THE UNIVERSITY OF TEXAS
HEALTH SCIENCE CENTER AT DALLAS

Southwestern Medical School

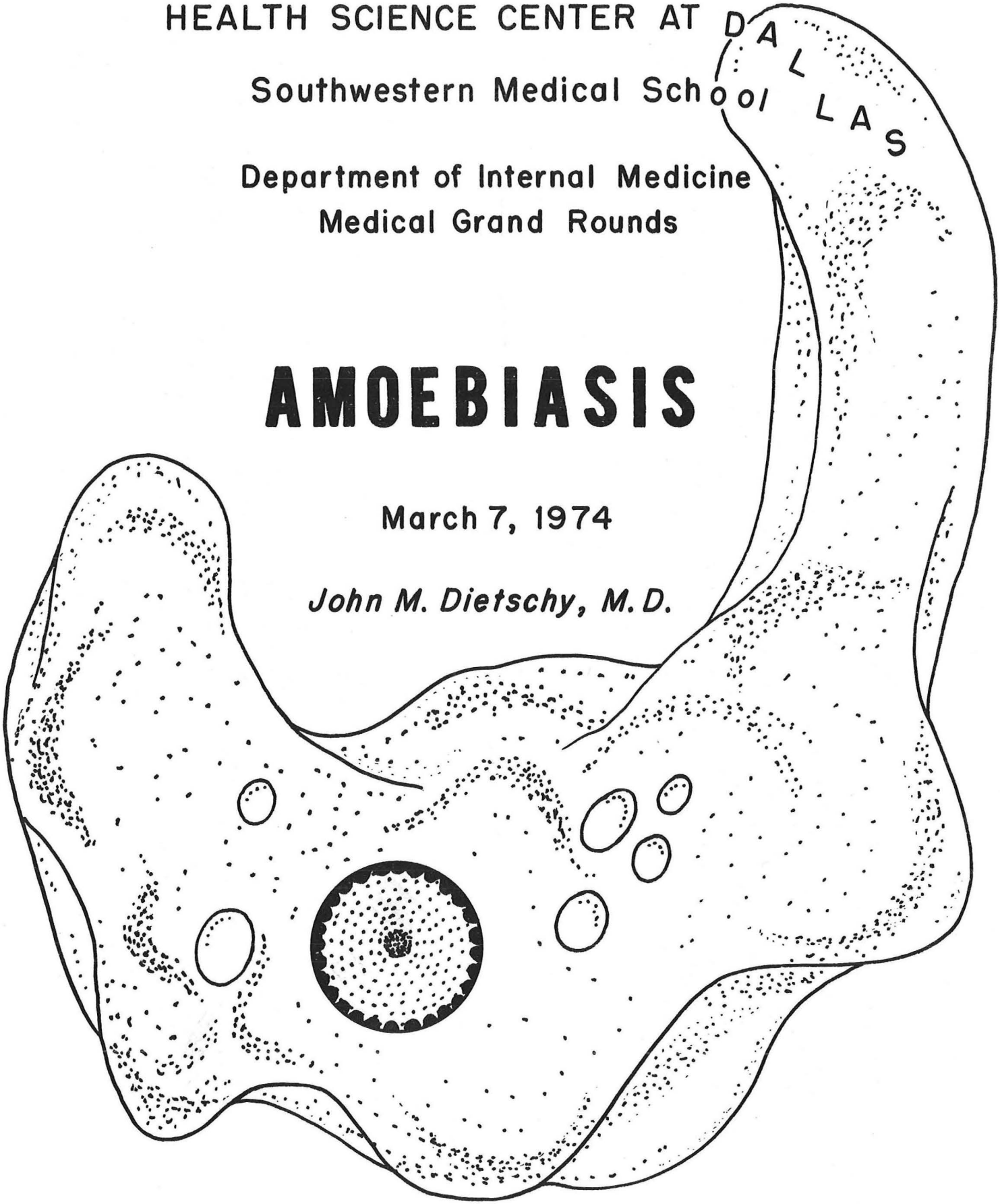
Department of Internal Medicine

Medical Grand Rounds

AMOEBIASIS

March 7, 1974

John M. Dietschy, M.D.



INTRODUCTION

Amoebiasis generally refers to a whole spectrum of diseases in which an amoeba with tissue invading capability, usually *Entamoeba histolytica*, colonizes a tissue producing tissue necrosis and abscess formation. In its most benign form, the infection may be clinically inapparent and will be recognized only because a particular patient is shedding cysts in the stool. Clinically apparent amoebic infection most commonly is manifest by diarrhea with or without gross bleeding. Less commonly the amoeba may produce a variety of clinical syndromes including single or multiple hepatic abscesses, an amoeboma of the cecum, acute appendicitis, brain abscess, or fistulas into the broncopulmonary system, the pericardium, the biliary tract, or externally to the skin. During the past ten years there has been a number of interesting advances in terms of pathophysiology of possible mechanisms for tissue invasiveness of the pathologic amoeba, the differentiation of various subtypes of *Entamoeba histolytica* and the introduction of several new drugs for the treatment of this disease.

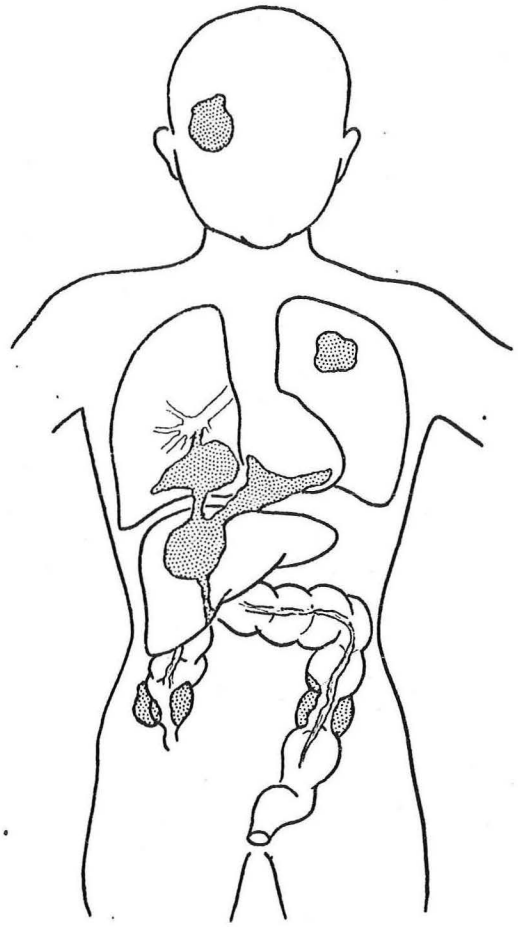


FIG. 1

GENERAL SCHEME OF CLASSIFICATION

All amoeba belong to the Class Rhizopodea and to the Order Amoebida. Characteristically, these organisms do not contain a cell wall and generally form pseudopodia by alternately shifting the cytosol from a gel to a sol phase. The Order Amoebida may be divided further into those organisms which are principally free-living and those organisms which are strictly parasitic. All parasitic forms are classified in the Family Endamoebidae.

The various genera of Endamoebidae generally are differentiated in terms of their morphologic characteristics and, in particular, in terms of the morphology of the nucleus on stained specimens. Those species of Endamoebidae listed in Fig. 2 all inhabit the large intestine of the host: the single exception to this being *Entamoeba gingivalis* that inhabits the mouth of man. Furthermore, of those species which are parasitic in man almost all lack the ability to invade tissue and, therefore, are principally lumen dwellers which

phagocytize food particles and bacteria from the intestinal contents; the principal exception to this generalization is, of course, *Entamoeba histolytica.*

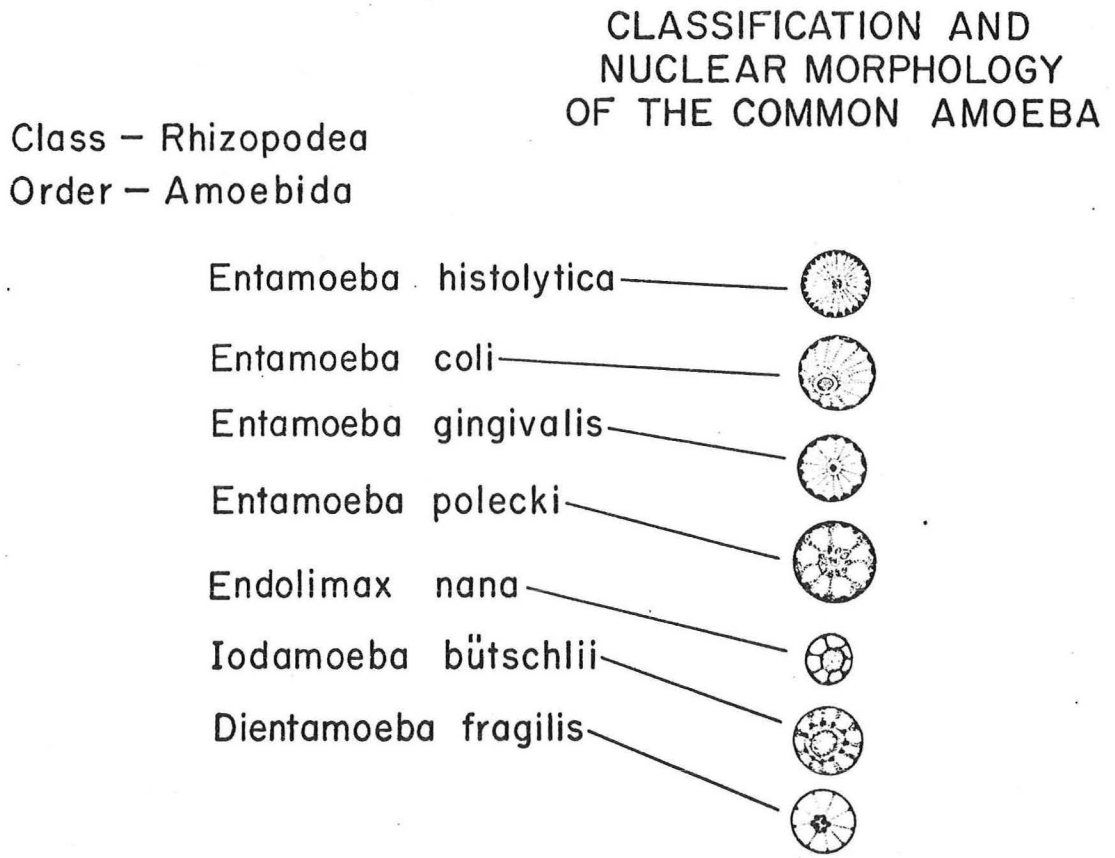


FIG. 2

The major phases in the life cycle of the parasitic amoebae are illustrated in Fig. 3. While the details of this scheme are derived from the cycle of *Entamoeba histolytica,* the general features apply to nearly all of the parasitic Endamoeba. The amoeba exists in the trophozoite form within the lumen of the large intestine. In the nonpathological strains the trophozoite exists by phagocytizing bacteria and other nutrients from the colonic contents. Under special circumstances the trophozoite of *Entamoeba histolytica* also is capable of cell invasion and destruction and actively phagocytizes cell components after dissolution of the cell membrane. In all species, the trophozoites reproduce within the luminal contents and in the colonic crypts by binary fission. The trophozoites are excreted in the feces only under circumstances in which a liquid, diarrheal stool is passed. During the process of formation of solid stools, however, encystation of the trophozoite normally occurs. During this process there is loss of cytoplasmic vacuoles and condensation of the cytosol and cell membrane. In several species there is reduplication of the nucleus so that in *Entamoeba histolytica*, for example, there is formation of precysts containing 1, 2 or 4 nuclei. The mature cyst is then excreted into the feces and enters into the environment where it may persist for long periods of time until

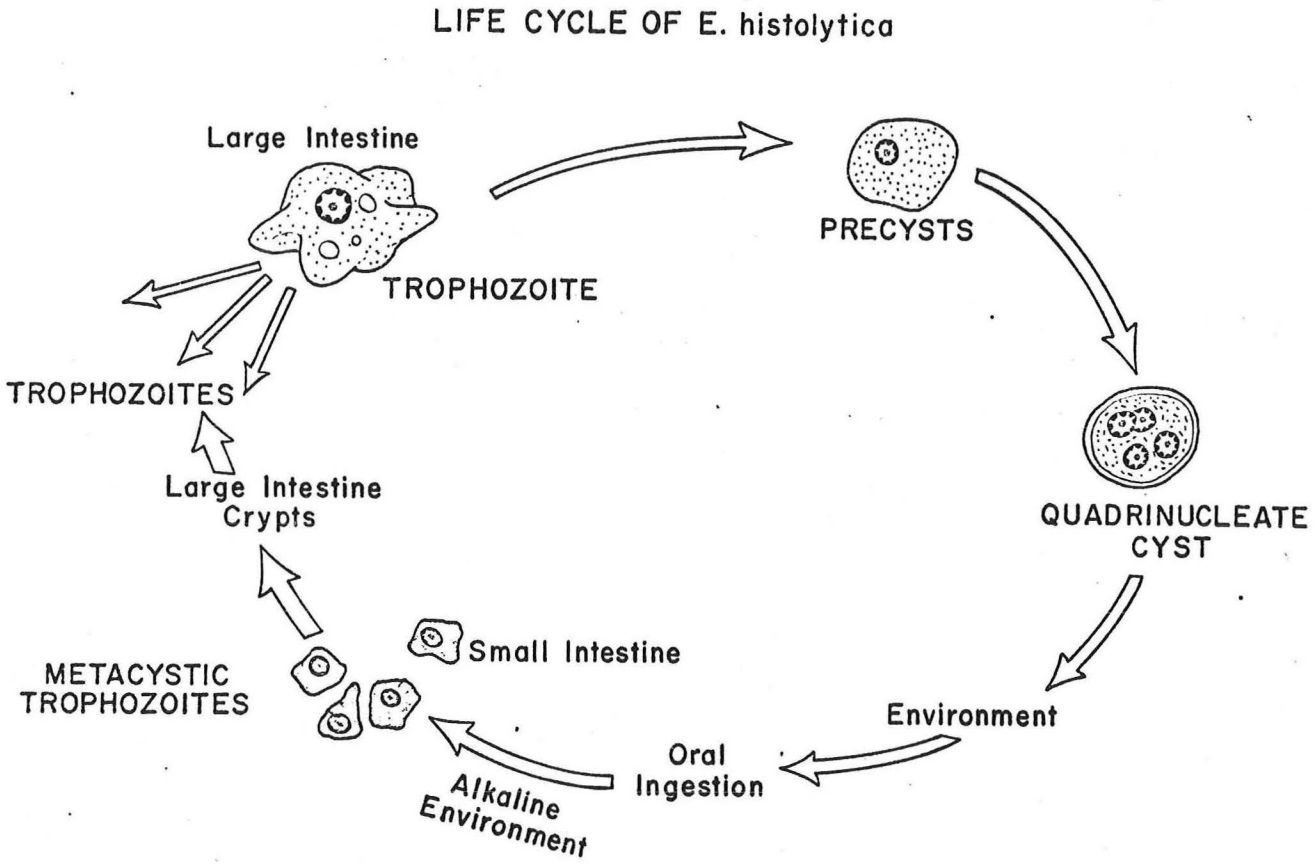


FIG. 3

ingested through the oral route into another host. Apparently, little happens to the ingested cyst during transit through the acidic environment of the stomach but as soon as the alkaline conditions of the small intestine are reached there is dissolution of the cell wall and excystation rapidly occurs. Generally, the cytosol undergoes fragmentation into as many pieces as there was nuclei in the mature cyst. The resulting organisms are referred to as metacystic trophozoites. These trophozoites do not undergo colonization within the small intestine but, rather, pass into the colon with the fecal stream. If conditions are appropriate, colonization may then take place within the large intestine with growth of the metacystic trophozoites into mature trophozoites. Thus, the cycle is complete and the parasite has established itself in a new host.

With the exception of *Entamoeba histolytica* and, rarely, several other Endamoeba, most species of amoeba are strictly parasitic and produce no symptoms in man. With minor variations they all undergo a cycle similar to that shown diagrammatically in Fig. 3 and so maintain themselves in the host.

Entamoeba coli forms rather large trophozoites 15 to 50μ in diameter. Specimens stained with iron hematoxylin show a spherical nucleus containing peripherally placed, course chromatin granules and a moderately large, eccentrically placed karyosome (Fig. 2). The cysts vary markedly in size and when mature usually contain 8 nuclei. Human infection occurs via ingestion of food contaminated with cysts. The infection rate is high varying from 10 to 50 percent in various parts of the United States and in Europe. However, *Entamoeba coli* is a nonpathogenic, lumen dweller which produces, in most instances, no symptoms. The principal importance of this organism is that it must be differentiated in stool specimens from pathogenic *Entamoeba histolytica*. This can usually be done in terms of a specific morphology of the trophozoites and cysts of these two species. The presence of *Entamoeba coli* is evidence of fecal contamination of food and, therefore, it is entirely possible that *Entamoeba coli* may coexist in the same patient with *Entamoeba histolytica*.

Entamoeba gingivalis is a parasite of the human mouth. It is said to be present in 10 to 50 percent of individuals with good dental hygiene and in as high as 71 to 95 percent of individuals with poor oral hygiene. It is likely to be present in dentures that are incompletely cleaned and in gingival tissue, particularly in the presence of suppuration such as pyorrhea alveolaris. Encystation has not been demonstrated with this species; therefore, it is assumed that transmission is via direct oral contact or by droplet dissemination.

Entamoeba polecki is a common parasite in the large intestine of the pig and monkey. It has a rather distinctive nucleus structure (Fig. 2). While it is considered an uncommon parasite in man, at least 19 cases of human infection have now been reported. The majority of these cases, apparently, are asymptomatic. One well documented case has occurred, however, in a Peace Corps volunteer in which the patient had recurrent bloody diarrhea. It is also significant that most of the antiamoebic drugs are not affective against *Entamoeba polecki* .

Entamoeba moshkovskii is an organism which also presumably parasitizes the human colon. Thusfar, however, it has only been isolated from sewage contaminated with human feces. It very closely resembles *Entamoeba histolytica* and, as will be discussed below, may well represent the infecting organism in patients who are allegedly carriers of asymptomatic *E. histolytica*.

Endolimax nana is another nonpathogenic lumen dweller that infects man by ingestion of contaminated food. It is nearly as common in various human populations as *Entamoeba coli*. Therefore, it is not uncommon to demonstrate *Endolimax nana* in the presence of diarrheal disease. It is doubtful, however, that this organism produces clinically apparent symptoms.

Iodamoeba bütschlii has a small to medium size trophozoite and is easily distinguished on morphological grounds by the distinctive pattern of chromatin in the nucleus (Fig. 2) and by the dense glycogen vacuole in the cytosol that stains intensely with iodine. Again, this organism is principally a non-invading parasite in the large intestine and, principally, in the cecum of man. While this is a common parasite of man, it is identified much less frequently than either *Entamoeba coli* or *Endolimax nana*.

Dientamoeba fragilis is a relatively small organism with active motility.

This organism apparently multiplies at all levels of the cecum and large intestine and is lodged normally in the crypt areas. While this particular amoeba is not capable of tissue invasion, there is some evidence that its presence in the colon may induce low-grade irritation which results in minor clinical symptoms and a low grade, diarrheal syndrome. However, as with the other parasitic amoeba, this organism may be isolated from asymptomatic carriers although in surveys it is usually found much less frequently than *Entamoeba coli* and *Endolimax nana*.

To summarize, as shown in Table 1, apart from disease produced by *Entamoeba histolytica*, the other amoebae produce clinical disease rarely, if at all.

TABLE 1

AMOEBA PRODUCING CLINICAL SYMPTOMS IN MAN

Entamoeba histolytica Group	-	Tissue Invasion
Entamoeba gingivalis	-	Oral Suppuration
Entamoeba polecki	-	Rarely Produces Bloody Diarrhea
Iodamoeba bütschlii	-	Rarely Tissue Invasion with Colitis or Dissemination to Lung or Brain
Dientamoeba fragilis	-	Irritation of Mucosa with Hypermotility, Abdominal Pain, Diarrhea

These include cases of diarrhea or tissue invasion by organisms such as *Entamoeba polecki* , *Iodamoeba bütschlii* and *Dientamoeba fragilis*. Even in these cases, however, (except in those cases where there is clearcut remote tissue invasion) it is very difficult to relate the presence of the organism in the stool to a particular clinical syndrome of diarrhea and bleeding. Since several of these amoebae are common parasites in man it is not unlikely that during an episode of chronic or acute diarrhea of any cause, one may be able to find and identify a specific amoeba in the stool specimen. This problem is illustrated by the data presented in Table 2 in which the incidence of *Entamoeba histolytica* and *Dientamoeba fragilis* was quantified in 1114 patients in a recent study from

TABLE 2

INCIDENCE OF *ENTAMOEBA HISTOLYTICA* AND *DIENTAMOEBA FRAGILIS*

IN 1114 PATIENTS IN TEL-AVIV

	Total Number	EH	DF	Both
Patients with GI Symptoms	1067	135 (13%)	53 (5%)	174 (16%)
Patients without GI Symptoms	47	5 (11%)	3 (6%)	5 (11%)

Tel-Aviv. As is apparent, in patients who manifest GI symptoms such as recurrent abdominal cramps, intermittent diarrhea, constipation or heartburn (??) 13% were found to harbor *Entamoeba histolytica* while 5% had *Dientamoeba fragilis*. On the basis of these findings the authors imply that the symptoms with which the patients presented were due to infestation with either *Entamoeba histolytica* or *Dientamoeba fragilis*. However, in this same study nearly identical percentage isolation rates for *Entamoeba histolytica* and *Dientamoeba fragilis* were found in a group of patients who were completely asymptomatic. Thus, it is difficult to ascertain whether, in fact, the somewhat vague and nonspecific complaints encountered in the first group of patients were due to amoebic infection.

AMOEBA CAPABLE OF TISSUE INVASION

From the foregoing comments it is apparent that there is considerable doubt as to the relationship between the production of clinical disease and infection with a number of parasitic amoebae that are not capable of tissue invasion. In contrast, however, it is clear that those amoebae which possess the unique ability to lyse and destroy host cells are capable of producing significant and, occasionally, fulminating disease. As shown in Table 3 there are three well recognized species of Entamoeba which are capable of destroying host tissue. These include *Entamoeba histolytica* in man, *Entamoeba ranarum* in the frog and *Entamoeba invadens* in reptiles. There is little doubt that in man the vast majority of all clinically significant disease is produced by *Entamoeba histolytica* which has the capacity to destroy host tissue and to invade the wall of the colon as well as more distant organ systems.

TABLE 3

TISSUE INVADING AMOEBA

Species	Host
E. histolytica	Man
E. ranarum	Frog
E. invadens	Reptiles

In recent years, however, it has become evident that the usual morphological criteria for the identification of the trophozoites and, more particularly, the cysts of virulent *Entamoeba histolytica* are totally inadequate. It is now apparent from several lines of evidence that organisms which essentially are indistinguishable from one another on morphological grounds are, in fact, members of different species of amoebae some of which produce disease and some of which do not. For example, as early as 1917 Wenyon and O'Connor reported that there was marked variation in the size of cysts excreted by different carriers of *Entamoeba histolytica*. Subsequently, this observation was repeatedly confirmed. In Fig. 4, for example, the range in the diameters of cysts isolated from 99 individuals harboring *Entamoeba histolytica* is plotted. It is readily apparent that these cysts fall into two statistically separable groups. The smaller group of cysts has a mean diameter of approximately 7μ whereas the larger cysts have a mean diameter of approximately 11μ. On the basis of these and a number of other similar observations it was concluded that *Entamoeba histolytica* could be divided into two types, i.e., a large race and a small race. Small race *Entamoeba histolytica* have now been segregated as a separate species by most investigators and is called *Entamoeba harmanni*. It has further been demonstrated that this small race of amoebae, while commonly infecting man, are of very low virulence or, according to some investigators, produce no clinical disease at all.

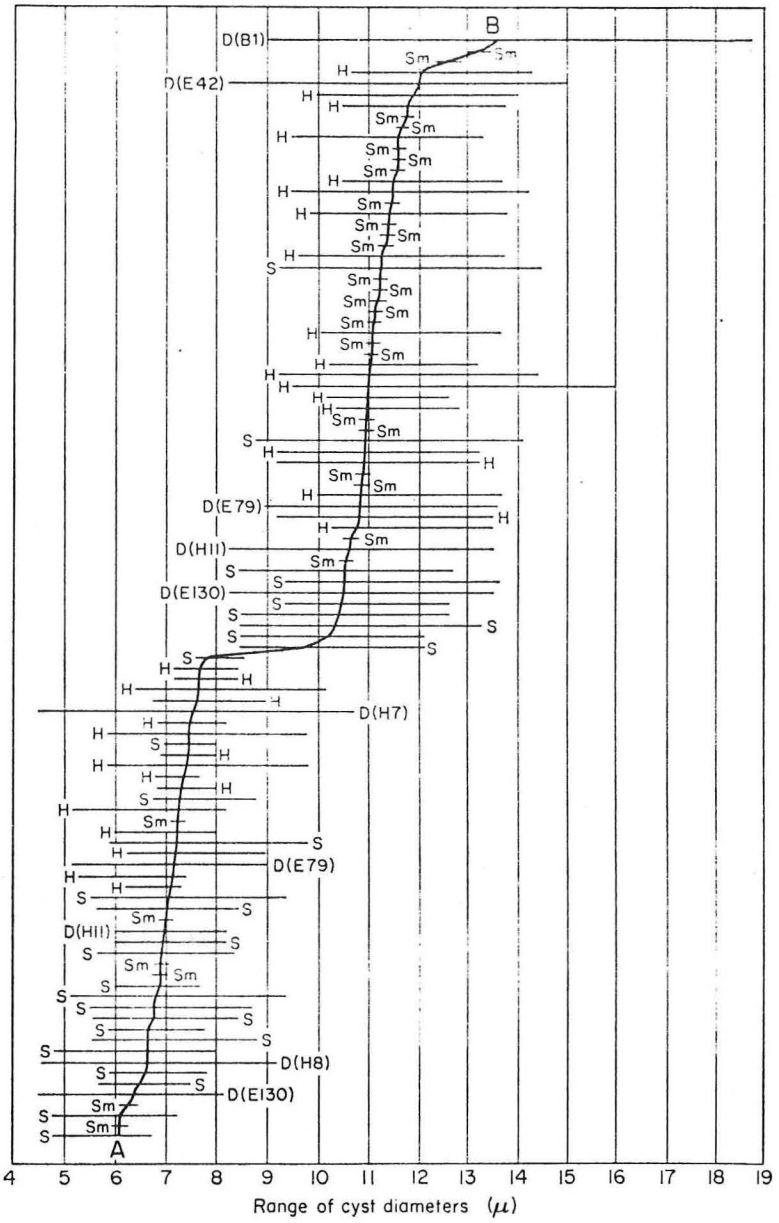


FIG. 4:

The range of cyst diameters isolated from 99 individuals is plotted. It is apparent that these cysts fall into two separate groups on the basis of mean diameters: 9μ separates the small race from the large race cysts.

More recent work has now demonstrated that even the group of amoebae designated as large race *Entamoeba histolytica* is not homogenous. In 1956 Connell reported a strain of *Entamoeba histolytica* which was capable of living and multiplying at temperatures much lower than tolerated by the usual strains of pathogenic *Entamoeba histolytica*. Subsequently, five additional reports appeared confirming the original observations. On the basis of these reports and as shown in detail in Table 4 it is apparent that one can clearly differentiate a species of *Entamoeba histolytica* (Laredo-type, Huff strain, Strain 403, Nelson strain, Messina strain) which is morphologically identical to pathogenic *Entamoeba histolytica* but which has quite different physical, cultural and biochemical characteristics. The Laredo-type can survive temperatures in the range of 0° to 41°C, can grow in culture at temperatures as low as 10°C and has an optimal temperature for growth in the range of 25° to 27°C. These temperature

TABLE 4

COMPARISON OF THE INFECTIVITY AND CULTURE CHARACTERISTICS OF THREE TYPES OF ENTAMOEBA

	Classical Large Population *E. histolytica*	Laredo-type *E. histolytica* (Laredo, Huff, 403, Nelson, Messina)	*E. moshkovskii*
Morphological Characteristics	-	Identical to *E. histolytica*	Identical to *E. histolytica*
Temperature, Survival Range	20-43°C	0-41°C	10-37°C
Temperature, Minimum for Growth in Culture	~30°C	~10°C	~10°C
Temperature, Optimum for Growth in Culture	37°C	25-27°C	? <37°C
Survival in Hypotonic Media	<1:2 dilution	1:64 dilution	1:64 dilution
Antigens	-	Deficient in Some Antigens Carried by Classical *E. histolytica*	Deficient in Some Antigens Carried by Classical *E. histolytica*
Pathogenicity	Produces Tissue Invasion in Man and Test Animals and Cell Death in Tissue Culture	Infects Man but Produces No Disease (see Table 6)	Does Not Infect Variety of Experimental Animals
Drug Sensitivity	-	Consistently More Resistant to Antiamoebic Drugs	?
Biochemical	2 Isoenzymes of Glucokinase	Single Glucokinase on Electrophoresis	Single Glucokinase on Electrophoresis

characteristics are all quite different from those manifest by classical pathogenic *Entamoeba histolytica*. Furthermore, when the Laredo strain is placed in incubation media diluted as much as 1:64 with water the organism will develop

secretory vacuoles and begin active division and growth. In contrast, classical pathogenic *Entamoeba histolytica* will not survive even a 1:2 dilution of the osmolality of the incubation medium. There are also antigenic differences between the Laredo strain and the classic strain of *Entamoeba histolytica*. Of particular importance is the fact that the Laredo strains were, for the most part, isolated from patients who were essentially asymptomatic. Furthermore, it is now evident that this cold resistance strain has very mild virulence when tested either in animals or in man. This latter point is illustrated in detail by the data presented in Table 5. The most important point illustrated in this table is that when the Laredo strain was administered to a 130 human volunteers, 81 volunteers became infected and shed cysts in the stool yet none of these developed symptomatic clinical disease.

TABLE 5

INFECTIVITY AND VIRULENCE OF LAREDO TYPE STRAINS IN EXPERIMENTAL RATS

Reference	Laredo-type Strain Used	Experimental Host	Route of Inoculation[a]	No. Infected/ No. Inoculated	Remarks
Beaver et al (1956a)	"H" = Huff, cultured at 37°C	Rats	IC (t)	10/10	Mild lesions in 4, no lesions in 6 animals
		Guinea-pigs	IC (t)	5/18	Severe lesions in 1, mild in 4 animals
		Dogs	Oral (c)	2/10	Severe lesions in 1, mild in 1 animal
		Humans	Oral (c)	81/130	No symptoms in all 81 infections
Rosas & Najarian (1965)	Laredo, cultured at 22°C and 30°C	Rats Hamsters	IC (t) IH (t)	0/25 0/63	
Healy & Gleason (1966)	Huff and Laredo, cultured at 37°C	Rats	IC (t)	1/21	No lesions in the single infection
Goldman & Cannon (1967)	Laredo, Huff, AG, JA, 403 cultured at 37°C	Guinea-pigs	IC (t)	0/51	
Neal & Johnson (1968)	Laredo, Huff, AG, JA, 403 cultured at 25°C	Rats	IC (t)	11/85	No lesion found in any animal

[a]IC = intracecal; IH = intrahepatic; t = trophozoite; c = cysts.

Earlier in this protocol it was pointed out that one of the parasitic amoebae of minor interest was an organism isolated from sewage and called *Entamoeba moshkovskii*. This Entamoeba has a morphological appearance which is similar to, if not identical with, that of classical *Entamoeba histolytica*. In addition, however, this organism also has the capacity to survive and multiply at low temperatures and to survive in the presence of a markedly hypoosmotic incubation media (Table 4). *Entamoeba moshkovskii* also appears to be nonvirulent in a variety of experimental animals. It is conceivable, therefore, that *Entamoeba moshkovskii* and the Laredo-type organisms are all members of the same group of Entamoeba and further, that this group is distinct from pathogenic *Entamoeba histolytica*.

In summary, Table 6 lists the current subclassification and virulence of the *Entamoeba histolytica* complex of amoebae. It should be emphasized that all of these amoebae are morphologically similar and all have quadrinucleate cysts. *Entamoeba hartmanni* is segregated from the main group primarily on the basis of the smaller mean diameter of the cyst; this organism produces only minor symptoms or, in the opinion of some investigators, no clinical disease at all. A second major category of amoeba can be segregated from the large race group on the basis of their resistance to cold and to hypotonic incubation media; this group also appears to be nonpathogenic. The obvious possibility raised by these observations is that many patients identified as asymptomatic carriers of amoebiasis on the basis of the finding of quadrinucleate cysts in the feces may not be carrying *Entamoeba histolytica* at all; rather, they may be infected with a separate, nonvirulent amoebae analogous to the carrier state for *Entamoeba coli*.

TABLE 6

SUMMARY OF THE CLASSIFICATION AND VIRULENCE OF THE

ENTAMOEBA HISTOLYTICA COMPLEX OF AMOEBAE WITH QUADRINUCLEATE CYSTS

Pathogenic *Entamoeba histolytica*	Large Race *Entamoeba histolytica*	Tissue Invading, Disease Producing
Entamoeba hartmanni	Small Race *Entamoeba histolytica*	Minor Virulence or Nonpathogenic
Cold Resistant *Entamoeba histolytica*	Laredo Strain, Huff Strain Strain 403 Nelson Strain Messina Strain *E. moshkovskii*	Nonpathogenic

In support of this possibility are a number of reports that amoebae isolated from symptomatic patients produce characteristic tissue invasion in a number of animal models whereas organisms isolated from asymptomatic carriers manifest little virulence in these same animals. This observation, of course, could derive from the fact that there is a relatively nonvirulent form of pathogenic *Entomoeba histolytica*. Alternatively, however, it is entirely possible that these carriers were infected with an organism of the Laredo-type which was only mistakenly diagnosed as *Entomoeba histolytica* on the basis of morphology. It

is clear that the laboratory methods for distinguishing these two possibilities are now available so that it is now probably not acceptable to diagnose an asymptomatic patient as a carrier of *Entamoeba histolytica* unless the organisms have been isolated from the stool and shown to possess the characteristics of growth in culture that one would expect for pathogenic *Entamoeba histolytica*.

MECHANISMS OF THE CYTOLYTIC ACTIVITY OF PATHOGENIC *ENTAMOEBA HISTOLYTICA*

There have been several excellent, recent studies into the mechanism of how pathogenic *Entamoeba histolytica* attack and lyse healthy cells and so are able to actively invade tissue. For example, as illustrated diagrammatically in the left panel of Fig. 5, when trophozoites of *E. histolytica* are overlaid on a layer of growing tissue culture cells there is fairly rapid lysis and phagocytosis of

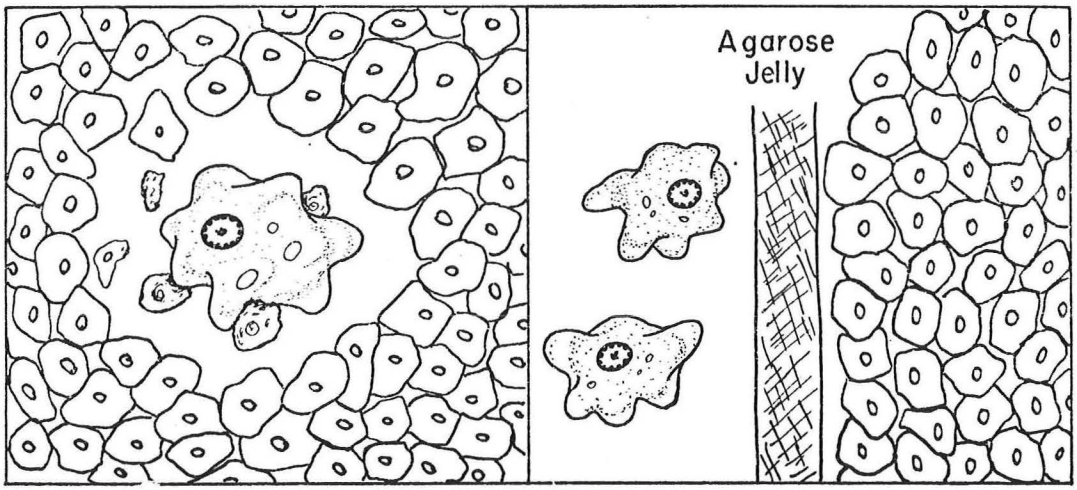


FIG. 5

those cells that are in immediate contact with the plasma membrane of the amoeba. In contrast, those tissue culture cells which are somewhat distant from the amoeba remain healthy and continue to undergo active cell division. This is very reminiscent of the picture seen in human tissues invaded by *Entamoeba histolytica* in that the amoeba commonly is surrounded by a halo of lysed cells while more distant cells manifest little inflammatory reaction and appear to be perfectly intact. Such findings in tissue culture and in the invaded tissue in vivo suggest that cell lysis is not dependent upon the liberation of some soluble toxic product from the amoeba. This conclusion is supported by experiments like that diagramed in the right panel of Fig. 5 in which trophozoites were separated from healthy tissue culture cells by an agarose gel barrier. This agarose barrier was freely permeable to protein molecules with molecular weights as great as 2×10^6, yet there was no evidence of lysis of the tissue culture cells under these experimental conditions.

When this process is studied in detail under phase microscopy, as shown in the left panel of Fig. 6, the first abnormality seen when an amoeba comes in contact with a tissue culture cell is condensation and change in the phase characteristics of the cell membrane that it is in contact with the plasmalemma of the amoeba. Cell organelles such as mitochondria become more prominent at this stage. Shortly thereafter (middle panel) the cell begins to shrink and pulls loose from the surface of the tissue culture plate. Coincident with this, blebs of cytosol are seen being excreted from that portion of the cell

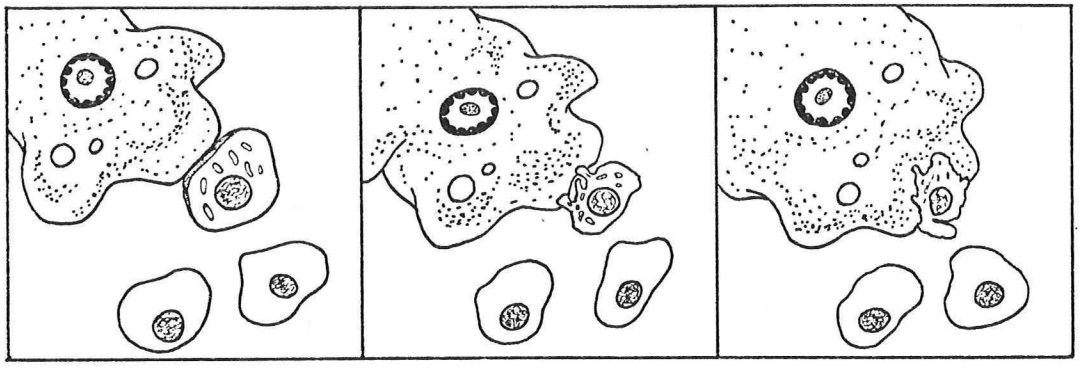


FIG. 6

membrane in contact with the amoeba and the amoeba then begins to engulf portions of the damaged cell piecemeal. The entire cell may be taken up intact or only portions of the cytosol and cell membrane may be engulfed while the nucleus is excreted into the incubation media.

Several recent studies that deal with the ultrastructure of pathogenic *Entamoeba histolytica* have provided a possible explanation for the mechanism of this process of contact lysis. Under electron microscopy trophozoites isolated from patients with active amoebic colitis show a definite "fuzzy" layer approximately 300Å wide surrounding the outer limiting membrane. The cytosol of the pseudopodia is usually less electron-dense than the central portion of the cell and contains few or no vacuoles. In contrast, numerous vacuoles are present in the central portion of the cytoplasm and each of these structures is surrounded by a limiting unit membrane resembling the plasmalemma suggesting that they are derived by phagocytic activity. It should also be pointed out that a fuzzy layer is seen lining the interior surface of the limiting membrane of these vacuoles. The endoplasmic reticulum consists of a number of small tubules and vesicles within the cytosol but no well-defined Golgi complex or mitochondria are seen in *Entamoeba histolytica* . There are, however, glycogen particles scattered throughout the cytosol. Of particular importance is the observation of peculiar structures, illustrated diagrammatically in Fig. 7, lying just beneath the limiting membrane on the periphery of the amoeba. These structures, surface lysosomes, consist of an amorphous background of material surrounded by a limiting membrane. The limiting membrane of the lysosome comes into immediate juxtaposition with the plasmalemma so that the contents of the lysosome are separated from the exterior of the amoeba by only the thickness of the double plasma unit membrane. On the interior margins of the lysosome one commonly sees tubular structures in close relationship to the lysosomes that resemble primitive Golgi tubular complexes. In addition, there commonly are collections of polyribosomes in the cytosol immediately adjacent

Surface lysosome in Entamoeba histolytica

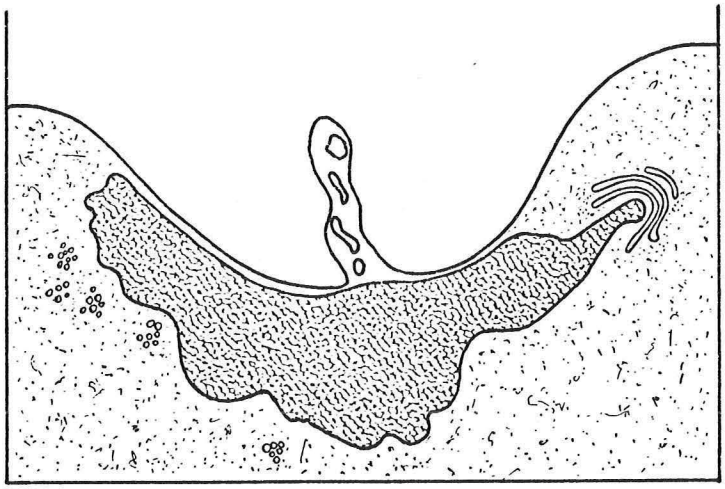


FIG. 7

to the lysosome. These structures suggest active protein synthesis is taking place and histochemical studies have shown the presence of various lytic enzymes in these surface lysosomes. Of particular interest, as also shown in Fig. 7, is the finding of an apparent "trigger" mechanism extending outward from the surface lysosome to the exterior of the amoebic cell. This "trigger" is a complex tubular structure and presumably in some manner results in rupture of the surface lysosome and discharge of the hydrolytic enzymes contained therein when the plasmalemma encounters a target cell. Scanning electron microscopy of *Entamoeba histolytica* reveals that the surface of the amoeba contains a number of these lysosomes that occupy small depressions in the limiting plasma membrane. One can visualize a single "trigger" projecting outward from the middle of each of these depressions.

Thus, as summarized in Table 7 the ability of pathogenic *Entamoeba histolytica* to attack and lyse living cells does not depend upon the liberation of a soluble toxin. The amoeba apparently must come into direct contact with the cell. Presumably this contact triggers the release of hydrolytic enzymes from surface lysosomes which are capable of dissolving the limiting membrane of the target cell. In some manner yet poorly understood this process then allows the amoeba to ingest the cell.

TABLE 7

SUMMARY OF THE CYTOLYTIC ACTIVITY OF *ENTAMOEBA HISTOLYTICA*

1. Cytolytic activity can be exerted in tissue culture in the absence of bacteria.
2. The effect depends upon contact between the amoeba and the target cell.
3. Toxic products are not liberated into the medium.
4. The primary effect is on the cell membrane.
5. The process presumably involves surface lysosomes in *E. histolytica*
6. These structures appear to possess a "trigger" mechanism.

Since pathogenicity appears to relate to the ability of a particular amoeba to invade living tissue it obviously is of great importance to know whether there are demonstrable morphologic differences that would account for the presence or absence of this invasive quality in different species of amoeba. There is a single report which indicates that the fuzzy coat is present on the trophozoites of *Entamoeba histolytica* obtained directly from a symptomatic patient but this fuzzy coat is lacking in *Entamoeba histolytica* maintained in culture. Unfortunately, the cultured amoeba was of the Laredo strain. Thus, it is currently unclear whether the presence or absence of this fuzzy coat is related to pathogenicity, to strain differences or to environmental factors. Furthermore, apparently no comparative studies have yet been made as to the presence or absence of surface lysosomes in pathogenic and nonpathogenic species of amoeba. It is apparent, however, that the biochemical and morphological techniques

currently are available that, in appropriate studies, would probably elucidate the reasons for pathogenicity in certain strains of Entamoeba.

GEOGRAPHICAL DISTRIBUTION OF *ENTAMOEBA HISTOLYTICA* AND OTHER SPECIES OF AMOEBA

There is voluminous literature covering many different human populations on the incidence with which cysts of the various strains of amoeba are found in the feces. These many reports were critically reviewed in 1964 by R. Elsdon-Dew for the World Health Organization of 1964 and are shown in Table 8. The data are reported in terms of the percentage of a particular population that was found to be excreting either the large race or the small race of *Entamoeba histolytica*. As Dr. Elsdon-Dew emphasizes, however, many observers tend to overlook the small race amoeba or to confuse them with *Endolimax nana* so that the percentage figures for this group probably represent underestimates.

TABLE 8

REPORTS OF LARGE AND SMALL *E. HISTOLYTICA* SEEN IN STOOL

Place, Etc.	Percentage	
	Large Race	Small Race
Africa		
Egypt	19.9	30.4
Liberia	37	32
Morocco	14.0	0.4
Bahr el Ghazal	24.1	21.5
Zambia, Africans	31.2	2.2
Italians *ex* East Africa	20	9
Asia		
Turkey	14.0	21.2
Bengal	13.9	27.8
Sarawak	1.6	4.7
Philippines	11.6	16.6
Formosa, U.S. soldiers	5.2	2.8
dependents	2.2	2.2
Leyte	6	10
Thailand, Chinese evacuees	3	14
North America		
New York, T.B.	1.5	1.9
Washington, D.C.	1.8	1.9
Indiana, survey	1.3	2.5
Indiana, mental hospital	17.3	11.8
college students	1.7	3.3
California veterans	2.0	1.6
military	2.9	3.2
Tennessee, Fayette	6.2	6.9
New Hope	17.1	6.2
Georgia (400 examined)	3.0	7.0
(377 examined)	3.7	7.8
Mississippi, Negroes	5.6	5.1

TABLE 8 (cont)

REPORTS OF LARGE AND SMALL *E. HISTOLYTICA* SEEN IN STOOL

Place, Etc.	Percentage	
	Large Race	Small Race
South America		
Argentine, La Plata	0.6	0.4
Australia		
New South Wales, children	0.2	0.5
soldiers	0.7	0.6
Europe		
London, patients (72 examined)	91.7*	22.2*
patients (20 examined)	85 *	35*
Marseilles	13.0	0.5
Bordeaux, children	9.7†	34.5†
Toulouse	8.3	0.5
Bonn 1922	3.9	2.1
Stade 1932	1.8	1.2
Beuthen 1932-35	1.4	0.9
Saar 1943-44	0.9	2.6
1947-48	6.6	1.4
Saar, foreign workers	8.3	4.8
Stade 1932, mental patients	9.5	2.2
Bonn, soldiers, 1944	26.4	30.9
soldiers, 1947	13.5	33.7
Bonn	9.5	12.1
Frankfurt, patients	2.4	0.6
Venice	4.8	3.5
Naples, amoebiasis	62.3*	40.6*
cyst-passers	58.3*	41.6*
Skoplje, children	40.0	12.7
Yugoslavia, children	30.6	0.6
Crimea, G.I. cases	6.0	3.9
children	16.8	12.9

*Presumably percentage of cases with *E. histolytica*.
†11/120 = 9.16%; 41/120 = 34.16%; 42/120 = 35.0%.

In view of the preceding discussion concerning the elucidation of subgroups within the complex of *Entamoeba histolytica* several comments are warranted concerning this type of statistical data. First, the small race *Entamoeba histolytica*, now known as *Entamoeba hartmanni*, probably are not associated with significant clinical disease and, therefore, finding of this organism in stool samples is probably of little more consequence than the finding of other parasitic amoeba such as *Entamoeba coli*. Second, it is completely unclear at the

moment what fraction of those quadrinucleate cysts identified as large race *Entamoeba histolytica* are, in fact, the cysts of pathogenic *Entamoeba histolytica* and what fraction are the cysts of nonvirulent strains such as the Laredo-type. Certainly there are instances in a number of areas in northern Europe and the northern states in America in which there is a significantly high incidence of apparent carriers of *Entamoeba histolytica*, yet clinical amoebiasis is essentially never seen. In view of the recent findings concerning the inadequacy of identification of cysts in the feces on morphologic grounds alone, one must view the incidence figures such as those reported in Table 8 with skepticism.

CLINICALLY APPARENT AMOEBIASIS

Clearly, amoebic dysentery represents one of the major debilitating illnesses in various populations of the world, particularly those in warm climates where sanitary conditions are relatively poor. All epidemiologic data, however, concerning the incidence of amoebic disease is relatively suspect since the data reported to a central agency can be no better than the physicians or parasitologists making the diagnosis at the bedside. With this reservation, the incidences of amoebic colitis reported to the World Health Organization from a sampling of countries is shown in Table 9. In general, the highest number of cases are seen from those countries located in the equatorial zones. Between 1946 and 1956 there was an average annual incidence of 4,135 cases of amoebic

TABLE 9

WHO REPORTS ON AMOEBIASIS 1946-1956

	Amoebiasis		Ratio
	Cases per annum	Case Mortality per 1000	Bacillary/Amoebic
Africa			
Angola	1,781	13.2	0.19
Ethiopa	5,465	2.3	0.79
Kanya	4,138	4.6	1.48
Tanganyika	1,647	11.6	1.70
Uganda	1,482	6.7	1.32
America			
Britsh Guiana	377	46.6	0.30
British Honduras	28	81.1	0.34
Canada	32	--	16.3
Ecuador	1,682	17.0	0.01
Mexico	17,221	25.7	0.12
U.S.A.	4,135	33.5	5.0
Venezuela	10,725	9.3	0.12
Asia			
Hong Kong	191	41.4	1.99
Iraq	14,719	0.6	0.04
Israel	1,614	--	1.70
Japan	410	56.5	217.1
Laos	13,384	3.6	0.01

TABLE 9 (cont)

WHO REPORTS ON AMOEBIASIS 1946-1956

	Amoebiasis		Ratio
	Cases per annum	Case Mortality per 1000	Bacillary/Amoebic
Europe			
Greece	439	0.6	0.66
Italy	300	97.6	0.53
Netherlands	76	15.7	11.82

dysentery reported in the United States. The mortality rate varies rather markedly from country to country, but in the United States is approximately 3.4%. Also shown in this table is the frequency of diagnosis of bacillary dysentery, principally Shigellosis, relative to amoebic dysentery. In general, perusal of these data suggests that in those countries with warmer climates bacillary dysentery and amoebic dysentery occur with relatively similar frequencies. In contrast, in countries farther from the equator such as the United States, Canada, Japan and the Netherlands, bacillary dysentery is reported much more frequently than amoebic dysentery. In the United States the incidence of the disease is more common in the southern, warmer portions of the country than in those states farther north. Clearly, amoebiasis is endemic in the Dallas-Fort Worth area.

THE SPECTRUM OF CLINICAL AMOEBIASIS

While amoebiasis commonly is manifest by a diarrheal syndrome, it is well established that the amoeba may widely involve a number of tissues in the body. In the vast majority of cases, however, dissemination takes place from clinically apparent or inapparent infestation of the gastrointestinal tract. The major migratory pathways for the tissue-invading amoeba are shown in Fig. 8. Very rarely there may be direct infection of the skin or genital mucosa in man. Much more commonly, however, the offending organism colonizes the lumen of the colon: direct contiguous invasion of the colonic mucosa then occurs with the production of superficial ulcers. In unusual cases there may be direct contiguous invasion of surrounding structures. This may occur via direct extension of the colonic infection to the perianal skin and perineal region. In other cases there may be direct invasion and perforation of the colonic wall with extension to the vagina and peritoneal cavity. The organisms metastasize to the liver by hematogenous spread and form micro- or macroamoebic abscesses. Once infection has been well established in the liver there may be widespread dissemination of the disease both by direct contiguous spread and by further metastases via the bloodstream. For example, direct spread by fistula formation may occur from the liver to various hollow organs, major blood vessels, the skin, the retroperitoneal space and kidneys, the pericardium, and the peritoneal and pleural cavities. Such complex fistula formation may, for example, form communications between the hepatobiliary tree and the bronchi. Hematogenous spread from the liver also may occur with the production of isolated amoebic abscesses in the lung parenchyma, brain, spleen and kidneys. The important point to be emphasized concerning this spread of amoebic infection is that

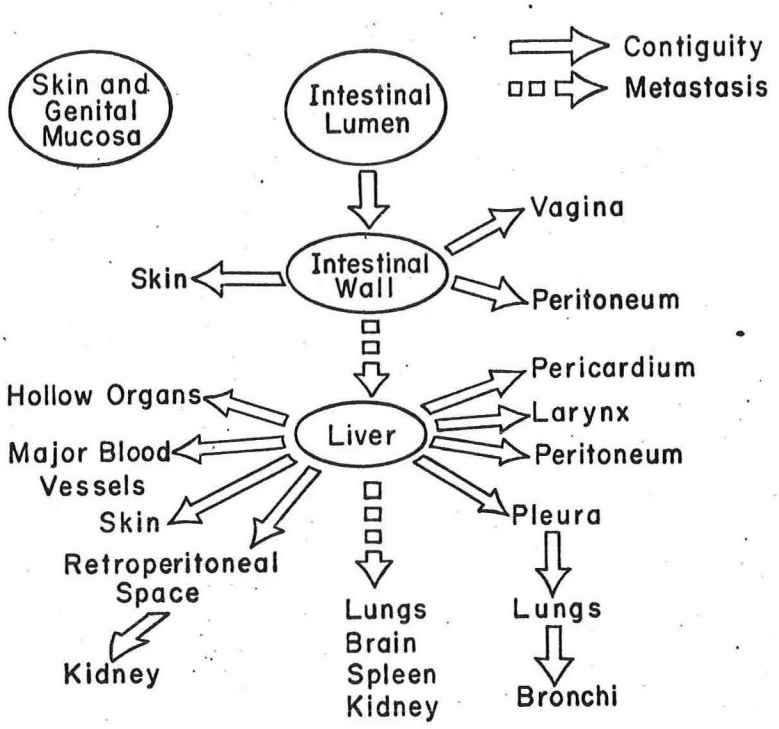


FIG. 8

distal involvement is uncommon in patients who have predominantly intestinal invasion whereas once the infection becomes established in the liver there is the potential for widespread involvement both by contiguous and metastic spread.

AMOEBIC COLITIS

In view of the comments outlined above concerning the inadequacies of morphological criteria for the diagnosis of infection with *Entamoeba histolytica*, it is nevertheless probable that there are relatively nonpathogenic strains of *Entamoeba histolytica* which flourish as non-invading parasites in a number of asymptomatic individuals. Currently, there is no definitive information as to whether such "nonvirulent" behavior is primarily a characteristic of a particular strain of *Entamoeba histolytica* or whether there is some host factor which determines whether tissue invasion and clinical disease will occur. There is a rather voluminous literature dealing with this latter possibility. For example, in certain experimental animals *Entamoeba histolytica* does not become invasive unless certain bacteria such as *Clostridium perfrigens* or *Bacterium subtilis* are also present. In addition, a variety of dietary manipulations in experimental animals and in man have been shown to increase the severity of the infection. Perhaps most intriguing in these latter studies has been the observation that in some experimental settings the amoeba appears to require an environment rich in cholesterol. A low-cholesterol diet inhibits tissue invasiveness while a high-cholesterol diet leads to widespread mucosal destruction.

Whatever the mechanism which triggers tissue invasion it is apparent that this process precedes the onset of clinical symptoms. The earliest lesion

identifiable in the large intestine is usually a tiny, pinhead-size ulcer with hyperemic margins and edema of the surrounding mucosa. This ulcer is very superficial and reaches only down to the muscularis mucosa. Commonly there is a small amount of whitish exudate within the ulcer region. In a smaller number of cases the earliest lesion appears as a more extensive superficial erosion with clearcut margins and irregular shape. Bleeding is uncommon in these early lesions. Later the ulcers enlarge and form more typical "buttonhole" lesions up to 1 cm in diameter with excavation of the ulcer base and irregular, overhanging margins. A "wet preparation" made from the discharge of these ulcers is commonly rich in living trophozoites. Histological sections taken of the margins of such ulcers also commonly show organisms invading the submucosal tissue. The polymorphonuclear leukocytic response to tissue invasion by the amoeba is relatively less intense than to bacterial invasion so that pus cells are relatively few in number in tissue sections and in these mucoid secretions from the ulcer region. In a number of cases the intact mucosa between ulcers is edematous but otherwise relatively normal. However, it should be emphasized that in many cases the mucosa may be diffusely involved and appear as an erythematous, granular, fragile mucosa which, for practical purposes, is indistinguishable from other forms of colitis such as acute Shigellosis or idiopathic ulcerative colitis.

Uncommonly, amoebic infection of the colon takes the histological form of an "amoeboma". This is usually present as a single lesion in which simultaneously there is destructive changes produced by the amoeba, inflammation and granulation tissue overgrowth but little if any fibrosis. Grossly the mucosal surface is ulcerated and covered with a greyish, nodular exudate. The wall of the intestine is very much thickened and is firm, inelastic and irregular. While most amoeboma occur as a solitary lesion found predominantly in the cecum or rectum, occasionally these lesions may be multiple.

The localization of amoebic ulceration in a total of 1,251 cases is shown by the data presented in Table 10. In 24 percent of these cases the entire colon was involved in ulcerating lesions. In the more localized form the principle sites of involvement were the cecum, ascending colon and sigmoid colon. It should be emphasized, however, that the data presented in Table 10 come from six separate series of autopsy cases. Obviously, these figures are therefore somewhat distorted since these patients all had overwhelming disease which proved fatal.

TABLE 10

LOCALIZATION OF INTESTINAL AMOEBIASIS

IN 6 SERIES OF AUTOPSIED CASES

Cecum	299 (24%)
Ascending colon	165 (13%)
Transverse colon	87 (7%)
Descending colon	98 (8%)
Sigmoid	155 (12%)
Rectum	25 (2%)
Appendix	103 (8%)
Ileum	20 (2%)
Whole colon	299 (24%)
Total	1,251

Clinical presentation of patients with amoebic dysentery or colitis varies enormously from patients with very mild or essentially no symptoms to patients who present with fulminating disease and overwhelming toxicity. Table 11 represents an attempt to classify amoebic invasion of the colon according to the

TABLE 11

CLINICAL SPECTRUM OF AMOEBIC COLITIS

	Distribution	Complications	Frequency
Acute Amoebic Colitis			
Mild	Rectal and Recto-sigmoid: Cecal Involvement not Clinically Approved	Rare, Occasional Amoeboma	Common
Severe	Often Diffuse with Right-sided Involvement	Local Perforation, Abscess, Occasional Amoeboma	Less common
Fulminating	Usually diffuse with Right-sided Involvement Including Terminal Ileum	Ileus, Mucosal Sloughing, Toxic Megacolon, Hemorrhage, Perforation	Infrequent
Chronic Amoebic Colitis			
Continuous or Intermittent	Usually Recto-sigmoid, may be diffuse	Debility, Protein Loss, Anemia	Infrequent
Amoeboma	Localized, usually Cecum or Recto-sigmoid	Confused with Carcinoma	Infrequent

the severity of clinical symptoms. Acute amoebic colitis usually begins abruptly and symptoms may be present for only a few days or few weeks at most. In patients with very mild symptoms there is usually involvement of the rectal or rectosigmoid areas but cecal involvement is not clinically apparent. Complications are rare although an occasional amoeboma is identified. This form of amoebic colitis is probably the most commonly encountered clinical syndrome. In more severe disease patients often present with diffuse colonic involvement. There may be marked fluid loss and ECF depletion, grossly bloody diarrhea with rare episodes of massive bleeding and occasional local complications such as colonic perforation or formation of amoeboma. In its most fulminating form diffuse amoebic colitis may present with the clinical picture of "toxic megacolon" which is indistinguishable from that similar syndrome seen in idiopathic ulcerative colitis.

Amoebic colitis also may present with a more chronic course in which there is a low grade, continuous or intermittent diarrhea, commonly without blood,

that occurs over many months or years. This type of chronic amoebic colitis may be localized or diffuse in its distribution in the colon and may lead to weight loss, general debility, protein-losing enteropathy and persistent iron deficiency anemia. If an amoeboma is present, the mass lesion in the cecum or rectosigmoid may be mistakenly diagnosed as carcinoma.

As will be discussed later in the section on the differential diagnosis of acute colitis, it is very often impossible on either clinical or sigmoidoscopic grounds to differentiate amoebic colitis from idiopathic ulcerative colitis. This is emphasized by the data presented in Table 12 in which similar findings appeared in patients with either disease. The critical differential point, therefore, becomes the direct demonstration of the invading organisms in colonic secretions or, alternatively, the demonstration of tissue invasion by appropriate serologic tests.

TABLE 12

SYMPTOMS OF 50 PATIENTS WITH AMOEBIASIS

AND 50 PATIENTS WITH NONSPECIFIC ULCERATIVE COLITIS

Amoebiasis		Nonspecific Ulcerative Colitis
28	Abdominal pain	38
45	Diarrhea	45
50	Blood and mucus	48
35	Tenesmus	33
22	Weight loss	38
6	Fever	18

LOCAL COMPLICATIONS FROM AMOEBIC COLITIS

The local complications that occur as a direct consequence of amoebic colitis fall into four categories: 1) acute perforation of the colon with peritonitis, 2) acute amoebic appendicitis, 3) amoebiasis cutis and 4) amoebiasis of the female genital tract.

One of the most dreaded complications of acute amoebic colitis is free perforation of the colon with subsequent development of peritonitis. This complication in most series carries a mortality rate in excess of 50% and in many instances equals 80 to 100%. As shown in Table 13, in two autopsy series of cases dieing of amoebiasis, perforation of the colon was the principle cause of death in 10 to 30% of the cases. The sites of perforation are most commonly in the cecum or sigmoid colon. This, presumably, occurs since these appear to be the major two anatomical sites for the invasion and proliferation of *Entamoeba histolytica*. Multiple perforations are also apparently very common: in one series, for example, in 6 of 8 patients there were two or more perforations. The incidence of colonic perforation varies markedly in different series: for example, values as low as

TABLE 13

PERFORATION AS A CAUSE OF

FATAL AMOEBIASIS

Total Autopsies	% Perforation of Colon
186	10.7
148	30.4

1.5% and as high as 21% have been reported in different groups of patients that manifest primarily colonic amoebiasis. A comparison is shown in Table 14 of the symptoms and findings present in patients with perforation as opposed to a control group of 100 cases of amoebic dysentery without colonic perforation. As

TABLE 14

SIGNS AND SYMPTOMS IN CASES OF AMOEBIC PERITONITIS

WHERE COLON ALONE WAS INVOLVED

Signs and Symptoms of Amoebic Peritonitis	With Perforation %	Control Group of 100 Cases of Amoebic Dysentery %
Dysentery	88.8	100
Abdominal distension	88.8	0
Diarrhea during hospital stay	88.8	100
Generalized abdominal pain	55	2
Fever	55	18
Ascites	55	0
Vomiting	44	15
Altered mental state	44	0
Generalized abdominal tenderness	33	8
Enlarged liver	33	13
Generalized rigidity or guarding	22	0
Local tenderness in one or both ileal fossa	22	30
Jaundice	11	2
Local abdominal pain in one or both ileal fossa	11	20

is apparent in this table, at the time of diagnosis most patients with colonic perforation had overt dysentery and marked abdominal distension. In contrast, no significant distension was noted in the 100 cases of simple amoebic colitis. In addition, the group with perforation manifested generalized abdominal pain, fever, ascites and vomiting. Generalized rigidity or guarding and localized tenderness was seen in only approximately one-fifth of the cases. Diagnosis of perforation secondary to amoebic colitis was often delayed because the patients commonly were considered to have some other lesion such as acute appendicitis, acute diverticulitis, intestinal obstruction, colonic carcinoma, etc. In the majority of cases a careful history will reveal the presence of an acute or chronic diarrheal syndrome preceding perforation: in addition, in the majority of cases pus obtained from the abdominal cavity by aspiration or exploration will reveal the presence of active trophozoites. If free perforation is suspected, surgical exploration is indicated. In the vast majority of cases, however, even with exploration the mortality rate is extremely high. This appears to relate to at least four factors: 1) there often is a delay in making the proper diagnosis since perforated amoebic colitis is seldom considered as the initial diagnosis. Appropriate drug therapy is therefore delayed. 2) The perforation most commonly occurs into the free peritoneal cavity rather than into the retroperitoneal space. 3) Characteristically there are multiple sites of perforation which make surgical correction difficult and hazardous. 4) The majority of patients with perforation are either very young or rather old and debilitated.

A small number of patients with amoebic infection may present with the clinical picture of acute appendicitis. Such amoebic appendicitis commonly ruptures and may be one of the specific causes for amoebic peritonitis. The preoperative diagnosis may be impossible since there are no distinctive clinical or laboratory features that would differentiate between amoebic and bacterial appendicitis. However, a preceding history of diarrhea should certainly alert the clinician to this possibility, and a detailed examination of stool specimens for amoeba should be undertaken. At laparotomy the appendix involved with amoebiasis is usually distended, and there is usually a marked periappendicitis with gross abscess formation. The wall of the cecum is commonly thickened and edematous. Since the cecum is grossly involved in the disease process, appendectomy is hazardous and may be complicated by formation of a fecal fistula, cutaneous amoebiasis or free perforation in the postoperative period. It is mandatory that the nature of the process be recognized at laparotomy so that the patient may be put on appropriate antiamoebic therapy in the postoperative period.

Amoebiasis cutis commonly results from direct extension of the infectious process to an adjacent skin surface. This dramatic but rare complication usually occurs in patients having severe dysentery. An ulcer appears in the perianal and perineal region with an irregular margin and base. Commonly, the ulcer is very painful. The lesion extends outward from the perianal region to the external genitalia. Amoebiasis cutis may also arise at the site of any operative or spontaneous fistula tract that is draining some portion of the abdominal viscera infected with *Entamoeba histolytica*. Again the lesion appears as a small, irregular, necrotic ulcer which rapidly spreads to involve large portions of the skin.

Amoebiasis of the female genital tract may be secondary to amoebiasis cutis which extends anteriorally to involve the vulva and vagina. However, several other cases also are reported in the literature in which the patients present with either a foul vaginal discharge or vaginal mass and are found to have either an ulcerated cervix and chronic cervitis or an actual fungating mass growing from the cervix. In most instances these lesions are initially misdiagnosed as carcinoma. However, appropriate tissue examination reveals trophozoites of *Entamoeba histolytica* invading the tissue, and the lesions respond promptly to antiamoebic therapy.

DIAGNOSTIC PROCEDURES USEFUL IN AMOEBIC COLITIS

Isolation and Identification of *Entamoeba histolytica*. In the patient with an acute colitis syndrome the most definitive diagnostic procedure is the isolation and identification of the active trophozoite from the stool or from colonic secretions. It should be remembered (Fig. 3) that the process of encestation depends upon the type of stool which is passed. As outlined in Table 15 in the presence of a dysentery syndrome when frank, liquid diarrheal bowel movements are being passed, usually only active trophozoites will be present. In less severe disease when the patient is passing semi-formed stool, one would expect to find the whole spectrum of forms including active trophozoites as well as uninucleate, dinucleate and quadrinucleate cysts. In patients who are essentially asymptomatic and passing formed stools only the mature quadrinucleate cysts will be found. In addition to examination of stool, even higher yields will be obtained by warm-stage, wet-prep examination of mucopurulent material or scrapings obtained directly from the infected colon at the time of

TABLE 15

FORMS OF *E. histolytica* IN STOOL

Liquid, Frank Diarrhea	-	Trophozoites
Semiformed Stool	-	Trophozoites Precysts, Uninucleate, Dinucleate and Quadrinucleate Cysts
Formed Stool	-	Mature Quadrinucleate Cysts

sigmoidoscopy. Identification depends upon finding the typical large trophozoites manifesting active motility and erythrophagocytosis. Staining will reveal the typical distribution of nuclear chromatin (Fig. 2). The cysts may contain from 1 to 4 nuclei depending upon the stage of encestation and, again, the stained preparations will show a very characteristic chromatin pattern. The typical characteristics of the trophozoite and cyst of *Entamoeba histolytica* given in Table 16. Obviously, it is of critical importance that *Entamoeba histolytica* be differentiated from the other common parasitic forms such as *Entamoeba coli*, *Endolimax nana*, *Iodamoeba bütschlii* and *Dientamoeba fragilis*.

TABLE 16

Entamoeba histolytica

Trophozoite Stage: Unstained

Size in microns	10 to 60μ
Motility	Active; progressive and directional
Pseudopodia	Finger shaped; hyaline and glass-like; rapidly extruded
Inclusions	Red blood corpuscles; no bacteria in fresh specimens
Nucleus	Invisible usually

Trophozoite Stage: Iron Hematoxylin Stain

Nuclear membrane	Delicate; inner surface has single layer of minute chromatin dots
Karyosome	Minute and in center of nucleus
Intranuclear chromatin	No chromatin between karyosome and nuclear membrane
Inclusions	Red blood corpuscles; no bacteria unless degenerated

TABLE 16 (cont)

Entamoeba histolytica

Cystic Stage: Iodine Smear Preparation

Size in microns	3.5 to 20μ
Shape	Usually spherical
Cytoplasm	Bright greenish-yellow
Glycogen mass	Diffuse and reddish-brown
Nuclei	1 to 4; minute central karyosome very refractive; nuclear membrane beaded and refractive

Although currently not being done at Parkland Memorial Hospital, it is possible to isolate and culture *Entamoeba histolytica*. As shown in Table 17 the yield of positive cultures depends, to some extent, on the method used to isolate the original inoculum. For example, in Table 17 positive cultures were obtained in only 16 to 39% of cases in which a spontaneously passed stool was used as the inoculum. Purged stools yielded a positive result in 44 to 78% of the cases while the highest rate of positive cultures was obtained by inoculating rectal scrapings.

TABLE 17

THE CORRELATION BETWEEN TYPE OF INOCULUM AND THE YIELD OF

ENTAMOEBA HISTOLYTICA (EH) IN CULTURE

Source of Culture Material	No. of Samples	Positive Spontaneous	EH Purged	Culture from Rectal Scrapings
Spontaneous Stool + Purged Stool	95	37 (39%)	74 (77.9%)	-
Spontaneous Stool + Rectal Scrapings	23	11 (48%)	-	18 (78.3%)
Purged Stool and Rectal Scrapings	31	-	17 (55.0%	23 (74.2%)
Spontaneous + Purged Stool and Rectal Scrapings	126	20 (16%)	56 (44.4%)	65 (51.6%)

Serologic Diagnosis of Amoebic Colitis. There are a number of different serological methods for the establishment of clinical amoebic infection. The three principle tests include indirect hemagglutination (IHA), complement

fixation (CF) and agar gel diffusion (Gel). Table 18 gives the reported ranges of values found in different types of disease and reported in a number of different reviews. In addition, the results obtained in a large and carefully performed study by Juniper et al also is shown for each group of patients. Several points concerning these data warrant emphasis. 1) In the Juniper series, in patients with no clinical symptoms approximately 1.1% of the population had a

TABLE 18

SEROLOGICAL TESTING FOR AMOEBIASIS

Disease	Indirect Hemagglutination IHA		Complement Fixation DF		Agar Gel Gel	
	Reported Range	Mean Juniper, et al	Reported Range	Mean Juniper, et al	Reported Range	Mean Juniper, et al
	%	%	%	%	%	%
No Amoebiasis	0.6-18	1.1	0-16	-	0-18	-
Invasive Colon	33-100	95	67-90	85	81-95	86
Symptomatic Colon	19-90	61	50-90	56	63-85	54
Asymptomatic Colon	0-66	58	11-87	58	1-51	52
Liver	47-100	83	83-100	83	80-100	83

positive IHA test. It is not clear that these patient represent false positives since old or inapparent amoebiasis may elevate the indirect hemagglutination test, and this elevation may persist for several years. 2) There is generally good correlation between the percent of cases that have a positive reaction and the severity of the disease present. Thus, with either hepatic amoebiasis or severe invasive amoebic dysentery the various serologic tests are positive in from 83 to 100% of the cases. The percentage of positive results is less in patients with milder symptomatic and asymptomatic colon disease. 3) For any type of clinical disease the indirect hemagglutination reaction generally has the highest rate of positivity. As shown in Fig. 9 there is general correlation between the titer obtained with the indirect hemagglutination test and the type of clinical disease that is present in a given patient population. In clinically mild colitis many patients manifest a relatively low titer while in more serious invasive colonic disease and in hepatic amoebiasis much higher titers are commonly encountered. Another point worthy of emphasis concerning these serologic tests is that once they become positive they may remain elevated for a number of months or years after succeesful treatment of acute amoebic infection. This is illustrated by the data shown in Fig. 10. In this diagram the titers obtained with the three major serologic tests at different periods of time after treatment of the primary amoebic infection have been averaged: it is apparent that significant elevations, at least in some patients, persist for greater than two years after therapy. Thus, in population surveys of asymptomatic patients

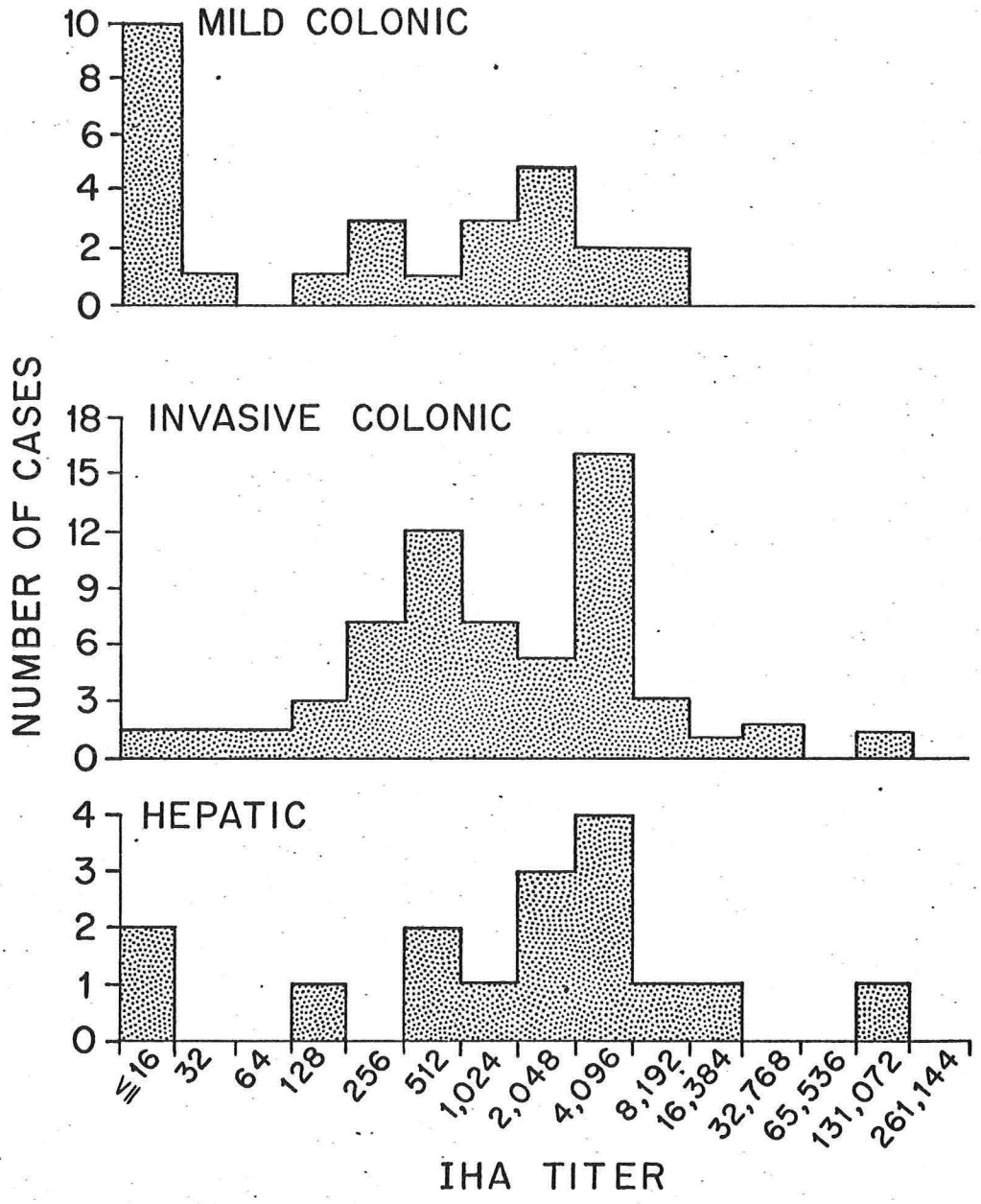

MILD COLONIC
INVASIVE COLONIC
HEPATIC
NUMBER OF CASES
IHA TITER
≤16
32
64
128
256
512
1,024
2,048
4,096
8,192
16,384
32,768
65,536
131,072
261,144

FIG. 9

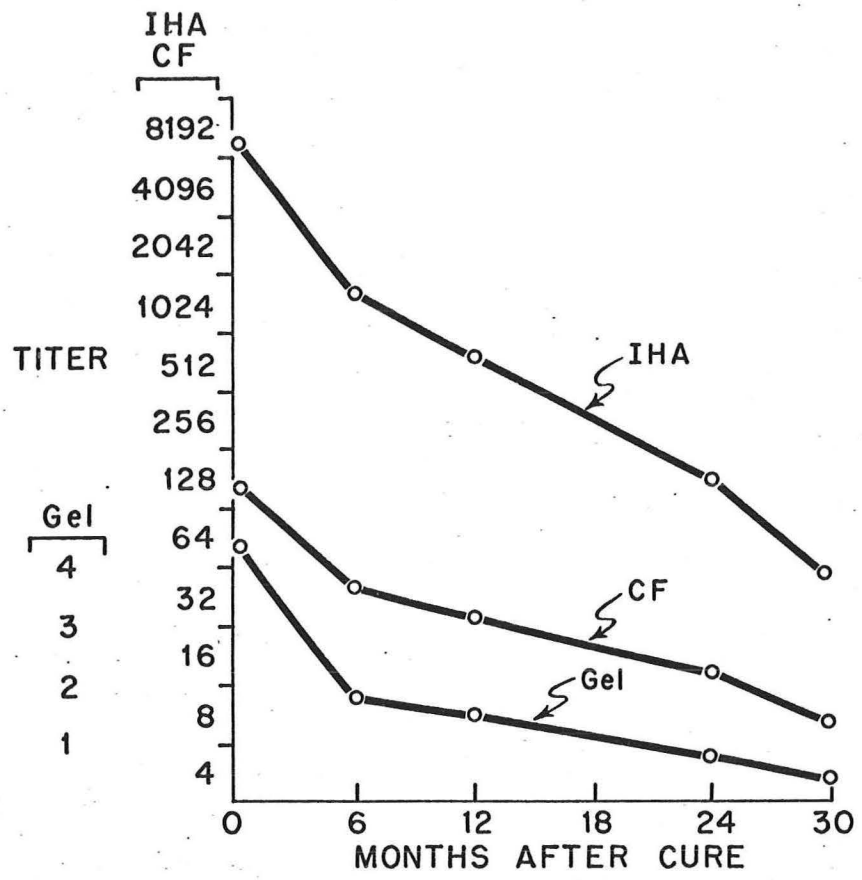


FIG. 10

it is entirely possible that some positive results reflect persistent elevations of the antibodies to amoebic antigens long after a symptomatic or asymptomatic infection has been overcome.

Proctoscopic Appearance and Biopsy. Classically, the proctoscopic appearance in patients with amoebic colitis is said to show relatively normal mucosa with small, discreet ulcers scattered irregularly over the colonic surface. It now seems very likely that this "classical" picture is fairly uncommonly seen. Rather, as outlined briefly in Table 19 amoebic colitis may present with a highly varied proctoscopic appearance. For example, in these 91 cases of proven amoebic colitis 19% manifest mucosal edema with mild friability, increased mucus production and very subtle pin-point depressions scattered over the colonic mucosa. The most common abnormality (52%) consisted of a generalized abnormality of the mucosa with marked edema, marked friability and hyperemia, superficial erosion-, pin-point hemorrhages and irregular, raised lobules with anastomosing clefts forming a reticular pattern. Twenty-two percent of the patients had irregular discreet ulcers varying in size from 2 mm to 30 mm with necrotic bases. Seven percent of the patients had ulcers of similar size but in this group the bases of the ulcers were filled with red granular tissue that bled easily when traumatized. Rarely, the entire mucosal surface was covered with a whitish pseudomembrane that occasionally was sloughed as a "mucosal

TABLE 19

PROCTOSCOPIC APPEARANCE IN AMOEBIC COLITIS (91 PATIENTS)

19% Mild Changes	Folds edematous with mild friability; increased mucus; pin-point depressions
52% Lobular Changes	Mucosa diffusely edematous, very friable and hyperemic; superficial erosions; pin-point hemorrhages; irregular, raised lobules with anastomosing clefts forming a reticular pattern
22% Necrotic Ulcers	2 mm to 30 mm irregular ulcers with necrotic bases; occasionally a large single irregular ulcer near anorectal junction
7% Open Ulcers	Open ulcers with clean, granular bases which bleed easily
2% Mucosal Cast	Entire mucosa covered with a whitish pseudomembrane

cast". Similarly, as shown in Table 20, there was marked variation in the histopathology of biopsies taken from these same patients. The "classic" picture of "flask" shaped ulcers with overhanging mucosal tissue was only seen in that group of patients who grossly manifested necrotic ulcers. More commonly those patients who grossly had mild or lobular changes at sigmoidoscopy or who

TABLE 20

BIOPSY HISTOPATHOLOGY

Gross Change	No. Biopsies	Amoeba Seen	Histopathology					
			Nonspecific	Mucopenic	Early Invasive	Late Invasive	Granulation Tissue	Acellular Debris
Mild	7	3	4	3	-	-	-	-
Lobular	26	13	12	12	3	-	-	-
Necrotic Ulcer	19	16	4	1	4	9	1	-
Open Ulcer	2	0	-	-				
Cast	1	0	-	-	-	-	-	1
All Types	55	32 (58%)	20 (36%)	16 (29%)	7 (11%)	9 (16%)	3 (5%)	1 (2%)

manifest open, non-necrotic ulcers or casts had relatively nonspecific histopathology. It should be emphasized, however, that in all of these groups

combined,amoebae were identified in the biopsy specimens in 58% of the cases. These studies emphasize that the appearance of the rectal mucosa at sigmoidoscopy and the histopathology of rectal biopsies is so variable and nonspecific as not to allow differentiation of amoebic colitis from other forms of specific and nonspecific colitis. Only the findings of *Entamoeba histolytica* trophozoites in the rectal secretions or invading the tissue in the biopsy specimens provides a definitive diagnosis.

DIFFERENTIAL APPROACH TO THE DIAGNOSIS OF AMOEBIC COLITIS

In the setting of the patient population seen at Parkland Memorial Hospital it is now apparent that the clinical picture of acute or chronic "colitis" with frequent liquid bowel movements and mucopurulent discharge with or without gross bleeding can be seen with five specific disease entities all of which occur with approximately equal frequencies. As shown in Table 21 this differential diagnosis includes idiopathic ulcerative colitis, idiopathic Crohn's disease involving the colon, Shigellosis, amoebic colitis and colitis induced by the antibiotics

TABLE 21

DIFFERENTIAL DIAGNOSIS OF "COLITIS"

1) Idiopathic Ulcerative Colitis

2) Idiopathic Crohn's Disease of Colon

3) Shigellosis

4) Amoebiasis

5) Antibiotic Induced

clindamycin and lincocin. Since the therapy and prognosis in each of these diseases is quite different it becomes of critical importance that a vigorous diagnostic workup be initiated immediately in each colitis patient in order to differentiate these five different diseases. Much less commonly, other illnesses such as vascular insufficiency to the colon and infiltrating malignancy may mimic these diseases but, for practical purposes, these possibilities can be ignored in the initial workup.

The specific procedures that should be undertaken in such patients are outlined in Table 22. All patients should be immediately sigmoidoscoped. This should be undertaken without any kind of preparation. The purposes of sigmoidoscopy are 4-fold. 1) It is first necessary to establish the diagnosis of "colitis" by evaluating the subjective appearance of the colonic mucosa, i.e., the presence of erythema, ulceration, exudation, friability, etc. 2) If the colonic mucosa appears relatively normal then a mucosal smear should be done to assess the presence or absence of polymorphonuclear leukocytes. The ability to express pus cells from the colonic mucosa is prima facie evidence of the existence of "colitis" regardless of the subjective appearance of the colonic mucosa. 3) Culture can be obtained at this time for pathogenic bacteria, i.e., shigella

and salmonella. 4) Mucopurulent material or scrapings can be obtained directly from the ulcerated mucosa for a warm-stage, wet-prep for amoebic trophozoites. If the diagnosis of amoebic colitis is not definitively made at sigmoidoscopy then barium enema with ileal spill commonly is the next indicated procedure, provided the patient is not seriously ill and toxic. The barium enema will allow the physician to evaluate the extent and anatomical distribution of the ulcerating disease in the colon and small bowel. If the diagnosis still remains uncertain then a small bowel series for evaluation of possible Crohn's disease of the small intestine and repeat sigmoidoscopy for biopsy of the rectal mucosa are indicated.

TABLE 22

DIAGNOSTIC PROCEDURES IN PATIENT WITH COLITIS

	Reason	Conditions
1) Sigmoidoscopy	Establish Diagnosis of Colitis a) Mucosal Appearance b) Mucosal Smear c) Culture for Pathogenic Bacteria d) Mucosal Scrapings for Amoeba	Do Not Prep Do Not Biopsy
2) Barium Enema with Ileal Spill	Delineate Distribution of Disease	Do Not Do In Seriously Ill Patient
3) Small Bowel Series	Look for Small Bowel Lesions	Do Not Do In Seriously Ill Patient
4) Biopsy of Mucosa	Look for Compatible Pathology Look for Granuloma Look for Amoeba	

The findings of major differential importance are outlined in Table 23. Generally, Shigellosis is an acute colitis which is self-limited so that the clinical course is usually less than one week. Furthermore, the patient can usually be safely maintained with appropriate attention to fluid and electrolyte balance for 24 hours until cultures establish the diagnosis of acute Shigella colitis. For practical purposes, however, if a patient presents with a history of continuous or intermittent diarrhea for a number of weeks or months then Shigellosis can be excluded as a likely diagnosis. Acute or chronic colitis due to clindamycin or lincocin can mimic in every way acute idiopathic ulcerative colitis. In this disease, however, at sigmoidoscopy one occasionally sees pearly, greyish patches scattered irregularly over the colonic mucosa. On histologic section these rather unique patches prove to be pseudomembranes; thus, the finding of such lesions at sigmoidoscopy should certainly arouse suspicion that one is dealing with an antibiotic-induced acute colitis and every effort should be made to elicit a history of antibiotic administration. This

TABLE 23

DIFFERENTIAL POINTS

Disease	Weak	Strong
1) Shigellosis		1) Length of Symptoms 2) Culture of Shigella
2) Antibiotic Colitis	1) Isolated Yellowish Gray Patches - Pseudomembranes	1) History of Antibiotic Administration
3) Amoebic Colitis	1) Isolated Ulcers 2) Few WBC's 3) Distribution of Disease	1) Identification of Amoeba 2) Positive Serology 3) Response to Specific Therapy
4) Idiopathic Colitis	1) Distribution of Disease	1) Exclusion of Other Forms of Colitis 2) Small Bowel Lesions in Crohn's Disease

form of acute colitis responds dramatically to the administration of steroids and rarely, if left untreated, may be fatal. The definitive diagnosis of amoebic colitis depends upon a) the identification of trophozoites of *Entamoeba histolytica* in the mucosal secretions, b) the presence of a diagnostically high serologic test or c) the prompt response to specific antiamoebic therapy. It should be emphasized that amoebic colitis can mimic in every way the proctoscopic and radiographic appearance of ulcerative colitis. Finally, idiopathic colitis, either ulcerative colitis or Crohn's disease involving the colon, basically are diagnoses of exclusion. The finding of typical small bowel lesions or of non-caseating granuloma in the rectal biopsies would strongly support the diagnosis of Crohn's disease but all other proctoscopic, radiographic and histopathologic findings may be seen in Shigellosis, antibiotic-induced colitis or amoebic colitis. It seems reasonable, therefore, that in the presence of a suspicious history all patients should either be treated for amoebiasis or, alternatively, serologic examination should be undertaken, before a final diagnosis of either idiopathic ulcerative colitis or Crohn's disease of the colon is assigned to the patient.

Finally, it should also be recognized that the clinical picture of "acute toxic megacolon" commonly associated with severe idiopathic ulcerative colitis can also be seen in antibiotic-induced colitis and in fulminating amoebic colitis. Therefore, if high-dose steroid therapy is to be initiated and if there is a reasonable possibility that one is dealing with amoebic rather than ulcerative colitis, then antiamoebic therapy also should be begun.

DRUG THERAPY OF AMOEBIC COLITIS

The therapy of amoebic colitis is outlined in Table 24. It is now clear from the literature that metronidazole (Flagyl®) is the drug of choice in treating both the luminal and tissue phases of amoebic disease. It is affective in both the asymptomatic carrier as well as in the patient with mild to severe

amoebic colitis. In the majority of cases the symptomatic patient becomes dramatically better within 24 to 36 hours of the administration of the drug and viable trophozoites disappear from the stool within this same period of time.

TABLE 24

THERAPY OF AMOEBIC COLITIS

Asymptomatic Carrier	-	Metronidazole (Flagyl®) Adults: 750 mg tid for 10 days Children: 40 to 50 mg/kg per day for 10 days
Symptomatic Carrier	-	A) Metronidazole (Flagyl®) 750 mg tid for 10 days b) Combination: Tetracycline: 250 mg q 6 h for 10 days Diloxanide furoate: 500 mg tid for 10 days Chloroquine: 150 mg bid for 14 days
Fulminating Colitis	-	Emetine HCl: 65 mg IM every day until can take oral medications Tetracycline: 250 mg IV q 6 h until oral medication can be taken Metronidazole given as soon as feasible
Post-colectomy for perforation	-	Same as above

Unfortunately, at present the drug must be taken orally so that in fulminating colitis due to amoebiasis or after emergency colectomy for perforation of the colon due to amoebic colitis metronidazole cannot be utilized. In this clinical setting it is probably best to administer emetine and tetracycline in combination until the patient is able to take oral medications; then metronidazole should be added to the regime.

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