Emerging from Obscurity: Biological, Clinical, and Diagnostic Aspects of *Dientamoeba fragilis*

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INTRODUCTION

Since the first description of *Dientamoeba fragilis* by Jepps and Dobell in 1918 (65) this ameboid organism has escaped the interest of most clinicians and diagnostic microbiologists. This is reflected in a variety of descriptions conferred on the organism, such as: “a neglected cause of diarrhea” (49), “an unusual intestinal pathogen” (12), “an emerging protozoal infection” (129), and “an enigma shrouded in the mysteries of clinical parasitology” (140).

Jepps and Dobell (65) considered the nucleus of *D. fragilis* to be the characteristic feature of the organism, since they observed that the predominant form was binucleate, a feature which readily differentiated it from other human intestinal amebas. Interestingly, although they had isolated *D. fragilis* from seven persons, six of whom had a history of dysentery or chronic diarrhea, they felt that this observation was of no clinical significance. This conclusion was based on their observation that *D. fragilis* had a similar mode of nutrition to the nonpathogenic organisms *Entamoeba coli* and *Endolimax nana*, in contrast to *Entamoeba histolytica*, which was then considered to be a “tissue parasite.” They proposed that humans were aberrant hosts, in which cysts did not develop, and suggested that *D. fragilis* had a true animal host in which it was capable of encystation. Unfortunately, there is still no evidence to support the existence of a natural host besides humans nor has a cystic stage of *D. fragilis* ever been convincingly demonstrated. Furthermore, the lack of a suitable animal model that is capable of supporting the life cycle of *D. fragilis* and that develops similar clinical symptoms has severely hindered more detailed studies of the biology of the organism.

The seeds of doubt concerning the pathogenicity of *D. fragilis* were unfortunately planted by Jepps and Dobell (65) and nourished by Dobell and O’Conner (38) and account at least partially for the lingering resistance in many clinical circles to accept the disease-causing potential of this organism. However, the evidence supporting the pathogenicity of *D. fragilis* is too convincing to justify the continued neglect of this parasite as a cause of diarrhea, abdominal pain, flatulence, fatigue, and anorexia, the symptoms most commonly observed in patients infected with this organism (68). Indeed, the organism has been isolated from and associated with clinical symptoms in patients from numerous countries throughout the world (140). Perhaps the most striking reason to consider *D. fragilis* a potential pathogen is that it can be easily treated and that the great majority of patients show significant clinical improvement thereafter (35, 49).
TAXONOMY

The name *Dientamoeba fragilis* refers to the fact that it is an enteric ameba with the curious characteristic of being binucleate and that it tends to degenerate rapidly after excretion in stool (65). It was also classified as an ameba by Chatton (20), who included it in the family Entamoebidae, where it remained for the best part of 50 years. However, doubts about its affinity were raised by Dobell (36), when he noted the presence of the “centrosomes” and the great similarity of *D. fragilis* at the light microscopic level to *Histomonas meleagridis* (see also “Morphology” below). *Histomonas* was, and is, accepted as a trichomonad flagellate despite its tendency to lose its flagellum in culture or when it invades tissues. Dobell (36) strongly suggested that *Dientamoeba* and *Histomonas* were closely related and that *D. fragilis* was an aberrant flagellate. This relationship was formalized when Grassé (51) removed *Dientamoeba* from the Entamoebidae and created the family Dientamoebidae to contain these two genera.

Further morphological support for the classification of *D. fragilis* within the trichomonad flagellates had to await the transmission electron microscopy studies by Camp et al. (13), who confirmed Dobell’s observations. Molecular support for the relationship was first obtained by Dwyer (39, 40), who showed substantial antigenic similarities among *Dientamoeba, Histomonas*, and *Trichomonas* to the exclusion of *Entamoeba* species.

Another 20-year gap ensued until DNA studies of *Dientamoeba* commenced. Phylogenetic analysis of the *D. fragilis* small-subunit rRNA gene sequence (115), using the same strain that had been studied at the morphological level by Camp et al. (13) (strain Bi/pa; ATCC 30948), unequivocally confirmed its trichomonad affinities; more recently, analysis of the same gene from *H. meleagridis* confirmed the close and specific relationship between *Histomonas* and *Dientamoeba* (48). The latter investigation hinted at a link between *Histomonas/Dientamoeba* and the genus *Trichomonas*, but this remains to be confirmed.

So where is *Dientamoeba* classified today? The organism presently resides in the phylum Parabasalida, class Trichomonadidae, family Trichomonadidae, and possibly the subfamily Trichomonadinaceae (48). The systematics of the parabasalids is, however, in need of revision, and the specifics of *Dientamoeba* classification may change. Its affinities to *Histomonas* and other trichomonads will not.

At the other end of the taxonomic scale, there are very different questions. How many species of *Dientamoeba* are there? 2. Is *D. fragilis* of human origin a single species? At the morphological level, amoeboid organisms present a serious problem because there are very few phenotypic characteristics on which to base a species diagnosis. Indeed, morphology has been superseded by molecular markers in the description and identification of species in some genera of amoeboid organisms, for example *Entamoeba* (34) and *Naegleria* (31). Although *D. fragilis* has been found in nonhuman primates, this diagnosis is based only on morphology (59, 73, 83). Whether these organisms represent distinct species within the genus awaits further information. However, attempts to infect a macaque intrarectally with human *D. fragilis* were unsuccessful (36). As far as we are aware, no other species in the genus *Dientamoeba* have been described.

Some molecular data do exist to address the second question. Using the approach of examining restriction fragment length polymorphisms of the *D. fragilis* small-subunit rRNA gene (riboprinting), Johnson and Clark (66) identified the existence of two genetically distinct types of *D. fragilis* among 12 isolates from humans. An additional 90 or more isolates have subsequently been studied using the same method (J. J. Wind- sor, unpublished data). In the latter set of samples only one genotype has been found. The rarer of the two genotypes was found in only two cases; one of these strains happens to be the isolate Bi/pa studied by Camp et al. (13) and Silberman et al. (115). This indicates that the results of studies using isolate Bi/pa may not be representative of the species as a whole. The degree of sequence divergence between the ribosomal genes of the two genotypes is estimated to be approximately 2% (66, 94). Whether this constitutes a species level of divergence in protozoa is a matter for debate, and no consensus exists among researchers in the field.

The significance of the existence of two genetically distinct forms of *D. fragilis* deserves to be investigated further. Is one form more virulent than the other, and could this possibly contribute to the differences in clinical perceptions regarding the organism’s pathogenicity? Results from the largest study so far have found only one genotype in both symptomatic and asymptomatic individuals (94). The rarity of the second genotype will make investigation of its role in disease difficult.

MORPHOLOGY

Most of the detailed light microscopic descriptions of *D. fragilis* date back to the early and mid-1900s. Using a camera lucida, the parasitologists of the time produced surprisingly detailed plates (8, 26, 65, 133, 134, 135, 136, 137). There are subtle differences in the descriptions since some workers prepared material direct from feces (57, 133, 134, 135, 136, 137) whereas others used material from culture (36). Dobell (36) considered *D. fragilis* in feces to be degenerate and therefore not a true morphological representation.

In direct saline preparations, *D. fragilis* usually appears rounded and shows a wide variation in size. In the original description by Jepps and Dobell (65), the size range given was 3.5 to 12 μm. Much larger sizes have been found in culture (20 to 40 μm) (114). The size range of *D. fragilis* in culture overlaps that of *E. histolytica, E. hartmanni*, and *Endolimax nana* (102, 107). The nuclei of *D. fragilis* are not visible in saline or iodine preparations, although food vacuoles or inclusions may be seen. *D. fragilis* moves by using thin, hyaline, leaf-like pseudopodia, which are irregularly lobed (Fig. 1) (65). Hakansson (55), examining his own freshly evacuated stool specimen, found rounded trophozoites of *D. fragilis*. Only after 5 to 10 min at room temperature did they recover from this temporary “paralysis” and display the characteristic fan-shaped motility, with lobes and indentations. Unlike *Entamoeba* trophozoites, no flow of endoplasm into the pseudopodia has been observed in *D. fragilis*, and while the edge is constantly changing, with sharp points appearing, no progression is seen (55, 56).

Jepps and Dobell (65) found that 80% of *D. fragilis* trophozoites in permanently stained fecal smears were binucleate and
20% were mononucleate. This percentage can vary considerably, even in stool samples taken from the same patient on different days (134). Although seen less frequently, some trophozoites have been described with as many as four or five nuclei (36, 81, 136). The diameter of the nuclei varies from 1 to 3 μm but depends largely on the size of the trophozoite (65, 137). Internally, the nuclei appear fragmented, usually containing four to eight granules, without peripheral chromatin (Fig. 2) (137). Often, one of the granules is larger than the others and stains more deeply (8, 65, 133). The binucleate form of *D. fragilis* is the typical stage observed. Mononucleated trophozoites of *D. fragilis* are therefore recently divided forms, produced by the process of binary fission, and are slightly smaller than the binucleates. The division is by simple constriction of the cell body. Nuclear division is found only in mononucleated trophozoites (36, 135). Dobell (36) described a “connecting thread” which joined the two nuclei together. He termed this a “centrodesmus” and could find no trace of it in mononucleated organisms. Wenrich (135) termed this structure a “post-division desmose,” believing it to arise from an intranuclear division centre. Dobell, however, thought that this organelle permanently linked the nuclei. Dobell’s review (36) of the morphology of *D. fragilis* and its comparison to that of the turkey pathogen *Histomonas meleagridis* was the defining publication of the era and was the first paper to acknowledge the flagellate attributes of *D. fragilis* and its morphological similarities to *H. meleagridis*.

Curiously, only a handful of papers have been published on the morphology of *D. fragilis* on the basis of transmission electron microscopy (13, 91, 112, 113). Camp et al. (13) published a comprehensive study of the binucleate stage of *D. fragilis*.

![FIG. 1. *D. fragilis* growing in Robinson’s culture, showing ingested rice starch and fine leaf-like pseudopodia. magnification, ×400.](http://cmr.asm.org/)

![FIG. 2. Iron-hematoxylin-stained smear of *D. fragilis* showing pleomorphic trophozoites. Note the characteristic fragmented nuclei and the very small mononucleated trophozoite in the center magnification, ×1,000.](http://cmr.asm.org/)

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strain Bi/pa. This paper confirmed many of the light microscopic observations of the flagellate characteristics of *D. fragilis* by Dobell (36). An extranuclear spindle is found between the nuclei, originating from polar complexes adjacent to one of the nuclei. These structures are homologous to the atractophores described in hypermastigotes (61). Parabasal filaments extend laterally to the external surface of the atractophores. Extensive Golgi complexes overlie the filaments and are very similar to the parabasal apparatus seen in trichomonads and hypermastigotes. This spindle is composed of two bundles of approximately 30 to 40 microtubules. One bundle appears at some distance from the nucleus, whereas the other is juxtanuclear and is often seen in a groove of the nuclear envelope. The nuclear structure of *D. fragilis* more closely resembles that of trichomonad flagellates rather than that of *Entamoeba* spp. Chromatin bodies or granules are often seen in the nucleoplasm (Fig. 3 and 4), and the nuclear envelope consists of two membranes (Fig. 5).

Electron microscopy revealed electron-dense rounded inclusions in the cytoplasm that were termed “microbody-like” and were presumed to be homologous to the paraxostylar granules of trichomonads (13) (Fig. 3). These inclusions were subsequently recognized as being hydrogenosomes (82). Digestive granules are also commonly found in the cytoplasm and may contain myelin, bacteria, or rice starch (Fig. 3 and 6). *D. fragilis* feeds by phagocytosis (Fig. 7), and waste products are released from the digestive vacuoles by exocytosis (Fig. 8).

**CLINICAL OVERVIEW**

Eighty-five years after its first description, although *D. fragilis* is accepted as a true pathogen in some countries and infected patients are treated, it is still struggling to gain acceptance as a legitimate pathogen in many others. This is a result of *D. fragilis* at times being found in patients who exhibited no apparent clinical symptoms (24) and often being identified
in patients coharboring other suspect pathogens. Robertson (100) described a young female patient who was admitted to hospital with a history of recurrent attacks of diarrhea and abdominal pain. *D. fragilis* was isolated from a series of her stool specimens, which also contained *E. histolytica*, Trichomonas spp., and Blastocystis hominis, as well as Endolimax nana and spirochetes. In the same year, Thomson and Robertson (128) reported a case involving a 38-year-old man who had dysentery. Although *D. fragilis* was isolated from this patient, other organisms were also found, including Entamoeba coli, *Trichuris trichura* and *B. hominis*. Due to the coinfections in these two patients, there was no sound basis to support a primary disease-causing role for *D. fragilis*. *D. fragilis* was subsequently found in stool samples from a healthy 3-year-old girl (125) and in epidemiological studies from Canada (96), England (64), and South Africa (42), but strong evidence suggestive of its pathogenic potential was not presented.

Interestingly, Wenrich et al. (138) reported that 4.3% of college freshmen were infected with *D. fragilis* in a professional school in Philadelphia. This incidence of infection was similar to that of *E. histolytica*, but, interestingly, more students with *D. fragilis* had clinical symptoms than did those with *E. histolytica*. Das Gupta (30) also identified *D. fragilis* in a Bengali man in India who had intermittent attacks of diarrhea, but Hakansson (55) was the first to propose that *D. fragilis* was more than just an innocent commensal organism. He reported a clinical case involving a 48-year-old physician who had severe colitis. *D. fragilis* was observed in large numbers at the onset of the illness, and variations in its abundance corresponded to the severity of clinical symptoms. After several treatments with

![FIG. 4. Electron micrograph of a binucleated trophozoite of *D. fragilis*. Digestive vacuoles (Dv) contain either myelin or rice starch (Rs). Magnification, ×25,000.](http://cmr.asm.org/)

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carbarsone, the patient's condition improved and the infection with *D. fragilis* disappeared. Subsequently, there have been numerous reports that have linked *D. fragilis* infections with clinical symptoms that subsided only after the elimination of the organism (25, 35, 56, 72, 80, 99, 103, 104, 137).

Yoeli (147) described nine patients who suffered from acute intestinal signs such as explosive diarrhea, severe abdominal pain, cramps, nausea, vomiting, mild fever, and general fatigue. In all of these patients, large numbers of *D. fragilis* organisms were observed in the absence of any other pathogens. Numerous reports from many different parts of the world continued to substantiate the association of *D. fragilis* with clinical symptoms, principally abdominal pain, diarrhea, nausea, vomiting, and fatigue (1, 3, 12, 27, 32, 53, 92, 113, 114, 143, 146).

Studies have also demonstrated links between this parasite and urticaria (146), biliary infections (127), pruritus (116), colitis (111), allergic colitis (28), irritable bowel syndrome (6), and diarrhea in people infected with human immunodeficiency virus (75, 78). Of particular significance is the observation that infections in children appear to be very common. Keystone et al. (71) reported that over 8% of 900 children studied in the Toronto area were positive for *D. fragilis*. Also, some authors have reported that infections in children are more often associated with clinical symptoms than are infections in adults (3, 54, 85, 117); children are also more often reported to exhibit peripheral eosinophilia (2, 93, 97, 98, 117, 134).

Despite the significant number of studies that have incriminated *D. fragilis* as a legitimate enteric pathogen, it is far too seldom included in the differential diagnostic repertoire of intestinal pathogens by both practicing physicians and diagnostic laboratories in many countries.

**PATHOLOGY**

In parallel with our poor understanding of the pathogenicity of *D. fragilis*, only a small number of studies have presented findings relevant to the pathological consequences of infections with this organism. The first reported pathological study performed on the appendixes of four patients (ranging in age from 20 to 28 years of age) infected with *D. fragilis* showed
distinct similarities (11). In each case there was marked fibrosis of the subserosa of the appendixes and trophozoites were seen in the lumen, with absence of tissue invasion. Of particular relevance was the observation that in each case there were ingested red blood cells within the *D. fragilis* organisms. The authors considered that this was a hallmark feature of pathogenic potential, since pathogenic amebae such as *E. histolytica* ingest erythrocytes whereas the nonpathogenic *Entamoeba coli* and *Endolimax nana* do not. A critical analysis of these pathological findings, however, did not permit *D. fragilis* to be discriminated as the causative agent of the fibrosis because in three of the four cases the appendixes also contained worm ova, larvae, or adults that also could also have potentially been responsible for these lesions. In a more extensive study of appendixes containing *D. fragilis*, Swerdlow and Burrows (124) examined an additional 11 organs and also reported extensive fibrous connective tissue of the submucosa. They found a variety of lesions, ranging from acute suppurative appendicitis to lymphoid hyperplasia to pure fibrosis. They felt that *D. fragilis* probably acted as a low-grade irritant, causing a chronic in-

FIG. 6. Electron micrograph of *D. fragilis* demonstrating digestive vacuoles (Dv) containing myelin. Magnification, ×21,000.
flammaratory reaction that resulted in fibrosis. However, only three of the appendixes from this study had pure *D. fragilis* infections, making it difficult to conclude that *D. fragilis* was solely responsible for the underlying fibrosis.

Shein and Gelb (111) reported finding multiple punctate ulcers on endoscopic examination of a female patient who had a history of chronic abdominal pain for 1 year and a sudden production of blood-streaked diarrhea. A biopsy of her rectum revealed shallow ulcerations with evidence of acute and chronic inflammation. Trichrome staining of mucosal aspirates revealed large numbers of *D. fragilis* organisms in the absence of any other known pathogens. However, more recently described pathogens such as the coccidia and microsporidia might have been overlooked. Three additional studies (92, 110, 120) reported inflammatory changes of the rectum and sigmoid colon in patients with *D. fragilis* infections. However, many of these patients had mixed infections and many had no pathological abnormalities.

A more recent study (28) reported a case of eosinophilic colitis in a female patient harboring *D. fragilis*. The patient was documented as having bovine protein allergy with intermittent episodes of diarrhea and abdominal pain, despite receiving an appropriate diet. After the patient was treated with iodoquinol and the parasite was eliminated, the symptoms disappeared.

In contrast to the findings of the above studies, Cerva et al. (15) were unable to establish any relationship between the presence of *D. fragilis* and any pathological abnormalities in the appendixes of the patients in their study, nor were they able to detect red blood cells in any of the *D. fragilis* organisms contained within the lumen of the appendixes. Certainly, the lack of an animal model hampers our ability to shed light on the exact pathological manifestations caused by *D. fragilis*.

FIG. 7. Electron micrograph of *D. fragilis* exhibiting phagocytosis. Magnification, ×6,600.
TRANSMISSION

The mode of transmission of D. fragilis has remained a mystery. Most intestinal protozoa that are transmitted via the fecal-oral route require a cyst stage in order for the organism to survive in the external environment. However, although a few authors have reported pseudocystic, precystic, or cystic stages of D. fragilis (52, 72, 95, 133), it is generally accepted that this parasite does not have a cyst form. It is likely that the cysts described by Kofoid (74) were merely rounded-up trophozoites. Wenrich (133) also described degenerating forms, which stained more intensely and were thought to be precystic forms or “pseudocysts.” In a later publication, Wenrich (136) found these forms to be no more frequent in older than in fresh material and subsequently dismissed the notion of “pseudocysts.” Piekarski (95) examined iron-hematoxylin-stained smears and reported the presence of precystic and cystic forms. However, closer scrutiny of the figures in that paper reveals that these forms are more likely to be degenerating trophozoites. Interestingly, Silard et al. (114) reported possible cystic forms of D. fragilis, with irregular, thick membranes, in cultured preparations examined by phase-contrast microscopy. These forms were not confirmed in permanently stained smears. It is still possible that a cyst form might be identified. Despite the larger body of research that has been undertaken on B. hominis, its cyst stage was confirmed only in 1991 (122), 79 years after its first description.

The lack of a cyst stage would cast doubt on the possibility of an effective direct fecal-oral route of transmission. Thomson and Robertson (128) stated that it appeared unlikely that an infection could occur via ingestion of the adult form of D. fragilis, since the life span of the parasite outside the body is very short. Furthermore, they doubted whether D. fragilis could survive the vicissitudes of its journey through the alimentary tract. Indeed, in a most dedicated fashion of scientific pursuit, Dobell (36) swallowed a culture containing thousands of active and healthy trophozoites of D. fragilis. After 10 years of examining his stools, he was unable to find the organism in a single sample. His attempts to infect macaques were similarly unsuccessful.

Dobell (36) compared D. fragilis with what he felt was its
closest biological relative, *Histomonas meleagridis*. He pointed out that since *H. meleagridis* was known not to have a cyst form and was transmitted via the eggs of the nematode *Heterakis gallinae*, it was highly likely that *D. fragilis* was also transmitted via the ova of nematodes, and suggested *Trichuris trichiura* and *Ascaris lumbricoides* as likely candidates. Circumstantial support for this hypothesis was found in reports by a number of researchers who described helminth coinfections in large numbers of patients infected with *D. fragilis* (58, 128).

Burrows and Swerdlow (10) agreed with Dobell (36) and Wenrich (136) that *D. fragilis* was probably related to *Histomonas*. This was based on the fact that neither organism formed cysts, each was pathogenic to its host to various degrees, each ingested red blood cells within the host as well as in culture, each showed a lag phase of about 24 h before multiplying in cultures, and each is transmitted in the egg of a species of roundworm. However, they disagreed with Dobell regarding the intermediate host. Whereas Dobell (36) thought that the intermediate host might be *Trichuris* or *Ascaris*, Burrows and Swerdlow (10) were convinced that the incriminating intermediate host was *Enterobius vermicularis*. This was based on pathological analyses of 22 appendixes from which *D. fragilis* was isolated. They found a 20-fold greater incidence of coinfection with the pinworm *E. vermicularis* than the calculated expected value. In addition, they observed small ameboid bodies, whose nuclei greatly resembled those found in uni-and binucleated *D. fragilis*, in the eggs of the pinworms. Further circumstantial evidence to support this assumption was provided by a number of other studies that showed a greater than expected incidence of coinfections with *D. fragilis* and *E. vermicularis* (11, 15, 19, 77, 86, 98, 124, 146). In addition, although Burrows and Swerdlow (10) were unsuccessful in their attempts to culture *D. fragilis* from pinworm eggs, Ockert (88, 89) not only successfully infected himself with *D. fragilis* by ingesting eggs of *E. vermicularis*, taken from a young boy who was coinfected with *D. fragilis*, but also successfully infected two other human subjects. It is noteworthy that the reported incidence of both *D. fragilis* and pinworms is higher in females than males (15, 53, 146).

Ockert and Schmidt (90) felt that they had found the definitive proof which confirmed that *Enterobius vermicularis* served as the vector for transmitting *D. fragilis*. They compared the isoelectric points of trophozoites of *D. fragilis* in culture with ameboid-like cells that they found in the eggs of *Enterobius*. Interestingly, their nuclei and their cytoplasm had almost identical electrostatic charges.

Based on morphological comparisons, Dobell (36) proposed that *Dientamoeba* was probably related to *Histomonas*. Similar assumptions have been expressed by Wenrich (136, 137), Camp et al. (13), and Dwyer (39, 40, 41) as a result of their studies. More recently, the specific phylogenetic relationship between *Histomonas* and *Dientamoeba* has been determined by the analysis of small-subunit rRNAs. Both species demonstrate reduced G+C contents and longer nucleotide chain lengths compared to other parabasalids (48). Based on the totality of these similarities, it appears plausible to make an assumption that both species may also have a common modus of transmission. Certainly, from the standpoint of the parasite’s ability to perpetuate itself and survive in the environment, it would be of benefit for *D. fragilis* to be transmitted by pinworms (23, 132).

Some authors have not been convinced of the role of *Entero-robustus* as the vector of *D. fragilis* since they were unable to establish any association between *E. vermicularis* and *D. fragilis* in any of their patients (69). Unfortunately, however, it is not always apparent how, or if, some of the researchers studying *D. fragilis* properly screened their patients for pinworms. Not only do pinworm infections often undergo spontaneous remissions, but also, unlike other nematodes that are diagnosed by the presence of fecal eggs, pinworms are diagnosed only by performing anal swabs or cellotape smears. These procedures are often not performed or are undertaken only when there are specific indications such as anal pruritus. It is therefore likely that some investigators may not have adequately looked for pinworms. However, it is also possible that other nematodes might serve as vectors. Indeed, Sukanahakutu (123) found *D. fragilis*-like structures in the ova of *Ascaris lumbricoides* in 38 patients in Thailand with *D. fragilis* infections. He did not find these structures in patients infected with *A. lumbricoides* without coinfections with *D. fragilis*.

Attention should be drawn to the fact that our inability to identify a cyst stage of *D. fragilis* does not guarantee its nonexistence. Indeed, *D. fragilis* infections are often associated with other intestinal parasites. Ayadi and Barri (3) investigated the incidence of both *D. fragilis* and the still unresolved issues pertaining to in vitro susceptibility testing is consequently undertaken in the presence of *D. fragilis*. Testing should be done properly screened their patients for pinworms. Not satisfied with the results of these studies, they were unable to determine whether the antimicrobial agent is active against *D. fragilis* or the accompanying bacteria supporting its survival. The argument, however, can be made that if bacteria are necessary for the survival of *D. fragilis*, testing should be done under conditions which most closely mirror those conditions. It has also been difficult to interpret minimal amebcidal in vitro concentrations of the drugs presently available, since their concentrations in the gastrointestinal tract have not been determined (17).

Advancements in our understanding of the effects of protozoicidal agents on *D. fragilis* is technically limited by our inability to maintain this organism in axenic cultures. Drug susceptibility testing is consequently undertaken in the presence of *D. fragilis*. There is only a limited amount of information available on the efficacy of therapeutic agents against this organism. Successful reported treatments for *D. fragilis* infections include diphenoxylate (70; S. S. Deser and Y. J. Yang, Letter, Can. Med. Assoc. J. 114:290, 293, 1976), tetracycline (29, 69), carbarsone (69, 72), metronidazole (28, 117, 120), iodoquinol (28, 79, 116, 117), erythromycin (98) hydroxycholine (98), paromomycin (28), and secnidazole (49).

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Despite the limited number of studies of the treatment of *D. fragilis* and the still unresolved issues pertaining to in vitro testing methods, it cannot be denied that a substantial body of information exists indicating that the elimination of *D. fragilis* from symptomatic patients results in clinical improvement. In-
deed, in a recent study performed in Australia, all 21 patients with a 2-month to lifelong history of irritable bowel syndrome symptoms (including diarrhea [2 to 15 motions/day], constipation, abdominal cramping, bloating, flatulence, nausea, fatigue, and anorexia) and concurrent *D. fragilis* infections who were treated with iodoquinol and doxycycline showed complete elimination of *D. fragilis*. Clinical improvement was achieved in 67% of these patients (6). Some of the patients, however, experienced side effects, including dizziness, headaches, nausea, lethargy, and pruritus. These findings, besides demonstrating the effectiveness of this treatment regimen, also suggested a possible pathogenic role for *D. fragilis* as a cause of irritable bowel syndrome.

Side effects have also been reported for other therapeutic agents used to treat *D. fragilis* infections. Transient liver function abnormalities were observed in several patients treated with diphetarsone (70). Tetracycline has limited usefulness in children because of its well-established deleterious effect on dental development. Presently, iodoquinol and tetracycline are the most commonly employed medications, but a recent study found the antiamebic drug mebendazole to be highly effective. *D. fragilis* was eradicated in 34 of 35 patients after receiving a single dose of mebendazole. A second dose was required only for one patient (49). Clearly, however, more work is required to establish effective and safe therapeutic protocols. In the United States, all therapy for *D. fragilis* is considered investigational by the Food and Drug Administration (94).

**EPIDEMIOLOGY AND DIAGNOSIS**

Studies from a large number of countries have substantiated the worldwide distribution of *D. fragilis* (139). Prevalence rates have been reported to vary from 0% in Prague (14) to as high as 42% in children in Germany (86, 87). Infection rates are probably influenced by population density and levels of hygiene, since rates of infection have been shown to be higher in mental institutions (56), among selected military personnel (105), among parasitology students (119), and in missionaries (127). In a semicommunal group of adults in the United States, an infection rate of 52.5% was reported (79). The diagnostic rates have been reported to vary from 0% in Prague (14) to as high as 42% in children in Germany (86, 87). Infection rates are probably influenced by population density and levels of hygiene, since rates of infection have been shown to be higher in mental institutions (56), among selected military personnel (105), among parasitology students (119), and in missionaries (127). In a semicommunal group of adults in the United States, an infection rate of 52.5% was reported (79). The diagnostic methods employed have a profound effect on the successful detection of *D. fragilis* and consequently on the accuracy and interpretation of such reports. In addition to the level of competence of the person evaluating the fecal samples, a number of other factors influence the diagnostic pursuit. The binucleate structure of *D. fragilis* cannot be appreciated if the sample is in a saline preparation (142), and so permanently stained fecal smears should be made (116). Grendon et al. (54) surveyed detection methods for *D. fragilis* in State Public Health Laboratories in the United States and found that permanent staining of all stools, rather than only loose and watery ones, resulted in a fivefold increase in the detection rate. The numbers of *D. fragilis* organisms shed in feces may vary considerably from day to day (146; Desser and Yang, Letter), as is the case for many intestinal protozoa. Yang and Scholten (145) examined the stool distribution of *D. fragilis* in one patient and found that more than twice as many organisms were present in the last portion evacuated. Also, increasing the number of fecal samples to three has been reported to increase the detection rate by over 30% (60). Detection rates have been reported to double when culture results were compared to stained smears from the same cohort of patients (141). It is clear that accurate diagnosis of *D. fragilis* requires the use of suitable staining or culture techniques and examination of more than one fecal sample.

**Microscopy**

Permanently stained fecal smears are commonly used in North America and are appreciated as an essential aid in the diagnosis of intestinal protozoa. In Europe, however, fresh unpreserved stool specimens are generally used for examination while stained smears are used in reference centers. Laboratories that examine stools by direct microscopy should be aware that *D. fragilis* trophozoites may be encountered as refractile, rounded forms, varying in size from 5 to 15 \( \mu \text{m} \) (142) (Fig. 9). The nuclear structure cannot be seen in saline or iodine preparations, and consequently the cells may be dismissed as artifacts. It is essential that permanent stained smears be performed on every stool sample to properly identify trophozoites of *D. fragilis*. The crystal violet hematoxylin method of Velat et al. (131) can be used to stain *D. fragilis* and other trophozoites of flagellates in fresh wet preparations. Although the preparation of the stain is complex, the method is simple and the results are excellent. Another simple method of staining without using specialized fixatives is to air dry a fecal smear, fix it in industrial methylated spirit, and stain it with either Giemsa stain (43) or Field’s stain (81). However, it is not possible to see the typical fragmented nuclei when using these simple, rapid methods since the nuclear contents often coalesce (86). Much better cytological results can be obtained by using a suitable fixative (see below) in combination with a permanent staining method. Concentration methods are not generally recommended for the recovery of *D. fragilis*, although trophozoites are sometimes found in concentrated stools (145).

**Fixatives**

Once *D. fragilis* trophozoites degenerate, they become harder to recognize. Therefore, for optimal results, fecal specimens should be placed in a fixative immediately (145). Dobell (36) employed Schaudinn’s fixative followed by staining with Heidenhain’s iron-alum hematoxylin. Generally, all fixatives used for intestinal protozoa are suitable for *D. fragilis*, these include polyvinyl alcohol (PVA) (50), modified Schaudinn’s fixative (109), phenol-alcohol-formalin (9), and sodium acetate-acetic acid-formalin (SAF) (145). SAF has the advantages that it is simple to make and relatively nontoxic (compared to other fixatives) and can also be used for concentration methods. The merthiolate-iodine-formalin method (106) is a combined fixative and stain technique; however, it is not very stable and does not stain the nuclei of *D. fragilis* well (146). Both Schaudinn’s fixative and PVA (which is a plastic powder dissolved in Schaudinn’s fixative) contain mercuric chloride. Concerns about safety and problems with the disposal of mercury led researchers to look for substitutes for mercuric chloride that are more environmentally friendly (47, 62). Studies using copper sulfate (CuSO\(_4\)) were controversial. Horen (62) found that copper sulfate gave results comparable to those obtained...
with the original formula, whereas Garcia et al. (47) described inferior results, for protozoa in general, with this substitute. They compared the original PVA with a formulation in which a zinc sulfate base was used to replace the mercuric chloride and found it to be a viable substitute, although the overall morphology was not as good. To date, none of the mercury substitute fixatives produce results equal to those obtained with mercuric chloride.

**Staining**

According to Dobell (36) “all good cytological methods yield good preparations of *D. fragilis* if they are employed with appropriate precautions.” Over the years, many different stains have been used to detect *D. fragilis*, after appropriate fixation, including iron-haematoxylin (36), Mayer’s haemalum (127), Lawless’ stain (76), Celestine Blue (144), and Wheatley’s trichrome (139). Ockert (86) found that fast methods of preparing permanent stained smears, such as Lawless’ technique, often gave poor results. This could be overcome by using thin fecal smears, rapidly overlaying the smear with a mixture of stain and fixative, complete rinsing out of the fixative, and using very pure reagents (86). The combination of fixative and stain is important since this can greatly affect the quality of protozoan morphology. Generally, the trend has been to use PVA or Schaudinn’s fixative with Wheatley’s trichrome (7, 53, 79, 116, 117, 118) or SAF fixative with iron-hematoxylin (67, 71, 145). SAF has been used with Wheatley’s trichrome (84, 143), but this may not give optimal results (46). Garcia and Shimizu (46) compared specimens fixed in a commercial zinc sulfate-based Schaudinn’s fixative (EcoFix) (Meridian Diagnostics, Inc.) and stained with either Wheatley’s trichrome or a commercial EcoStain (Meridian Diagnostics, Inc.). The commercial stain produced a gray-green or gray-blue monotone, with very little pink tone, and the contrast was lower than that achieved with trichrome stain. Nevertheless, the combination of EcoFix and EcoStain provided a better alternative than EcoFix and Wheatley’s trichrome. For optimal results, without the inherent safety problems associated with mercuric chloride fixatives, we favor the combination of SAF and iron-hematoxylin.

Van Gool et al. (130) in the Netherlands described a “triple feces test” that combined the sampling of patient stools collected on three consecutive days, the use of SAF fixative, and the use of the stain chlorazol black. The test was described as highly effective in identifying intestinal protozoa, including *D. fragilis*, was relatively fast and easy to perform, and did not require dehydration steps with xylene. Although this technique was found to be a better detection method for fecal protozoa than direct microscopy and concentration methods, it has not been compared to the more established permanent staining methods used in North America.

Technical difficulties can arise when *D. fragilis* chromatin

![Image](https://example.com/image.png)
granules are covered with stain deposits, when the nuclear fragmentation is not obvious, or when the majority of trophozoites are mononucleated (145). In these circumstances, they can be confused with trophozoites of *Endolimax nana* (44, 45, 145).

**Immunological Diagnosis**

Specific immunological tests for *D. fragilis* are not currently available. Chan et al. (18) were the first to develop an immunofluorescence assay to identify *D. fragilis* trophozoites in preserved fecal specimens. They produced anti-*D. fragilis* antiserum in rabbits by using the dixenic *D. fragilis* strain Bi/pa. After absorption of the antiserum with *Klebsiella pneumoniae* and *Bacteroides vulgatus*, the two bacteria present in the culture, it was used in an indirect fluorescent-antibody assay to detect *D. fragilis* in preserved fecal samples. There were no cross-reactions with any of the 4 species of helminthes or 10 species of protozoa encountered in their study. The authors considered the indirect fluorescent-antibody assay to be highly specific for *D. fragilis* and were able to identify the organism in seven of nine confirmed positives. Two samples with only very small numbers of *D. fragilis* trophozoites gave questionable results.

In a later study, Chan et al. (16) employed an immunoblot assay and found that serum samples from patients with confirmed *D. fragilis* infections reacted with a 39-kDa *D. fragilis* protein. It is unclear what this protein may be, what significance it may have in the pathogenesis of the disease, and whether it has any immunoprophylactic properties.

**DNA-Based Diagnosis**

Only one study to date has investigated the potential of detecting DNA in feces for diagnosis of *D. fragilis* infection (94) by amplifying a portion of the small-subunit rRNA gene by PCR. However, the sensitivity of this PCR cannot be compared directly to microscopy because different samples were used for the two detection methods. The development of a quick and accurate immunodiagnostic test would be of great benefit to the diagnosis of *D. fragilis* infections.

**CULTIVATION**

**General Considerations**

The culture methods used for *D. fragilis* are xenic, in which the parasite is grown in an undefined bacterial flora. The balance required in controlling the bacterial flora while providing for the needs of the parasite is crucial for the successful culture of intestinal protozoa (21). The intestinal bacterial flora provides *D. fragilis* with a food source. In all xenic culture media, rice starch provides the carbohydrate essential for bacterial growth. Antibiotics such as erythromycin, penicillin, and streptomycin are often used to suppress gram-positive organisms (101, 108, 114). Usually xenic culture media are biphasic, with
a slant of agar or egg and a liquid-phase overlay, although monophasic media have also been successfully used. Many different xenic media can be used to grow *D. fragilis* from clinical specimens, although we prefer to use Robinson’s medium (101).

**Historical Background**

Some early parasitologists maintained that *D. fragilis* was easily isolated and grew abundantly in certain media (22, 36). This is in contrast to the experiences of many present-day investigators (21). Dobell (36) credits Thomson and Robertson with the first culture of *D. fragilis*, using Boeck and Drbohlav’s *E. histolytica* medium (Locke egg serum) (5). It was heavily contaminated with *Blastocystis hominis*, and they were able to maintain it for only a short period. Dobell and Svensson are credited with having produced the first culture of *D. fragilis* free from other protozoa in 1929 (36).

Dobell (36) used two different biphasic media, one (HSre + S) with a solid slope of inspissated horse serum and the other (Ehs + S) made with an inspissated egg slope. The former gave the best results when overlaid with dilute egg white in Ringer’s fluid and supplemented with rice starch. Interestingly, Dobell (36) found an optimum growth temperature of 40°C for some strains of *D. fragilis*. Cleveland and Collier (22) isolated *D. fragilis* while attempting to improve their cultivation of *E. histolytica*. The medium they used was Loeffler’s dehydrated beef serum slants covered with fresh horse serum saline.

Balamuth (4) described a monophasic liquid medium containing dehydrated egg yolk and liver infusion. This medium permitted the growth of amebae (*Entamoeba histolytica*, *Entamoeba coli*, *Isodamoeba bütschlii*, and *Endolimax nana*), flagellates (*Trichomonas* spp. and *Chilomastix mesnili*), and *D. fragilis*. Balamuth used this medium to study the effects of drugs, amebicides, and antibiotics on amebae and *D. fragilis*. While experimenting on a xenic culture of *D. fragilis*, Balamuth inadvertently eliminated all but two of the bacterial species and produced a dixenic culture. Balamuth designated the subline B1/p/a, and the bacteria were identified as *Clostridium perfringens* and *Aerobacter aerogenes*. *Klebsiella pneumoniae* and *Clostridium perfringens* are now listed as the bacterial species, although Chan et al. (18) used this strain and found the anaerobic species to be *Bacteroides vulgatus*.

Jacobs (63) performed many experiments on the cultivation of *D. fragilis* but was unable to support its growth without viable bacteria. Using crude xenic cultures transplanted into a medium containing *Clostridium perfringens* with penicillin, streptomycin, and sulfadiazine, he was able to produce a monoxenic culture of *D. fragilis*. However, all attempts to produce an axenic culture (without other organisms) of *D. fragilis* have failed to date (17, 18, 21).

Robinson (101) formulated a biphasic medium for the diagnosis of human parasitic amebae that had a saline agar slope as the solid phase. The liquid phase was complex and included *Escherichia coli* growing in a defined medium (R). Other additives included erythromycin, horse serum, potassium phthalate, Bacto Peptone, and rice starch (21). Robinson’s medium supported the growth of all intestinal amebae including *D. fragilis*. This medium was used by Johnson and Clark (66) to grow *D. fragilis* prior to performing ribo-printing. Diamond (33) developed a monophasic medium, TYSGM-9 (Trypticase, yeast extract, serum, gastric mucin), which supported the growth of lumen-dwelling protozoa, including *D. fragilis*.

**Surveys Using Culture**

The most sensitive method of detecting *D. fragilis* is by using culture techniques, compared directly to stained smears (86, 141). Although cultivation of intestinal protozoa is not usually attempted in routine diagnostic laboratories (21, 140), recent data have shown that it can be successfully employed outside a research environment (141). The use of Robinson’s medium in a small diagnostic laboratory doubled the detection rate from 1.3 to 2.6% compared with the rate for trichrome-stained smears. It was considered less laborious than staining and required a smaller amount of feces, and culture lysates can provide material for subsequent genotyping. On the negative side, cultures take 48 h or longer and cannot be used for fecal samples that are submitted in fixative. They are unlikely to replace staining methods in diagnostic parasitology laboratories because they do not detect all intestinal protozoa. However, cultures may play a role in laboratories that do not have the expertise to detect *D. fragilis* in stained smears but want to exclude this parasite. Positive cultures were even obtained from stool samples stored at room temperature or 4°C for 24 h. A previous study (108) had reported that *D. fragilis* could be cultured from feces stored for up to 24 h at room temperature but only for 10 h at 4°C.

Ockert (86) reported two studies undertaken in his research laboratory where all stool samples were both stained and cultured. In the first study, involving 576 children, 3% of the stained smears were positive whereas 35% were positive in culture. In the second study, involving 1,066 persons, 1.97% of the stained smears were positive while 39.3% were positive in culture. The medium used was a modification of that of Dobell and Laidlaw (37), using coagulated human serum for the solid phase. The primary cultures were subcultured twice into fresh medium. Of the *D. fragilis*-positive samples identified, only 40% were detected by the primary culture whereas over 80% were detected following the first subculture and the rest were detected after the second subculture. Silard et al. (114) also favored the use of Dobell and Laidlaw medium and isolated *D. fragilis* from 2.8% of clinical samples. Using culture over a 10-year period in Israel, Talis et al. (127) detected *D. fragilis* in 30,609 (15.2%) of 201,750 specimens. The medium used was a diphasic egg medium formulated in their laboratory. Using samples from a patient with known *D. fragilis* infection, Sawangjaroen et al. (108) compared three media, modified Boeck and Drbohlav (BD) medium, TYSGM-9 (33), and Cleveland and Collier medium (22). Modified Boeck and Drbohlav medium was found to be the most suitable since it was the only medium that supported the initial growth and subculture of *D. fragilis*. The authors then surveyed consecutive samples from patients with diarrhea. A surprisingly low incidence of 1.5% (4 of 260) was found; however, culture doubled the detection rate.
Appearance in Culture

Trophozoites of *D. fragilis* initially appear as rounded refractile bodies, containing many rice starch granules, in freshly mounted culture preparations after 48 h of incubation (Fig. 10). After approximately 10 min at room temperature, they begin to produce sluggish, small, irregular pseudopodia (108) (Fig. 1). The size of *D. fragilis* in culture varies considerably, and some workers have described a range of 6 to 40 μm (114). Robinson and Ng (102) found that with experience, this size range became a good guide to the presumptive identification of *D. fragilis* after addition of a drop of iodine. Moreover, the appearance of circular brown-red forms filled with starch grains aided this diagnosis. Several intestinal amebae grow well in culture media used for *D. fragilis* and also ingest rice starch. Although none of the amebae demonstrate the characteristic motility of *D. fragilis*, it is nevertheless recommended that the diagnosis be confirmed by using a suitable staining method (101). Robinson (101) fixed culture-positive amebae in a mixture of acetic acid and phosphotungstic acid and stained them with a hematoxylin stain. Windsor et al. (141) confirmed the positive amebae by fixing in Schaudinn’s fixative and staining with trichrome, whereas Silard et al. (114) preferred a simpler method of fixing in methanol and staining with Giemsa. Diagnostic uncertainties can arise for cultures containing granular forms of *B. hominis* and *D. fragilis* (J. J. Windsor, unpublished data). However, these species can be differentiated on the basis that the granules of *B. hominis* are distinctly rounded (Fig. 11) whereas *D. fragilis* simply contains ingested rice starch. Blastocystis grows freely in nearly all xenic media suitable for *D. fragilis* and *Entamoeba* spp. This was of considerable concern for investigators attempting to isolate these parasites (21, 37).

CONCLUSIONS

Significant progress has been made in the biological classification of *D. fragilis*. Although *D. fragilis* was initially thought to be an ameba, phylogenetic analysis of small-subunit rRNA gene sequences has confirmed morphological observations and antigenic analyses showing that it has an extremely close relationship with *Histomonas*, and today *D. fragilis* is classified as among the trichomonads.

Unfortunately, there still exists a difference of opinion regarding the clinical significance of this organism as a human pathogen. The overwhelming circumstantial evidence, however, strongly suggests that *D. fragilis* is a bona fide pathogen. This is based primarily on the observation that there are a large number of case reports from many parts of the world that describe patients whose clinical symptoms subsided only after...
therapeutic intervention and elimination of the organism. However, it cannot be denied that there are patients who harbor this organism but do not exhibit clinical signs. Recently undertaken molecular studies may help to shed light on these differing observations. Using ribotyping, two genetically distinct types of *D. fragilis* have been identified. It certainly would not be unreasonable to speculate that different genetic types may demonstrate different degrees of virulence.

The accuracy of diagnostic testing for *D. fragilis* has traditionally relied on permanently stained fecal smears, since the characteristic binucleate appearance of the organism cannot be appreciated in saline or iodine preparations. A number of studies have substantiated the necessity of using this methodology. The fairly recent introduction of fecal cultures for diagnostic studies have substantiated the necessity of using this method-

It would be remiss of us not to extend our sincerest thanks to Alan Curry at the Manchester Royal Infirmary for his inexhaustible generosity in providing the micrographs used in the present review article.


