CLINICAL SEMINAR

Amebiasis

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William A. Sodeman, Jr, MD

This discussion concerns amebiasis in the temperate portion of the United States. There is really no substantial qualitative difference between the behavior of the ameba in the tropics as compared with the behavior of amebae in temperate climates. There is a quantitative difference in the expression of the various syndromes in these two environments. Almost every carefully performed epidemiologic survey in the United States shows an incidence of *Entameba histolytica* infection which exceeds 2% (1). There is an additional 1–2% infection with *Entameba hartmannii* and an additional fraction of a percent incidence of the other protozoan organisms which can infect the intestinal tract. Most of the *E histolytica* infections in our environment are silent or carrier infections. Occasionally, the ameba finds a vehicle for expression of itself. This is usually a dramatic, localized outbreak of epidemic, fulminant, intestinal and systemic amebiasis. Interestingly, in all of these epidemic outbreaks there is good evidence of waterborne sewage contamination. This discovery carries the interesting implication that the 5% general incidence of protozoan parasites which we find in an epidemiologic survey represents a relatively constant breach of environmental hygiene.

In an epidemic outbreak of disease wherein we assume that there is a single strain of virulent parasite which is operative, the ratio of clinical illness to carrier state is still remarkably low. The best illustration of this is the outbreak of amebiasis that occurred in South Bend, Indiana (2). A careful epidemiologic study of the group at risk was carried out. There were 800 isolates of *E histolytica* among the population of South Bend that could have been affected, but only 31 clinical cases of amebiasis. There were 2 liver abscesses and 4 deaths in that particular epidemic, hence the impression that the ratio of clinical disease to carrier state is low even with a virulent parasite.

In addition to epidemic experience, we observe the steady but infrequent appearance of single cases of clinically significant systemic amebiasis, usually liver abscess. These are truly indigenous cases. They turn up at the University of Michigan at the rate of about one a year. The last one we had was a 25-year-old musician from the Ann Arbor-Ypsilanti area who presented with weight loss, right upper quadrant discomfort and night sweats. His only history of travel was a visit to New York State. He had not been out of the country or to the Southern United States. His illness was prolonged, and at the time he was seen, he had been sick for 3 months. A month

From the Department of Internal Medicine, University of Michigan, Ann Arbor, Mich.

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Address for reprint requests: William A. Sodeman, Jr, MD, Associate Professor, Department of Internal Medicine, University of Arkansas Medical Center, Little Rock, Ark 72201.

before he came to the University of Michigan he had been hospitalized in New York City with similar though somewhat less marked complaints and had been released without a diagnosis. He gave no history of dysentery or diarrhea. If anything, he felt that he had had some constipation in the recent past. Physical findings were limited to a remarkable hepatomegaly with liver tenderness. Barium studies were not helpful. Liver function tests showed only mild abnormalities. Scans and selective angiography delineated space-occupying lesions in the liver, which on aspiration, contained typical pus. The patient responded promptly to metronidazole. He encountered some complications later, but has since recovered and has done extremely well.

These sporadic kinds of outbreaks, whether they are epidemics or single cases, keep alive in our own environment the clinical speculation that amebiasis is some kind of sleeping evil which, given the proper circumstance, can be released into the population. There are less frightening alternative observations that can be made in the light of a 2% incidence of ameba.

Intestinal amebiasis presents in several different ways. When there is amebic dysentery, with passage of exudate and blood rather than feces in the stool, the laboratory will report that there are large, motile, red-cell containing trophozoites that have a characteristic *E histolytica* nuclear morphology. If there is diarrhea with stools containing exudate and blood in addition to feces and again characteristic ameba on microscopic examination, it is relatively easy to make a diagnosis of amebic colitis. Few if any supplementary studies would be necessary before beginning treatment. These presentations are rare in our environment. When a patient presents with symptoms that are nonspecific or functional and the laboratory reports only the presence of characteristic cysts, then a diagnosis of a carrier state can be made. The decision to treat the patient may not be so easily justified. Most hospital laboratories report the finding of amebae far less frequently than a 2% incidence of infection would suggest they should, in part because cysts in a carrier state may be few in number and may be shed irregularly in the stool, and in part because of unfamiliarity or inexperience in the laboratory. This review of the current status of our understanding of ameba infection will hopefully offer some perspective for deciding how zealously one should look for amebae in any individual and what to do when a carrier is found. This is a story which is still developing and I will try to indicate the areas which remain speculative.

Classically in the United States, physicians are taught that all strains of *E histolytica* have pathogenic potential and are all morphologically distinct from other amebae which are parasitic in man. Thus *E histolytica* can be identified solely by looking at it under the microscope. There have been workers in this country and abroad who have taken issue with one or both of those statements, yet the statements survive in the standard texts (3, 4). The clinical derivative from the dictum that all *E histolytica* are potentially pathogenic is that when true *E histolytica* is diagnosed in a patient, he must be treated; he harbors a pathogen. The clinical derivative of the second dictum is that microscopic examination of the stool is an adequate mode of diagnosis. Both of these statements have been more rigorously challenged and it is the development of this challenge that I would like to present. If the newer understanding of amebiasis is confirmed, it will change the clinician's response to a patient with ameba infection. By chance this has occurred at a time when a new and success-

ful therapy for amebiasis has been introduced which completes the change in the clinical picture of this disease.

The early history of amebiasis was discussed by Elsdon-Dew (5) from whose work this summary was drawn. Chronologically, clinical amebiasis began with Losch who was a clinical assistant in St. Petersburg, Russia. In 1875, he observed motile amebae containing red cells in the stools of a Russian peasant who had diarrhea. He later performed an autopsy on this patient and he was able to identify active amebae in colonic ulcers. He inoculated dogs with ameba-containing stool and produced dysentery in 1 dog. When the dog died, there were motile amebae present in colonic ulcers. After Losch's description, there was a host of descriptions of *E histolytica*; after some stress among investigators, it was finally differentiated from the other protozoan parasites of the GI tract—*E coli, I butschii, E nana* and *D fragilis*. Initially, *E histolytica* was thought to be an invasive parasite that lived only in the presence of host tissues. Kunen and Swellengrebel postulated in 1913 that there could be a commensal phase of *E histolytica* called the minuta stage.

A second investigator, Dobell (6), took an opposite view. He wrote a book entitled *The Amoeba Living In Man*, published in 1919. It is this particular document that is carried through the literature to us today. It was Dobell's impression that the ameba, as an obligate cellular parasite, was in a kind of balanced equilibrium with the intestine—ie, cellular dissolution was balanced by regeneration and replacement of intestinal tissue. This impression of the ameba as an obligate cellular parasite in equilibrium with its host was strengthened by the failure to establish ameba in an in vitro culture. In 1925, however, a reproducible culture technic was found, and it was seen that ameba could grow in the absence of mammalian cells.

There emerged from these findings a divergence of opinion about the pathogenic potential of amebae, in particular, concerning virulent and avirulent strains. Craig and Faust (7), who exemplified an American school of thought, felt that ameba infection always implied a mucosal lesion somewhere in the intestinal tract. A less extreme, but similar view, sometimes called the Unicist school, proposed that at times *E histolytica* could live as a commensal. (A commensal is defined as a messmate, as someone who eats with you and doesn't eat you.) However, all *E histolytica* belonged to a single race of potentially pathogenic amebae. The differences in behavior in ameba infections were explained by these investigators as being due to extrinsic factors such as host resistance, diet and the microenvironment in the intestinal tract. This viewpoint remains represented in the standard medicine textbooks to this day (3, 4). It should be clear that the external environment, the intestinal microenvironment, and the host response play a very fundamental role in the outcome of any experience with *E histolytica*, but this is distinct from or adds to the question of intrinsic pathogenicity. Elsdon-Dew justifies the role of external factors in South Africa where there is a single pool of ameba to which three culturally distinct groups are exposed. The urban Bantu come down with a fulminating infection, amebic dysentery. The East Indian population presents essentially as carriers and the Europeans in this environment have rather infrequent clinical infections. They all have a relatively heavy common exposure. These Promethean and Unicist schools, as they were called, recognized a single strain of ameba that had a pathogenic potential.

Since 1925 there has been another group

called the Dualists (9). This group differentiates what we call *E histolytica* into two species. One species they called *E dispar,* and the other, *E dysenteriae. E dispar* is thought to be a commensal parasite. The second parasite, *E dysenteriae,* could live as a commensal, but it retains a pathogenic potential. Brumpt (9), whose ideas these were, founded these conclusions on clinical and epidemiologic experience and it is a system that fits the picture. Unfortunately, *E dysenteriae* in its commensal phase and *E dispar* are, or were said to be, morphologically identical, thus being indistinguishable by exclusively microscopic methods of diagnosis. This concept was impossible to apply clinically, though it did stimulate other workers, particularly Neal (10), who developed Brumpt's thesis, by using animal inoculation to test the virulence of ameba. They felt that they could demonstrate two types or races of *E histolytica,* one which was invasive and would cause disease and the other which was not. These characteristics were, they thought, related to human disease. The strain characteristics were partially fixed and bred true in culture, but it was not felt that these characteristics alone were of sufficient status to separate the two kinds of amebae into two different species. They postulated in effect morphologically identical races, one which was invasive and one not, but could not exclude transitions from one group to the other.

In 1957, Burroughs (1) established the now well accepted separation of *E hartmanii* from *E histolytica. E hartmanii* is thought to be a nonpathogenic ameba, though its pathogenicity has not been subjected to exhaustive testing. It is distinguished from *E histolytica* on the basis of cyst size, nuclear morphology and also by serology. Superficially, it is similar to *E histolytica* in appearance, and prior to its separation from *E histolytica,* was probably always identified as *E histolytica.* This obscures much of the epidemiologic work prior to 1957 which undoubtedly lumped these two together. We emerge with three incompatible, mutually exclusive clinical pathologic identifications of ameba, each one vigorously defended by competent and well recognized authorities. In the last 10 years, a substantial body of new information tends to support the Dualist school of interpretation. The new information is preliminary; it is an evolving story and still needs to be verified. The following is a bit speculative.

In 1956, Connel noted that an isolate of *E histolytica,* which he called the Laredo strain (2), was capable of being cultured at room temperature in addition to the customary 37°C. This was felt to be an oddity but elicited little other comment. This ameba morphologically resembled conventional *E histolytica.* The patient who provided this isolate had a history of diarrhea for 3 years, but also had a villus adenoma in the transverse colon; when this was resected, his diarrhea subsided. There was no evidence that he suffered invasive intestinal amebiasis. Over the last 10 years, five more isolates of this low temperature strain of parasite (13–15), all from human stool, have been reported. The Huff strain clearly came from a carrier (16). The AG and JA strains came from patients whose historic background was inadequately described, but who clearly did not have invasive intestinal disease (14). The isolate of Nelson and Jones (13) and the 403 strain (15) were stated to have come from carriers. All have been subjected to careful scrutiny and are morphologically identical with classic *E histolytica,* though they are readily separable in the laboratory by manipulating the culture temperature.

This group of amebae was awarded the designation of Laredo-like *E histolytica* at

the Eighth International Congress of Tropical Medicine and Malaria. There have accumulated extensive studies that demonstrate a number of points of difference between the classic ameba and the Laredo-like ameba. These have been reviewed by Goldman (17) and are summarized below.

The temperature differential has been reconfirmed. True *E histolytica* in continuous culture has a minimum temperature requirement of at least 30°C. The Laredo-like ameba can be continuously maintained at temperatures as low as 10°C. The optimum temperatures are 37° for the classic and 25–30° for the Laredo-like ameba. Classic *E histolytica* cannot be maintained in any but an isotonic medium. Dilution of the medium by as little as 1 to 2 with distilled water will cause the cultures to die out. The Laredo-like ameba can not only survive but it can grow in a medium diluted as much as 1 to 64 with distilled water. This offers another differential test that could be used in a bacteriology laboratory.

There are substantial numbers of other biochemical differences between these two amebae. Drug sensitivity studies reveal that the Laredo-like ameba is more resistant to the usual amebacidal drugs than the classic strain. There are quantitative differences in the free amino acid composition and there have been differences demonstrated in the isoenzyme patterns.

In addition to the biochemical differences, there are a number of immunochemical comparisons of classic and Laredo-like amebae. All of them show the presence of shared antigens, but there are a sufficient number of antigenic differences to permit the separation of classic from Laredo-like ameba by immunologic means. The methods of comparison have included fluorescent antibody technics, agar diffusion studies and hemagglutination studies.

Not all information has been in support of differences of the two groups. No difference in carbohydrate utilization in culture has been found. There is a single unpublished report of the laboratory demonstration of exchange of genetic material between amebae of the two groups based on drug resistance as a genetic marker (18). Presumably, amebae capable of exchanging genetic material cannot be separated as species. It should be noted that the negative studies are unconfirmed and the evidence continues to support the differences between these two groups.

What elevates these apparent biologic differences between classic and Laredo-like ameba to clinical significance are studies of the pathogenicity of the parasite. As indicated, no isolate of Laredo-like ameba has clearly come from an individual who suffered acute intestinal or systemic amebiasis. All have come more or less clearly from carriers. One strain was subjected to the most strenuous clinical test of pathogenicity that any ameba has been subjected to. Prior to its identification as a Laredo-like ameba, the Huff strain was selected for a clinical trial of an induced ameba infection in human volunteers (16). The Huff strain was given to 130 prisoner volunteers by oral ingestion of cysts. Eighty-one of the volunteers were successfully infected, as evidenced by the continuous passage of cysts in their stools. Of the group of 81, no individual was symptomatic in spite of the passage of ameba in the stool. The clinical evaluation of these patients included sigmoidoscopy. A variety of animal inoculations have been carried out with uniform demonstration of a low or negligible pathogenicity of the Laredo-like ameba in all cases. There is some overlap between the classic and Laredo-like ameba strains in terms of their virulence in animals other than man.

Considering the criteria which distin-

guish the Laredo-like ameba from the classic strain, the differences are substantial enough to suggest that the Laredo-like ameba may be a separate species. This would in effect make them the equivalent of Brumpt's nonpathogenic *E dispar*. This has not been done because of the paucity of isolates of low temperature strains. There have been only six isolates thus far reported. In terms of numbers, the evidence is not yet overwhelming enough to permit the establishment of a new species. The next step from the clinical standpoint is reasonably clear. The parasitologist has handed the clinician an attractive pathophysiologic scheme which could explain the clinical appearance of ameba infection; this scheme needs to be validated. In effect, it is ready for clinical trial. An exhaustive re-evaluation of the efficiency of cultural methods for the diagnosis of ameba infections is required. We are currently no more justified in accepting microscopic examination of the stool for the diagnosis of *E histolytica* than we would be in restricting ourselves solely to the Gram stain of a sputum smear for the diagnosis of pneumonia. Clinical microscopy is useful and has a place in both circumstances, but it may be insufficient when taken alone. As a second step, we will need to identify the biologic characteristics of the amebae that infect at least 1–2% of our local population. Thirdly, we need to confirm the consistency of the various biologic characteristics among more isolates of the low temperature strain. Finally, we need a clinical correlation between the presence of symptoms and the strain of ameba apparently responsible in an attempt to confirm avirulence of the Laredo-like group. With the information incomplete, it is difficult to justify not offering treatment if one finds somebody with an *E histolytica* infection. However, there is a revolutionary new approach to the diagnosis and clinical decision about therapy which is clearly just before us. If the information which I have reviewed is validated, then amebiasis will be stripped of its mystery and permitted to find its own proper place among gastrointestinal infectious diseases.

If and when it finds this place, one expects that there will remain some infections to treat. Just as there is currently a new understanding that threatens our current concept of clinical amebiasis, there are significant, virtually revolutionary advances in therapy which press in on our established regimens. Here the contest is less emotionally charged, since truly effective therapy of low toxicity, until now, has been lacking. What follows is a review of the efficiency and tolerance of the commonly employed intestinal and systemic amebacides to provide the framework within which the new offerings may be judged. As the spectrum of pharmacologic behavior unfolds, the rationale for acceptance of the newer preparations should become much clearer.

Emetine is the baseline drug against which other drug activities are measured. It is the oldest known effective amebacide. It has been found useful in both intestinal and systemic ameba infection. Its earliest use was in the treatment of dysentery (19). An alkaloid extracted from a plant which is indigenous to South America, it was taken to Europe in 1658. It led a rather checkered and secret career, and at one point, was sold to the French government as a secret remedy. It didn't achieve significant use until 1858 and the classic paper describing its effectiveness was that of Leonard Rogers in 1912 (20). The drug remains in widespread use today.

Emetine affords prompt relief of symptoms in acute amebiasis although it is ineffective in terms of parasitologic cure. It

seems to be more effective in extraintestinal amebiasis, perhaps because it is concentrated in the liver, which is the usual site of extraintestinal involvement. Representative results in invasive intestinal amebiasis—ie, amebic dysentery—are 50% successes, 28% absolute failures and 22% probable failures (21). A success is a patient that is symptom-free and parasite-free. An absolute failure is a patient whose symptoms have not improved, and in whom parasites and ulcers in the rectum are still present. A probable failure is a patient whose symptoms have improved but whose ulcers remain.

These results are drawn from the work of the Amebiasis Research Unit in Durban, South Africa (21). Although various amebacides have been widely tested and reported, the subsequent discussion will be based on the data reported by this single unit. The compilation is based on a number of separately published studies, but they have all been performed by a single group of investigators who explicitly attempted to achieve maximum uniformity in their drug test program. They represent, then, the most reliable body of comparative information concerning amebacidal drugs.

The clinical dissatisfaction with emetine stems not only from its rather mediocre performance in terms of parasitologic cure but also from its substantial toxicity. Emetine is a protoplasmic poison with a direct effect on muscular and nervous tissue (22). It is excreted slowly, and the effect may be cumulative if its administration is too rapid. The incidence of reported untoward reactions is high if the dosage is adequate and the observation complete. In one study, 91 of 96 patients experienced some side effect (23). Within the safe dose range, one may expect myocardial changes such as increase in heart rate, decrease in systolic pressure and evidence of T wave inversion on the electrocardiogram (24). In addition, encephalitis, paralysis and Herxheimer-like reactions have been reported. With this drug as a scale, it is possible to turn to the comparative value of the other common amebacides that we use.

Some classes of drugs are active in intestinal infection; diodoquin (5, 7 diiodo 8 hydroxyquinoline) is representative of the first general category of these. It has low toxicity, most frequently manifested as iodine sensitivity. It has no better effect than emetine, with 58% success, 24% failure and 18% probable failure rate (21). It is a contact or luminal drug. The dose is 0.65 g orally three times daily for 21 days.

Representative of the next class of drugs are the arsenic preparations, carbarsone and Milibus. Carbarsone's dosage is 0.25 g three times a day for 10 days. This is the largest dose which can be tolerated without a prohibitive incidence of reactions. The toxicity is arsenic poisoning and includes nausea, vomiting, convulsions, skin rash, exfoliative dermatitis, cramps, diarrhea and occasionally jaundice (25). It is not significantly more effective than emetine (21).

Milibus contains arsenic and bismuth. Its dosage is 0.5 g three times a day for 7 days. The toxicity is generally similar to carbarsone, although the margin of safety may be a little greater (24). It is substantially less effective than emetine (21).

Entamide furoate (diloxanide furoate) is not available in this country, but has been widely used in tropical Africa. The dosage is 4 g daily for 10 days. It seems to be an effective drug in both the acute intestinal disease and in the carrier state. Cure rates in some hands have ranged as high as 80%, though the South African group's success rate in amebic dysentery was in the range of 40%. Toxicity is mild—mostly tingling and the development of flatulence. It com-

pares favorably with emetine and is a simpler drug to administer (26).

Chloroquine is not usually thought of as an intestinal drug; it is a systemic amebacide. It has little effect on intestinal infections. The South African group could only get a 10% cure rate with this particular drug (21).

Paromomycin is a nonabsorbale, broad spectrum antibiotic isolated from streptomyces. In addition to its antibacterial properties, it is thought to have an antiamebal action. The use of antibiotics in amebiasis is based on an indirect effect of the antibiotic altering the bacterial flora and modifying the apparently delicate ameba-bacterial relationship. Any direct amebacidal action of this particular drug then is a further valuable factor. Two grams a day for 10 days is the usual dose. The toxicity is that common to all antibiotics that disturb the intestinal flora. It is an effective therapy with a success rate of 80%. It is more effective in acute intestinal disease than in the carrier state (26, 27).

Tetracycline is representative of the absorbable broad spectrum agents. The dosage commonly employed was 0.5 g four times a day for 10 days. Until recently, the broad spectrum antibiotics have been the drugs of choice for acute intestinal amebiasis in most tropical areas. They are oral agents with a high degree of effectiveness in comparison with emetine, and with a low toxicity. However, their cost may be high. Relapse rates are also high. This is a late relapse, suggesting that the drug effect may be more suppressive than curative. For this reason, it is often given in conjunction with a contact amebacide such as diodoquin. The immediate success rate is 97% in the hands of the South African group (26). Toxicity includes alteration of the intestinal flora and sensitivity reactions. There are also problems with the deposition of the drug in the enamel of the teeth in children, though a short course should not cause this problem. The use of outdated tetracycline should be completely preventable. Clearly, tetracycline is the drug that any new preparation will have to beat in terms of its utility. Factors of cost, mode of administration and toxicity are its weak points.

This brings us to metronidazole. At this time, metronidazole is the most effective, least toxic intestinal amebacide available (28–32). It is coupled with a potent systemic action. The effective dose remains to be clarified. For acute intestinal infection, the current recommendation is 750 mg, three times a day for 5 days (29). A number of regimens using fewer, larger doses have been studied, and are of particular interest when patients will not return for follow-up. In our environment, this particular approach, a 5-day course, has so few side effects that it is probably the program of choice. The toxicity includes nausea, vomiting, dizziness, urticaria and a metallic taste in the mouth usually with higher doses of the drug. There is an untoward reaction after ingestion of alcohol, which has been reported in some patients who receive the drug. It is a nitroimadozole derivative, it does cause leucopenia, and at this time it is contraindicated in blood dyscrasias. It has a central nervous system toxicity in some animals; at this time, it is felt that it should be withheld in patients with organic central nervous system illness until this area of the drug activity is better understood. Since the range of toxicity is different, it is difficult to compare it with that of emetine and tetracycline; however, the impression is that the toxicity of metronidazole is milder. As an amebacidal therapy, it appears to have a clear advantage since relapse rates seen with tetracycline have not been reported. Success rates from 86 to 95% have

been consistently reported. Currently it is the drug of choice.

In the treatment of amebic abscess, emetine is once again the yardstick against which the other drugs must be measured. The success rate of emetine in the hands of the South African group from whom these data have been derived is 100% (33), but it should be noted that these figures are for a double course of the drug. With a single course of the drug, they have a relapse rate of 7.7%. The toxicity is the same as for its use in intestinal disease.

Chloroquine is a highly effective drug in systemic amebiasis with a substantial advantage over emetine because of its lower toxicity. Given as a single course of 150 mg two times a day over 30 days after a loading dose, the success rate for chloroquine alone is 72% (33). An absolute failure here is an instance where a patient's condition deteriorates during the 30-day course on the drug, and it is thought that one must then intervene with emetine. The toxicity includes retinal lesions, skin eruptions, including a psoriatic-like eruption, nausea, dizziness, and in a few people, psychic stimulation. However, chloroquine used in this manner rarely presents a significant toxicity problem.

The failure of either emetine or chloroquine alone to give 100% success with a single course of administration prompted the South African group to try combination therapy. With this approach, a single course of each drug is administered concurrently. Success with this regimen is excellent, and until recently, this has been the therapy of choice for amebic abscesses (34).

Metroniadazole then came on the scene. Once again this proved to be the most potent, least toxic drug for systemic ameba infection that has yet been described. The minimum dose in systemic amebiasis has yet to be determined. The South Africans use 800 mg three times a day for 5 days. The best local equivalent is 750 mg since the drug is formulated in a 250-mg tablet in this country, and in a 200-mg tablet outside of this country. In the South African group, and in others, this has been 100% effective in treating amebic abscess (28, 29). In the few patients we have had an opportunity to treat at the University of Michigan, we also have had 100% success. Single large doses have been tried with success but with some attendant toxicity, usually in the form of vomiting at the dose level administered.

In summary, what we treat, whom we treat, with what agent we treat ameba infection all have undergone recent and substantial change. For those who have been separated from the evolution of recent information, amebiasis presented in this way at this time has the appearance of a new disease.

REFERENCES

1. Burrows RB: Prevalence of amebiasis in the United States and Canada. Amer J Trop Med Hyg 10:172–184, 1961
2. Brooke MM, Melvin DM, Sappenfield R, et al: Studies of a water borne epidemic of amebiasis in South Bend, Indiana. III. Investigation of family contacts. Amer J Hyg 62:214–266, 1955
3. Bennett IL: Amebiasis in Harrison, T. R. et al, Principals of Internal Medicine. Fifth edition. New York, McGraw-Hill, 1966, p 1762
4. Shookhoff HB: Amebiasis in Beeson, P. B. and McDermott, W., Textbook of Medicine. Twelfth edition. Philadelphia, W. B. Saunders Co, 1967, p 345
5. Elsdon-Dew R: The present status of amoebiasis. Proc. 6th Internat. Cong. Trop. Med Mal. 3:340–349, 1958
6 Dobell C: The Amoeba Living in Man. A Zoological Monograph. New York, William Wood, 1919

7. Faust EC, Russell PF: Clinical Parasitology. Seventh edition. Philadelphia, Lea & Febiger, 1964
8. Elsdon-Dew R: Endemic fulminating dysentery. Amer J Trop Med 29:337–340, 1949
9. Brumpt E: Etude sommaire de l'Entamoeba dispar n. sp amibe a kystes quadrinuclees, parasite de l'homme. Bull Acad Med (Paris) 94:943–952, 1925
10. Neal RA: The pathogenicity of Entamoeba histolytica. Proc. 6th Internat. Cong. Trop. Med Mal 3:351–359, 1958
11. Burrows RB: Differentiation of Entamoeba hartmanni from the dwarf strain of Entamoeba histolytica. Proc. 6th Internat. Cong. Trop. Med. Mal 3:360–368, 1958
12. Dreyer KA: Growth of a strain of Entamoeba histolytica at room temperature. Texas Rep Biol Med 19:393–396, 1961
13. Nelson EC, Jones MM: Cultivation of Entamoeba histolytica in carbon dioxide-bicarbonate buffer system media. Amer J Trop Med Hyg 13:667–673, 1964
14. Entner N, Most H: Genetics of Entamoeba: characterization of two new parasitic strains which grow at room temperature (and at 37° C). J Protozool 12:10–13, 1965
15. Richards CS, Goldman M, Cannon LT: Cultivation of Entamoeba histolytica and Entamoeba histolytica like strains at reduced temperature and behavior of the amebae in diluted media. Amer J Trop Med Hyg 15:648–655, 1966
16. Beaver PC, Jung RC, Sherman HJ, et al: Experimental Entamoeba histolytica infections in man. Amer J Trop Med Hyg 5: 1000–1009, 1956
17. Goldman M: Entamoeba histolytica-like amoebae occurring in man. Bull WHO 40: 355–364, 1969
18. Entner N: Mating in Entamoeba histolytica. Presented at the Annual Meeting of the American Society of Tropical Medicine and Hygiene, Washington, DC, Nov 4, 1969
19. Walters J: Amoebiasis past and present. J Trop Med Hyg 67:133–136, 1964
20. Rogers L: The rapid cure of amoebic dysentery and hepatitis by hypodermic injections of soluble salts of emetine. BMJ 1: 1424–1425, 1912
21. Wilmot AJ, Armstrong TG, Elsdon-Dew R: The comparative value of amoebicidal drugs. J Trop Med Hyg 54:161–165, 1951
22. Manson-Bahr P, Walters JH: The Chemotherapy of Tropical Disease. Springfield, Charles C Thomas, 1961, p 31
23. Klatskin G, Friedman H: Emetine toxicity in man. Ann Intern Med 28:892–915, 1948
24. Hunter GW, Frye WW, Swartzwelder JC: A Manual of Tropical Medicine. Philadelphia, W. B. Saunders Co, 1966, pp 300–302
25. Shaffer JG, Shlaes WH, Radke RA: Amebiasis: A Biomedical Problem. Springfield, Charles C Thomas, 1965, pp 126–129
26. Wilmot AJ, Powell SJ, McLeod I, et al: Some newer amoebicides in acute amoebic dysentery. Trans Roy Soc Trop Med Hyg 56:85–87, 1962
27. Wilmot AJ, Powell SJ, McLeod I, et al: Paromomycin in acute amoebic dysentery. Ann Trop Med Parasitol 56:383–385, 1962
28. Powell SJ, Wilmot AJ, MacLeod I, et al: Metronidazole in amoebic dysentery and amoebic liver abscess. Lancet 2:1329–1331, 1966
29. Powell SJ, Wilmot AJ, Elsdon-Dew R: Further trials of metronidazole in amoebic dysentery and amoebic liver abscess. Ann Trop Med Parasitol 61:511–514, 1967
30. Minvielle L, Elorriaga FA, Castellanos CC, et al: An evaluation of the amebacidal properties of metronidazole. Dis Colon Rectum 12:412–416, 1969
31. Scott F, Miller MJ: Trials with metronidazole in amebic dysentery. JAMA 211:118–120, 1970
32. Blechman MG: Metronidazole in the treatment of amebic liver abscess. J Med Soc NJ 67:29–31, 1970
33. Wilmot AJ, Powell SJ, Adams EB: The comparative value of emetine and chloroquine in amebic liver abscess. Amer J Trop Med Hyg 7:197–198, 1958
34. Wilmot AJ, Powell SJ, Adams EB: Chloroquine compared with chloroquine and emetine combined in amebic liver abscess. Amer J Trop Med Hyg 8:623–624, 1959