evidence that *B. hominis* was a cause of colitis, and Garavelli and Scaglione (22) reported new blastocystosis cases in the general population in Italy. In 1989, Garavelli et al. (24) furnished another report on blastocystosis in Italy. Qadri et al. (58) reported a series of cases from Saudi Arabia successfully treated with metronidazole. Their success rate with this drug exceeded that reported in the United States. Llibre et al. described *B. hominis* in chronic diarrhea in patients with AIDS (40). Cross (14) reported that *B. hominis* was gaining acceptance as an agent of protozoan intestinal disease, and Shikiya et al. provided a case report of colitis (67).

Several reports appeared in 1989. Tsang et al. reported the first patient with terminal ileitis secondary to *B. hominis*. Treatment with metronidazole resolved the radiographic abnormalities and the symptoms improved (75). Guglielmetti et al. reported a family outbreak of blastocystosis (26), and Narkewicz et al. reported *B. hominis* gastroenteritis in a hemophiliac with AIDS (52).

In 1990, Garavelli et al. (23) reported that blastocystosis is a significant problem in AIDS patients. Five patients (two with AIDS and three with AIDS-related complex) presented with diarrhea, abdominal pain, nausea, fever, and pruritis. *B. hominis* was diagnosed in stool samples at >5 *B. hominis* per OIF. No other parasites were found and bacterial pathogens were ruled out. Metronidazole treatment (2 g daily for 12 days) eliminated the *B. hominis*. The patients' bowel function returned to normal. Doyle et al. (18) completed a prospective study of 143 patients with *B. hominis* in Vancouver. The most common symptoms were watery diarrhea, abdominal pain, and gas. Of 143 patients, 19 were asymptomatic carriers and 21 were diagnosed as having chronic gastroenteritis. About half had a history of recent travel. The distribution of symptoms was "similar to that seen in patients with *G. lamblia*." Also in 1990, Wilson and Winget (78), in a military hospital, studied 115 patients with *B. hominis* infection. Forty-nine patients had *B. hominis* alone: 35 experienced diarrhea, 21 acutely and 14 chronically with diarrhea lasting several weeks to years. Other symptoms included abdominal pain, cramping, nausea, fever, bloating, weight loss, vomiting, and heartburn. Of 47 patients, 20 had positive tests for occult blood. Metronidazole therapy was successful in patients with acute diarrhea.

Two recent reports present a different viewpoint about the importance of *B. hominis* in gastrointestinal disease. In 1986, Markell and Udkow (45) studied five patients with *B. hominis* only, but concluded that the organism was not a pathogen because diiodohydroxyquinolone treatment did not eliminate the organism. Studying other patients who had *B. hominis* plus a "recognized" protozoan pathogen, they stated, "*Blastocystis hominis* persisted after treatment with iodoquinol (25 patients), metronidazole (12 patients), quinacrine (9 patients) and paromomycin (1 patient). . . . On the basis of our findings in this study, we believe that when a symptomatic patient in whom *B. hominis* has been found responds to therapy, that response represents elimination not of *B. hominis* but of some undetected pathogen, such as *Giardia*, *Dientamoeba*, or *E. histolytica*." Their findings are unique because none of the previously cited manuscripts that deal with treatment reports complete failure of therapy with iodoquinol, and particularly with metronidazole. Attempts to cure blastocystosis with quinacrine or paromomycin have not been reported elsewhere. Markell and Udkow attributed the symptoms of the five patients with *B. hominis* alone to irritable bowel syndrome (45). In 1988, Miller and Minshew (51) provided a review and another negative report about the pathogenesis of *B. hominis*. However, all 11 of their patients were compromised by other underlying gastrointestinal disease.

Most of the early studies were done in geographic areas with a high level of endemic blastocystosis and crowded living conditions, areas that also experience fulminant epidemics of blastocystosis. North America is not a good geographic area in which to study *B. hominis* disease. The current incidence in the United States is ca. 4 to 12%, down from the 18% reported in the 1960s, whereas the incidence reported from tropical countries ranges from 20 to 50% (34). As might be expected, the incidence of *B. hominis* in hospital as well as community populations varies widely. The incidence in the National Institutes of Health Clinical Center was reported as 18% in 1983 but has declined to ca. 6% in 1989. Other laboratories have reported 11.6% incidence (45), 35.5% incidence among, 2,391 troops returning from duty in a tropical country (34), and 16% (14). *B. hominis* was found in 93 (52%) of 180 homosexual men in south Florida (46). In the same study, only 8 (17%) of 47 heterosexual women and 7 (16%) of 45 heterosexual men had *B. hominis*. The reduced incidence in the United States in the past two decades apparently reflects epidemiologic factors. Many reports of infections now originating from North America include immigrants from countries (usually tropical) known to have a high incidence of gastrointestinal and other disease, but there are not enough of these patients to affect the overall incidence of blastocystosis. Much remains to be learned about the epidemiology of *B. hominis* infections and transmission, but overcrowding, malnutrition, and poor hygiene must be important factors. The fecal-oral route, including contaminated food and water, appears to be a factor.

EFFECT OF DISEASE ON LARGE BOWEL MUCOSA

Experimental Animal Studies

When germfree guinea pigs were orally infected with axenic cultures of *B. hominis* and bacterial flora from the human bowel (57), 14 of 43 animals developed *B. hominis* infections. Those with particularly heavy infections devel-

oped diarrhea of >1-week duration. After intracecal inoculations, 13 of 28 animals developed infections. Gross cecal hyperemia was observed in those animals with heavy infections. Microscopic examination revealed frequent penetration by *B. hominis* cells of the epithelium (Fig. 4C) but not the lamina propria, where a slight increase in cellularity was noted. Infections did not result from inoculation of axenic strains alone, and only one of eight guinea pigs developed an infection after inoculation with a monozenic culture that included *Proteus vulgaris*.

Fractions of axenic *B. hominis* cultures were injected into isolated segments of rabbit ileum (ileal loops) (83a). The fractions tested were heated and unheated whole cultures, culture filtrate, and column-purified filtrate fractions. After 24 h, the segments were excised (Fig. 4D) and the fluid volume was measured in the test and control segments. The ratio of test segment fluid volume to that in the control segment was used to measure the presence of a diarrheagenic toxin. Unheated whole culture, culture filtrate, and some purified protein fractions were positive, with high ratios of test loop to control loop fluid volumes, suggesting the presence of a diarrheagenic toxin.

Effects in Humans

Inflammation of the intestine in blastocystosis has been described by Shikiya et al. (67), Tsang et al. (75), Lynch (44), and Russo et al. (62). In the study by Kain et al. (32), 13 (26%) of 50 patients with *B. hominis* infections showed abnormalities at endoscopy and 17 (34%) of 50 showed abnormalities on histological examination. However, none of 14 patients in whom other possible causes of gastroenteritis were absent showed abnormalities on endoscopy, and on biopsy, only 1 of 13 showed mild acute inflammation in the lamina propria. That patient had high numbers of *B. hominis* but no invasive disease was present.

The most usual complaint of blastocystosis patients is of intense abdominal discomfort accompanied by pain. Diarrhea is not standard, and constipation is common. The symptoms gleaned from the literature include abdominal pain, discomfort, anorexia, bloating, cramps, diarrhea, constipation, alternating diarrhea and constipation, watery diarrhea, mucus diarrhea, vomiting, dehydration, sleeplessness, nausea, weight loss, inability to work, lassitude, dizziness, flatus, pruritis, and tenesmus. Blood in the stool as well as excessive mucus and leukocytes have been reported. Moderate to severe eosinophilia is not uncommon and was reported in 8 of 19 patients in one study (66).

TREATMENT

Early treatment for *B. hominis* infections was the same as for *E. histolytica*. Purging with salts followed by an enema was considered the standard treatment. Large doses of dilute hydrochloric acid by mouth were prescribed and one or more of several arsenilic acid derivatives, such as Stovarsol, Narsenol, and, later, Carbarsone (4, 10, 15, 39, 46, 53, 54, 62, 64, 67, 76, 81, 82). The arsenicals resulted in permanent clearance of *B. hominis* from the entire gastrointestinal tract, for in the early literature, *B. hominis* was often reported in the jejunum and duodenum as well as the cecum and colon.

Diiodohydroxyquinoline, iodochlorhydroxyquinoline (Entero-vioform), and emetine have been used, the last extensively by the British (39, 77). Emetine is still available but needs further evaluation (79). In our experience, Entero-vioform was notably successful for permanent clearance of *B. hominis*, but this drug has now been banned in the United States following an adverse report in a Japanese study (29). After World War II, physicians in Japan prescribed the drug freely for a variety of ailments, usually at doses of 1 g per day for indefinite periods. Thousands of patients developed neurologic disease but almost all returned to normal when they stopped using the drug. Entero-vioform undoubtedly was misused; therefore, the ban seems premature. If diiodohydroxyquinoline had been used in the same way, similar problems might have been encountered.

In vitro tests (86) of 10 antiprotozoal drugs found 6 to be effective against *B. hominis*. In order of effectiveness, they are emetine, metronidazole, furazolidone, trimethoprim-sulfamethoxazole, 5-chloro-8-hydroxy-7-iodoquinoline (Entero-vioform), and pentamidine. Moderately effective were two other quinolines, chloroquine and 5,7-diiodo-8-hydroxyquinoline (Floraquin). Diloxanide furoate was not inhibitory nor were paromomycin and other antimicrobial agents. These in vitro results have been confirmed in patients, except that trials of pentamidine and diloxanide furoate have not yet been reported.

Through anecdotal reports, it has become evident that there are a significant number of treatment failures with metronidazole and trimethoprim-sulfamethoxazole. When adult patients can tolerate metronidazole (essentially the drug of choice), the dose is increased from 250 to 750 mg three times daily. More primary cures are achieved with the higher dosages administered for 10 days and recurrences of the parasite are prevented. Intolerance to the elevated dosage is a common problem, however. Some patients do not respond to treatment and suffer a painful and debilitating chronic infection. Additionally, more effective drugs are needed. Trimethoprim-sulfamethoxazole has been used to stop an outbreak of blastocystosis among primates at the San Diego zoo; however, insufficient data are available to assess its efficacy in humans.

Many individuals suffer from chronic blastocystosis, infections refractory to metronidazole and other drugs. Dehydroemetine is available as an alternative. It has been used for treating amebic dysentery and amebic abscesses when metronidazole fails. Tetracycline could be coadministered when treating blastocystosis to remove bacterial support essential to *B. hominis* survival (79). Complete information about precautions, adverse effects, dosage, and intramuscular administration of dehydroemetine should be obtained, and the drug should always be used in a hospital setting.

With the growing problem of resistant infections, another look might be given at the arsenilic acid derivatives reported to be effective in the early literature. Thus far, in vitro tests have not been done on members of this group. Carbarsone is available in the United States, and judging from the success reported in older studies, a retrial might be in order. Treatment information for parasitic disease (81) is available from the World Health Organization.

SUMMARY AND CONCLUSION

The history of *B. hominis* is unique. Few infectious agents have provoked the many misconceptions that plague this enigmatic parasitic ameba. Conflicting descriptions of its nature and pathogenesis have continued throughout the 20th century.

As seen by the greatly expanded number of reports in recent years, *B. hominis* is now a major subject of study, particularly for evidence of disease causation. Physicians are treating patients with intestinal disease caused by *B. homi-*

nis. Many mild cases resolve in about 3 days without treatment, but others are acute and chronic disease is common. As with *E. histolytica*, the carrier state is often seen without symptoms. Treatment is usually with metronidazole, but emetine (for refractory infections), trimethoprim-sulfamethoxazole, and pentamidine are also effective.

In fecal samples, this complex protozoan appears in a variety of cell forms which makes microscopic diagnosis difficult. As yet, no specific fluorescent-antibody test is available for diagnosis. A culture method to demonstrate the more easily recognized CB form is available, but probably not feasible for most diagnostic laboratories. The common cell forms are the CB form, the granular (mitochondria) form, and the ameba form. The unexpected size range of these forms in clinical material, from yeast size (ca. 7 μm) to giant cells of 20 to 40 μm, makes diagnosis difficult. Pseudopodia may be demonstrated by the ameba form in heated microscope stage culture chambers.

The anaerobic *B. hominis* has no cyst form. Its mitochondria are uniquely anaerobic and have no cytochrome protein or oxidative mitochondrial enzymes. Because of its many cell forms and anaerobic mitochondria, *B. hominis* is an organism of great interest for morphologic and biochemical study.

Reproduction is asexual, usually by binary fission. Shizogony occurs in cultured cells. The CB appears to be an organelle whose specific purpose is for reproduction by shizogony. From 2 to 30 progeny are derived from schizogony. The ameba form reproduces by plasmotomy; it has no CB.

The pathology of *B. hominis* infections has been studied in gnotobiotic guinea pigs in which inflammation of the intestinal mucosa and invasion of the superficial layers were seen. Only limited studies of human pathology are available. Those who have studied mucosal histopathology report inflammation and cellular changes that resolve after treatment. More study in this area is strongly indicated (32, 44, 57, 62, 67, 75).

Ultrastructural details of *B. hominis* major forms, except for the schizont, are complete. The organism has no cell wall. The concentric CB takes up as much as 95% of the cell. The major organelles, which include multiple nuclei, Golgi apparatus, mitochondria, endoplasmic reticulum, fat, and other inclusions, are confined in two or four opposed pods in a thin band of peripheral cytoplasm between the spherical entire plasma membrane and the CB membrane. The pods buldge the CB membrane inward. There is evidence of a bacteroid endosymbiont.

Education about *B. hominis* is needed. Entry of recent findings into new textbooks is imperative for its understanding among medical practitioners. Laboratory workers need to be aware of it for many reasons. The College of American Pathologists includes *B. hominis* in its proficiency testing samples and requires that it be reported from clinical samples.

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