The Relationship of Clinical Amoebiasis to Various Strains and Growth Requirements of *Endamoeba histolytica*[1]

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INFECTIONS WITH *Endamoeba histolytica* present many interesting questions with reference to the occurrence of clinical manifestations. The large majority of persons infected with this amoeba have no symptoms referable to the infection, but a small proportion develop mild abdominal symptoms or severe amoebic dysentery, and a few, liver abscesses that may lead to secondary infections or abscesses in the lung, brain, skin, or other parts of the body. The proportion of infected persons developing severe clinical manifestations varies widely in different parts of the world. In the tropics, some areas have a high incidence of severe infections while others present a very few clinical cases even though the incidence of symptomless infections is high. The reasons for these variations must be related either to the parasite itself, to the condition of the host, to environmental influences, or to a combination of these factors.

LIFE HISTORY AND ACTIVITY OF *Endamoeba histolytica*

Let us first review the life history of *Endamoeba histolytica* and its possible activities from the time it is introduced into the mouth of the host, as a cyst, until it is excreted in the feces, also in the form of a cyst. The cyst apparently passes unharmed through the stomach and enters the small intestine. During its passage through the small intestine or after arrival in the cecum, the four-nucleated amoeba within the cyst becomes active and emerges from the cyst capsule through a small pore, which is formed either mechanically or chemically by the action of the amoeba. The process of excystation requires from a few minutes to an hour or more and is accomplished by alternate extrusion and retraction of the amoeba in order to equalize the pressure inside and outside of the cyst capsule.

After excystation, the four-nucleated amoeba apparently undergoes further nuclear division and single nuclei, with a portion of cytoplasm, separate into eight small motile amoebae. This process

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usually takes place in the lumen of the cecum. The amoebae feed upon the cecal contents, grow to full size, and then undergo mitotic and cellular division. They may be carried in the fecal contents toward the anus, undergoing excystation, and thus preparing for survival outside the body until they enter the mouth of the same host or of a new host. If conditions in the cecum or other parts of the large intestine are favorable for tissue invasion, the motile amoebae may penetrate the wall of the intestine. This occurs when stasis of the intestinal contents permits time for its accomplishment. It may occur on the surface of the intestinal mucosa, but it is more likely to take place first in the natural depressions, or rugae, or in the lumens of glands.

The penetration into the tissues is accomplished by combined physical and chemical action. Amoeboid movement of the parasite may permit penetration between epithelial cells; the loss of a few epithelial cells by trauma may permit penetration beneath the epithelium, or the lytic toxin of the amoeba may digest these cells before penetration takes place. Sometimes the amoeba proceeds beneath the epithelial cells to the base of the glands and there multiples, outside the basement membrane, before penetrating into the connective tissue stroma of the mucosa. Sometimes it invades the stroma more superficially and gains access to blood or lymph capillaries through which it may proceed to the submucosa. In these various situations, the lytic toxin may produce necrosis of the delicate superficial tissue while the amoebae are penetrating to deeper layers, so that they often appear beyond the areas of visible necrosis.

Thus, we may have superficial erosion of the mucosa, or the development of minute abscesses in the submucosa, after the amoebae have penetrated the muscularis mucosae and have advanced laterally and perpendicularly in the submucosa. The development of lesions in the submucosa leads to interference with the nutrition of the mucosa above it and to the formation of the so-called bottleneck ulcer with extrusion of blood, necrotic material, and amoebae into the lumen of the intestine. Advancing beyond the area of necrosis, the amoebae in the submucosa may enter lymph or blood vessels, or may penetrate through the muscular coat of the intestine either directly or by way of connective tissue septa, ultimately causing perforation into the peritoneal cavity, or adhesions to other peritoneal surfaces. If they enter blood vessels, they are carried as emboli by the blood stream to the liver, where they cause necrosis of

the capillary walls and continue their multiplication, and spread until they form a liver abscess.

With the development of lesions in the intestine, bacteria follow the amoebae into the tissues producing secondary infection. The type of bacteria invading the tissues probably determines how this secondary infection will affect the pathological and clinical picture. If the bacteria are nonpathogenic, this picture will remain essentially that of an amoebic infection. If they are highly pathogenic, however, the bacterial infection may dominate the picture, or there may be a fulminating infection in which both amoebae and bacteria combine to produce results. Undoubtedly, bacteria often accompany the amoebae to the liver, but we do not know whether bacteria are necessary to the formation of a liver abscess. This seemed to be the case in experimental amoebic abscesses produced in cats by Cleveland and Sanders.[2] In human cases, amoebic liver abscesses are sometimes contaminated with bacteria but they sometimes are bacteriologically sterile. Thus we see a close association between the amoebae and bacteria, and one of the interesting questions is to what extent the amoebae depend upon bacteria for their survival and multiplication.

REASONS FOR VARIATION IN CLINICAL MANIFESTATIONS OF INFECTION

One of the reasons for variation in the clinical evidence of infection with *Endamoeba histolytica* is an apparent difference in the pathogenicity of individual strains of the parasite. Brumpt[3] created the species *Endamoeba dispar* for strains of the large race of *Endamoeba histolytica* that do not produce symptoms in man and which, in his observations, invaded only the mucosa of the intestine of kittens without producing deeper ulcers. He showed that these strains are widely distributed throughout the world, particularly in the temperate zones and also in certain sections of the tropics, including Rio de Janeiro and other parts of South America. Simic,[4] in Yugoslavia, accepted Brumpt's nomenclature and found that *Endamoeba dispar* predominated in southern Yugoslavia, whereas strains producing dysentery were more common in Macedonia. Marín[5] and Poindex-

2. L. R. Cleveland and E. P. Sanders, The virulence of a pure line and several strains of *Entamoeba histolytica* for the liver of cats and the relation of bacteria, cultivation, and liver passage to virulence. Am.J.Hyg., 12:569–605, 1930.

3. E. Brumpt, Differentiation of the human intestinal amoebae with four-nucleated cysts. Tr.Roy.Soc.Trop.Med.& Hyg., 22:101–124, 1928.

4. T. Simic, Présence de *l'Entamoeba dispar* Brumpt dans le sud de la Yougoslavie (Macedoine Serve). Ann.de parasitol., 9:289–302, 1931.

5. R. A. Marín, Preliminary note on the morphology and pathogenicity of *E. histolytica* in Puerto Rico. Puerto Rico J.Pub.Health & Trop.Med., 6:429–433, 1931.

ter[6] reported that the strains in Puerto Rico had a low degree of pathogenicity for kittens. Most protozoölogists, however, have refused to accept Brumpt's establishment of a new species for the mildly pathogenic large amoeba or for the small race that he called *Endamoeba hartmanni*. Evidence indicates that both of these strains, though usually producing no symptoms in man, sometimes produce severe lesions in kittens.

In Tennessee, the writer and Dr. William W. Frye[7] studied many strains of *Endamoeba histolytica* with reference to their pathogenicity in man and kittens. The amoebae were established in uniform culture media and the cultures injected directly into the ileum of numerous series of twenty or more kittens. A pathogenic index for each strain, based upon the average severity of the lesions and the duration of the infection before death of the kittens, was calculated. Some of the experiments were repeated at intervals over a period of as long as five years; they indicated that the individual strains retained approximately the same degree of pathogenicity. Strains obtained from a rural community in the hill country of Tennessee, where a high incidence of infection in man was associated with practically no active amoebic dysentery, showed a low pathogenic index in kittens. Those from another community in the low country of Tennessee, where there was closer contact with commerce from the Gulf of Mexico and the tropics and where there were many cases of active amoebic dysentery, showed a much higher pathogenic index. Strains obtained in the city of Nashville, Tennessee, varied in conformity with the clinical picture in the human host. The strains from Chicago, obtained soon after the epidemic of amoebic dysentery in 1933, showed the highest pathogenic index of any of those studied. One strain of the small race of *Endamoeba histolytica* produced very mild but definite lesions in kittens.[8]

In studying these strains of *Endamoeba histolytica*, we wondered whether or not the bacteria accompanying the amoebae in culture influenced their pathogenicity in kittens. To test this hypothesis we interchanged the bacteria accompanying a more pathogenic strain with those of a less pathogenic strain. We found that this procedure did not alter the pathogenic index of the two strains in kittens but that it seemed to indicate that the difference in pathogenicity was caused by the amoebae themselves rather than by the bacteria.

6. H. A. Poindexter, The Puerto Rican strain of *Endamoeba histolytica*. Puerto Rico J.Pub. Health & Trop.Med., **9**:31–36, 1933.

7. H. E. Meleney and W. W. Frye, The pathogenicity of *Endamoeba histolytica*. Tr.Roy. Soc.Trop.Med.& Hyg., **29**:369–379, 1936.

8. W. W. Frye and H. E. Meleney, The pathogenicity of a strain of the small race of *Endamoeba histolytica*. Am.J.Hyg., **27**:580–589, 1938.

Another cause of differences in clinical manifestations is the individual harboring the amoebae. This fact is universally recognized and was particularly apparent in the Chicago epidemic where all degrees of severity of clinical manifestations occurred—from no symptoms at all to fulminating infections with gangrenous ulceration of the intestine and liver abscesses. We also saw this individual variation in our kitten experiments. Each series contained some kittens that did not become infected; the lesions produced by a single strain usually varied widely in their severity in individual animals. Only by using a large number of kittens in each experiment and by estimating the average severity of the lesions could a comparison of the pathogenicity of strains be made.

This difference in the response of the individual host to the infection has never been satisfactorily explained. It may involve many factors, the first of which may be the general condition of the host—an unsatisfactory term but one that implies nonspecific resistance to tissue invasion. A second factor may be the bacterial flora of the intestine which may influence the hydrogen-ion concentration of the intestinal contents, the nutrition of the amoebae, and the condition of the intestinal mucosa. Still a third factor may be the diet of the host, which may, in turn, affect the bacterial flora, the nutrition of the amoebae, and the condition of the host's tissues. McCarrison[9] reported that monkeys in India, fed on a vitamin B-deficient diet, developed active amoebic dysentery while those on a normal diet did not. Faust and Kagy[10] reported that experimentally infected dogs, fed on raw liver, controlled and sometimes eradicated the infection.

A fourth factor may be physical or chemical injury to the intestinal mucosa, possibly combined with bacterial infection, as reported by Nauss and Rappaport.[11] A fifth factor may be the rapid passage of the amoebae from host to host, which undoubtedly occurred in the Chicago epidemic by means of water polluted with hotel sewage. Baetjer and Sellards[12] found that rapid passage from

9. R. McCarrison, The pathogenesis of deficiency diseases. VIII. Effects of autoclaved rice dietaries on the gastro-intestinal tract of monkeys. Indian J.M.Research, **7**:283–307, 1919.

10. E. C. Faust and E. S. Kagy, Studies on the effect of feeding ventriculin, liver extract and raw liver to dogs experimentally infected with *Endamoeba histolytica*. Am.J.Trop.Med., **14**:235–255, 1934.

11. R. W. Nauss and I. Rappaport, Studies on amebiasis. I. Pathogenesis of mucosal penetration. Am.J.Trop.Med., **20**:107–127, 1940.

12. W. A. Baetjer and A. W. Sellards, Continuous propagation of amoebic dysentery in animals. Bull.Johns Hopkins Hosp., **25**:165–173, 1914.

kitten to kitten increased the severity of the infection, but they attributed such increase to the transfer of hemolytic streptococci. Faust and Swartzwelder[13] also reported an increase in severity by direct transfer in dogs. Meleney and Frye[14] obtained similar results. After six direct transfers in dogs, they found that the two strains, previously studied in kittens, had a considerably higher pathogenic index immediately after this dog-to-dog transfer, but that they soon returned to their previous pathogenic index after reestablishment in culture. These findings led us to suggest that the increase in pathogenicity might be due to a greater phagocytic power of the amoebae, temporarily acquired by continual feeding on host tissues.

A sixth factor may be the influence of highly pathogenic bacteria producing the initial lesions through which the amoebae gain entrance to the tissues. Many workers believe this last factor important. Spector,[15] Deschiens,[16] and Nauss and Rappaport[17] reported it in kitten and cat experiments, respectively. Cleveland and Sanders[18] also reported that, in order to produce amoebic liver abscesses in cats, it was necessary to use cultures containing bacteria recently obtained from the intestine. A seventh factor, often mentioned, is the climatic environment of the host, particularly the tropics or the warm season, which is believed to influence the diet and the general resistance of the host, the bacterial flora of the intestine, and the ease of transmission through flies and unsanitated surroundings. It is interesting to note, however, that some tropical areas such as Puerto Rico appear to have a low incidence of active amoebic dysentery.

INTESTINAL INFECTION WITHOUT INVASION OF TISSUES

Another interesting question is whether or not *Endamoeba histolytica* can multiply indefinitely in the lumen of the intestine over a long period of time without any invasion of the tissues. Authorities are divided in their opinion on this point. Some believe that *Endamoeba histolytica* requires tissue fluids and cells for its multiplication.

13. E. C. Faust and J. C. Swartzwelder, Effect of continuous passage of *Endamoeba histolytica* through experimental dogs. Proc.Soc.Exper.Biol.& Med., **32**:954–958, 1935.

14. H. E. Meleney and W. W. Frye, The effect of direct animal passage on the pathogenicity of *Endamoeba histolytica* for kittens. Am.J.Hyg., **25**:313–326, 1937.

15. B. K. Spector, Pathological changes produced in intestines of kittens by *Endamoeba histolytica*, with and without certain bacteria. Am.J.Hyg., **22**:366–375, 1935.

16. R. Deschiens, Le rôle de la flore bacterienne, associée a l'amibe dysentérique, dans l'amibiase. Ann.Inst.Pasteur, **61**:5–32, 1938.

17. R. W. Nauss and I. Rappaport, *op. cit.*

18. L. R. Cleveland and E. P. Sanders, *op. cit.*

Human autopsies sometimes reveal small lesions that give rise to no clinical manifestations during life.

Meleney and Frye[19] reported one case in which no macroscopic lesions were found at autopsy but invasion of the mucosa and submucosa, without definite ulceration, was discovered in routine sections of the colon. Faust[20] reported thirteen autopsies on cases of sudden death, among which five showed convincing evidence and three suggested signs of tissue invasion, whereas five demonstrated no gross or routine microscopic evidences. Frye and the writer examined the colons of a number of *Macacus rhesus* monkeys, infected with monkey or human strains of *Endamoeba histolytica*, but found no macroscopic lesions or microscopic evidence of tissue invasion in sections taken at random. Kessel[21] studied pigs in Peiping, China, infected with this amoeba, and also found no macroscopic lesions.

The complement fixation test has afforded evidence suggesting that *Endamoeba histolytica* may reside entirely in the lumen of the intestine. Although Craig's[22] complement fixation test, performed with a human hemolytic system, yielded reactions on almost all the symptomless carriers tested, Meleney and Frye,[23] using a sheep hemolytic system, obtained a low proportion of positive reactions on carriers and no positives on a series of infected persons residing in the hill community of Tennessee, where no clinical evidence of amoebiasis was present.

Endamoeba histolytica is easily cultivated in the test tube, where no living material is present except the accompanying bacteria that are also present in the lumen of the intestine. As will be shown later, the culture medium need not even contain egg or blood serum.

These facts make it easily conceivable that *Endamoeba histolytica*, like the other intestinal amoebae, can multiply indefinitely in the lumen of the intestine as a commensal without invading the surrounding tissues.

19. H. E. Meleney and W. W. Frye, Studies on *Endamoeba histolytica* and other intestinal protozoa in Tennessee. VII. The histopathology of intestinal amoebiasis in the kitten and in man. Am.J.Hyg., **20**:84–105, 1934.

20. E. C. Faust, Amoebiasis in the New Orleans population as revealed by autopsy examination of accident cases. Am.J.Trop.Med., **21**:35–48, 1941.

21. J. F. Kessel, Intestinal protozoa of the domestic pig. Am.J.Trop.Med., **8**:481–501, 1928.

22. C. F. Craig, The technique and results of a complement fixation test for the diagnosis of infections with *Endamoeba histolytica*. Am.J.Trop.Med., **9**:277–296, 1929.

23. H. E. Meleney and W. W. Frye, Practical value and significance of the complement-fixation reaction in amebiasis. Am.J.Pub.Health, **27**:505–510, 1937.

NUTRITIONAL REQUIREMENTS OF *Endamoeba histolytica*

In order to understand the activity and pathogenic action of any microorganism, it is important to know its nutritional requirements. *Endamoeba histolytica* differs from the other intestinal amoebae of man in that the motile forms in the feces usually do not contain bacteria but often do contain red blood cells. These facts have been advanced as evidence that this amoeba is an obligatory tissue parasite, but this last assertion cannot be true since cultivation is possible in media not containing living tissue. There are, however, certain interesting associations of the amoebae with both tissues and bacteria.

Bacteria are sometimes found in *Endamoeba histolytica* from the intestine and are usually present in the amoebae in cultures. In sections of amoebic ulcers of the intestine or skin, secondary bacterial invasion of the tissues is usually present and the amoebae are generally found invading the tissue in advance of the bacteria. Amoebic liver abscesses are often contaminated with bacteria; Cleveland and Sanders[24] were unable to initiate amoebic liver abscesses in cats without accompanying bacteria. Although *Endamoeba histolytica* is easily cultivated in artificial media, no one has yet succeeded in cultivating it continuously in the absence of living bacteria.

The foregoing facts indicate that this amoeba is usually dependent upon bacteria for its multiplication. However, the existence of amoebic liver abscesses in man without bacteria and the advance of the amoebae beyond bacteria in other lesions suggest that bacteria themselves are not essential food requirements. It may be that some bacterial enzymes or other products are necessary. The portal blood, supplying the liver, probably carries bacterial products from the intestine and these may comprise the nutritive substances that support the amoebae in the liver. Najjar and Holt[25] recently demonstrated that certain intestinal bacteria apparently synthesize thiamine, one of the essential food factors of man; this, or other factors, may also be essential to the amoebae. On the other hand, the required nutritive substances in the portal blood may be products of digestion of the food of the host.

In order to determine its nutritional requirements and to permit the study of its biological characteristics and toxin, free from contaminating bacteria, we became interested about six years ago in

24. L. R. Cleveland and E. P. Sanders, *op. cit.*
25. V. A. Najjar and L. E. Holt, Jr., The biosynthesis of thiamine in man and its implications in human nutrition. J.A.M.A., **123**:683–684, 1943.

attempting to cultivate *Endamoeba histolytica* without bacteria. It seemed essential to obtain amoebae free from bacteria so as to initiate bacteria-free cultures. Cleveland and Sanders[26] and others had shown that amoebae, recovered from sterile liver abscesses, would not multiply continuously in culture without the addition of living bacteria. Difficulty in producing sterile liver abscesses in animals precluded the use of this method of separating amoebae from bacteria. The isolation of single motile amoebae from culture also seemed impractical because of probable damage to the amoeba during manipulation. We decided, therefore, that the most practical method was to obtain large quantities of cysts in culture; these would be washed as free as possible from bacteria, the remaining bacteria to be killed by a chemical agent without harm to all the cysts. Mercuric chloride in a dilution of 1 to 50,000 in contact with the washed cysts for forty-five minutes was used as the bactericidal agent. This procedure often produced cysts entirely free from bacteria, although anaerobic cultures sometimes revealed resistant bacteria. We are not sure that the washing alone was not responsible for eliminating them, when bacterial sterility was attained.

The next step was to obtain excystation of the bacteria-free cysts.[27] At first, we were unable to obtain such excystation unless we again added living bacteria to the culture. It was found, however, that if the oxygen were removed from the media, either through replacement by nitrogen, by absorption with pyrogallol, or by the use of a reducing agent, such as cysteine, the amoebae would excyst not only in nutritive media but even in normal saline. After excystation, however, they failed to multiply even in nutritive media to which was added a filtrate of the bacteria with which they had previously been growing. The addition of killed bacteria caused somewhat longer survival of the excysted amoebae, but there was no evidence of multiplication unless living bacteria were added.

Dr. Thomas L. Snyder, who entered the study about four years ago, decided that a better approach might be to determine the simplest medium in which the motile forms would multiply in the presence of bacteria. Reardon and Rees[28] had already shown that, if a solid egg-Ringer base were used, normal saline alone as a super-

26. L. R. Cleveland and E. P. Sanders, The production of bacteria-free amoebic abscesses in the liver of cats and observations on the amoebae in various media with and without bacteria. Science, **72**:149–151, 1930.
27. T. L. Snyder and H. E. Meleney, The excystation of *Endamoeba histolytica* in bacteriologically sterile media. Am.J.Trop.Med., **21**:63–73, 1941.
28. L. V. Reardon and C. W. Rees, The cultivation of *Endamoeba histolytica* without serum. J.Parasitol. (Suppl.), **25**:13–14, 1939.

natant fluid, with rice starch added, would support multiplication of the amoebae and cyst formation. Snyder[29] found that an infusion, prepared by incubating the coagulated whole-egg base with normal saline, could be substituted for the egg itself. Even rice starch was not necessary, although it produced more abundant multiplication. He then proceeded to determine what portion of the egg was essential and found that the white of the egg could be replaced by horse serum or peptone, and that the essential part of the egg yolk was contained in its ether-soluble portion. Since this portion was essentially lipoidal in nature, Snyder substituted cholesterol for it and found that a dilution of one to one million was sufficient to support growth, whereas no growth occurred in the absence of cholesterol. This procedure established cholesterol, or some closely allied substance, as a necessary growth requirement of *Endamoeba histolytica*. That cholesterol is not the only substance of this kind, which may be used by the amoebae, is indicated by the fact that Snyder was able to replace it by an ether extract of rice flour, suggesting that a plant sterol also satisfies the requirement.

Having found that an anaerobic condition was necessary for excystation and, assuming that the living bacteria in the culture consumed the free oxygen in the medium, Snyder next established anaerobic cultures with bacteria and found that a medium consisting of cholesterol, peptone, Ringer's solution, cysteine, and rice starch would permit continuous cultivation under anaerobic conditions. The living bacteria were still present but were much less numerous in the anaerobic cultures. In order to simplify the problem of determining what substance, or substances, were provided by the living bacteria, either directly or through their action on peptone or on some other constituent of the medium, Snyder then attempted to establish a culture in this medium from cysts that had been freed from bacteria and to which a pure culture of an anaerobic bacterium from the original amoeba culture was added. In order to accomplish this, it was necessary to add horse serum. Snyder found that the necessary factor in the horse serum was heat-labile. Since the anaerobic bacterium grew luxuriantly in the absence of cholesterol and serum, these two requirements appear to be related directly to the amoeba.

In summary, growth requirements of *Endamoeba histolytica*, thus far determined, consist of water, certain inorganic salts included in Ringer's solution and, possibly, in peptone, cholesterol, a heat-

29. T. L. Snyder and H. E. Meleney, Anaerobiosis and cholesterol as growth requirements of *Endamoeba histolytica*. J.Parasitol., 29:278–284, 1943.

labile factor contained in horse serum, and protection from free oxygen. In addition, it is probable that the amoeba requires certain amino acids present in peptone, some form of carbohydrate represented by starch, and certain other undetermined factors supplied directly, or indirectly, by bacteria. This is as far as our study has progressed up to the present time, but it appears possible that, when a method is perfected for obtaining viable cysts entirely free from all bacteria, the nutritional requirements can be completely determined and cultivation accomplished without bacteria. It may be that portal blood and liver tissue contain all the necessary growth requirements; however, this last possibility has not yet been explored.

THE TOXIN OF *Endamoeba histolytica*

It is obvious from the microscopic pathology of amoebic lesions that *Endamoeba histolytica* possesses and, apparently secretes, a substance that causes a lytic type of necrosis of the tissues without causing any cellular response on the part of the host. By extraction in absolute alcohol, Craig[30] obtained from cultures of *Endamoeba histolytica* a heat-labile substance, which dissolved both intestinal epithelial cells and red blood cells *in vitro*. Apparently no further observations have been made on this toxin, but it is an interesting field for study. The isolation of this substance and its chemical study might be achieved even without accomplishing a pure culture of the amoebae without bacteria, since repeated washing of motile amoebae will remove nearly all the bacteria in the culture. It would be advantageous, however, to study the substance from a culture entirely free from bacteria. This substance might be used for a diagnostic skin test, and it might be used as an immunizing agent. Craig's original work suggested that this substance comprises the antigen for the complement-fixation test. If different strains of *Endamoeba histolytica* could be shown to possess different concentrations of the toxic substance, this finding would strengthen the point of view that there is an actual difference in the pathogenicity of the various strains.

CONCLUSION

A review of the relationship of *Endamoeba histolytica* to the clinical manifestations, which it produces, indicates that this amoeba is a species of parasite which, like many others, contains many strains

30. C. F. Craig, Observations upon the hemolytic, cytolytic and complement binding properties of extracts of *Endamoeba histolytica*. Am.J.Trop.Med., 7:225–240, 1927.

that vary in pathogenic activity; that the pathogenic activity of any particular strain seems to depend upon many factors present in its immediate environment, and that its nutritional requirements are very definite and closely related to its intimate association with the living bacteria among which it usually lives. This review also gives evidence that there are many unanswered questions with relation to the nutritional requirements and to parasitic activity of this amoeba.

However, I believe that a foundation has been laid for the solution of these unsolved problems and I believe that, as our knowledge of nutrition advances and as new technics are developed, we shall acquire a complete understanding of this parasite, which will enable us to deal with it in a more intelligent manner. Meanwhile, physicians and laboratory workers should continue to search for *Endamoeba histolytica* as a possible cause of disease. Physicians should eliminate it by intelligent treatment of all persons whom they find infected, and public health workers should promote the personal and environmental hygiene which will prevent its spread. Even though *Endamoeba histolytica* often produces no clinical disease, it should always be considered as an important enemy of man.