Problems In AMOEBIASIS

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An evaluation of presently held concepts on the role of Entamoeba histolytica and the probable participation of intestinal bacteria in the etiology of this disease, with descriptions of methods of approach to the problems.

Written for students and investigators on various aspects of amoebiasis WITH EMPHASIS ON TECHNIQUES.

An added chapter -- CLINICAL NOTES: ON THE ETIOLOGY, PATHOLOGY, CLINICAL MANAGEMENT AND THERAPY OF AMOEBIASIS by Clarence A. Imboden, Jr., M.D.

By combining a SPECIAL TECHNIQUE OF MICROISOLATION with methods developed from use of antibiotics, it is shown how to eliminate all other species of microorganisms from fecal suspensions containing E. Histolytica, from other situations where its cysts may be deposited, as well as from cultures.

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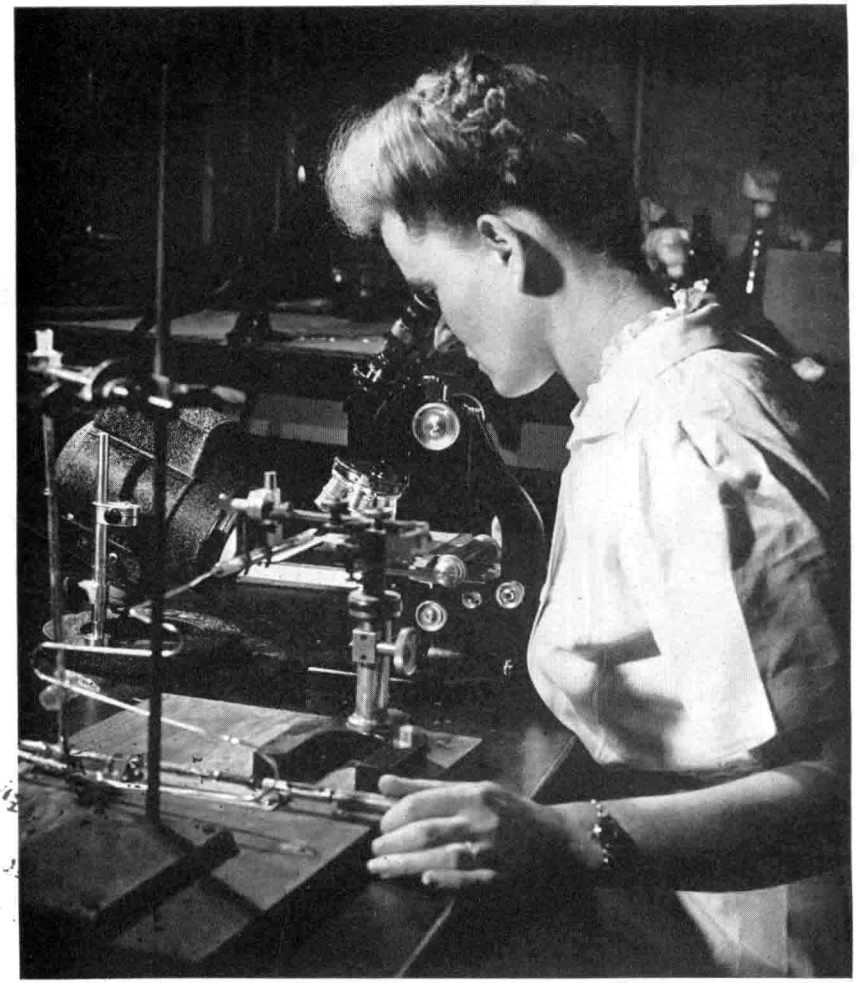

Expert at work on the microisolation of cysts of *Entamoeba histolytica*.

PREFACE

THIS TREATISE was written in the hope that it might be useful to other investigators of problems in amoebiasis, since data available at the present time do not warrant many statements currently made concerning the growth requirements of *Entamoeba histolytica*, factors of its pathogenicity, its relationship to bacteria and/or other microorganisms in the intestinal flora, its metabolism, or concerning chemotherapy. Although in problems of enteritis the importance of studies on *E. histolytica* can in many cases hardly be overemphasized such studies have not received attention of many prominent biochemists and physiologists, probably because the parasite has never been cultivated by itself, and the complications inherent in the activities of associated microorganisms or metazoan cells are formidable. As pointed out by Stephenson* in her excellent book on bacterial metabolism studies on the nutrition of "difficult" pathogens are complicated by the complex and peculiar media, "that are rendered necessary owing to the inability of many parasitic organisms to synthesize for themselves certain molecules essential for growth." However, these difficulties have not proved insurmountable in cases where the pathogens have been obtainable *in vitro* in pure culture. In the case of *E. histolytica* all attempts to find adequate combinations of essential molecules, in the absence of other living things, have proved unsuccessful. It appears, therefore, that a treatise on methods of approach is in order at the present time.

In view of the complex interrelationships between amoebae and species of bacteria in the intestinal tract and in the test tube, a working knowledge of bacterial metabolism is a prerequisite for progress in problems of amoebiasis. Such knowledge is obtainable only by those having had considerable training in

*Stephenson, M. S.: *Bacterial Metabolism*, 3rd Edition. London, Longmans, Green, 1949, 398 pp.

organic chemistry and physics. In addition, investigators of any disease, whether in man or other animals, must have training in clinical and pathological fields, or collaboration with clinicians and pathologists.

In the absence of experimental data by which certain observations on cases of amoebiasis might be explained, investigators have had recourse to hypotheses and theories. However, a number of theories on the relationships of *E. histolytica* to the host have furnished incentives for much diligent work which justifies advancement of the theories even though it may lead to their eventual abandonment. In her discussion of this method of approach to problems, Stephenson has translated a statement by Pasteur with approval, to the effect that adoption of ideas that cannot be rigorously proved may be the best way of looking at things. She was discussing the controversies on the ideas of the organic chemists, Berzelius, Wohler, and Liebig, and of Pasteur, concerning factors of fermentation, and investigations which were forerunners of our present-day concepts of the causation of disease. However, when by abuse of this method the investigator places greater emphasis on proving his theory than in evaluating all of the evidence *pro* and *con* he becomes partner in a situation wherein progress may be arrested rather than advanced. As pointed out later in the present treatise there appear to have been illustrations of such abuse in the history of amoebiasis.

The writer wishes to express appreciation for suggestions offered by fellow workers, particularly to Drs. Victor H. Haas and Willard H. Wright, Director and Assistant Director of the National Microbiological Institute, National Institutes of Health. Drs. Harry S. Eagle, Theodor von Brand, John E. Tobie, Clarence A. Imboden, Jr., Edmund J. Talbott, Harry D. Baernstein, Joseph Greenberg, Mr. John Bozicevich, Miss D. Jane Taylor, and Mrs. Ida L. Bartgis have read and criticized all or parts of the manuscript. My associates Miss Lucy V. Reardon and Dr. Leon Jacobs have also read and criticized the manuscript and rendered invaluable service in preparing the indices and references to

literature. Dr. Elizabeth Verder of the Laboratory of Infectious Diseases, National Microbiological Institute, Dr. Floyd S. Daft, Director, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, and Dr. Leslie Hellerman of the School of Medicine of Johns Hopkins University have also contributed valuable information and suggestions. Under the direction of Miss Inez Demonet of the Medical Arts Section of the National Institutes of Health, Mrs. Frances Rose prepared drawings, and the Photographic Section under the direction of Mr. Roy Perry prepared the photographs. Dr. Clarence A. Imboden, Jr., now at the United States Hospital of Public Health at Carville, Louisiana, with whom the writer collaborated at Bethesda in researches on experimental amoebiasis, has kindly contributed Chapter 7, which is of especial interest to physicians.

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This Book

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By

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CHAPTER I

INTRODUCTION

REGARDLESS of discrepancies among viewpoints of investigators at the present time concerning the host-parasite relationships in amoebiasis, whether *Entamoeba histolytica* invariably produces lesions, whether it may occasionally live within the lumen of the intestine as a commensal, whether symbiosis of amoebae and bacteria is required for pathogenicity, or whether in some cases the occurrence of *E. histolytica* may have nothing to do with the patient's symptoms, there is general agreement that the majority of infected individuals are "carriers." They do not have symptoms that are demonstrable by methods usually employed. On the other hand, amoebic dysentery and/or manifestations of extraintestinal amoebiasis, such as abscess of the liver, lungs, or other organs, are not of infrequent occurrence. The disease is one of the most serious of those caused by protozoan parasites, and its distribution is cosmopolitan.

A Brief Résumé of Investigations on Amoebiasis

In a preliminary analysis of the history of observations and experiments on amoebiasis the writer felt that a clearer discussion might be provided by dividing the period from Loesch (1) 1875, to the present into three epochs based on what appeared to be the dominant opinion of each epoch. In the first epoch extending from 1875 to about 1913 there were grave doubts among investigators that amoebae may cause dysentery. Many of these doubts were removed by the outstanding experiments of Walker (2), and Walker and Sellards (3), on human volunteers. Since some of these volunteers developed dysentery, with amoebae in their stools, following ingestion of material from cases of amoebiasis it appeared that the etiological relationship between *E. histolytica* and dysentery was established. In 1919 the situation was further

clarified in the monograph by Dobell (4) in which the distinguishing characteristics of four species of commensal amoebae and the pathogenic *Entamoeba histolytica* were so clearly described and illustrated that even at the present time, students of the problem having access to the monograph hardly need other guidance. The commensal species include *Entamoeba coli, Endolimax nana, Iodamoeba bütschlii, and Dientamoeba fragilis.* It is not improbable, however, that *D. fragilis* may be implicated in certain cases of enteritis. From extensive work on infections with this amoeba in Panama, Hakansson (5, 6) obtained some evidence for mild pathogenicity. This amoeba does not produce cysts and the questions concerning its pathways of transmission, and sites of proliferation within the intestinal tract are open for future investigations.

Some general statements in the above monograph are mainly of historic interest at the present time but nevertheless they had a profound influence on the initiation of the second epoch during which *E. histolytica* was regarded as an absolute pathogen that, according to the dominant viewpoint, obtained sustenance wholly from tissues of the host. Dobell stated that the amoeba obtained nourishment only through absorption, that within the host it never ingests bacteria, and despite a previous demonstration of its growth by Cutler (7) that *E. histolytica* would never be cultivated *in vitro.* However, Loesch (1) described cocci, vibrios, and bacilli, in amoebae from the first documented case of dysentery, although on the basis of this observation and Dobell's dictum, Hegner and Taliaferro (8) expressed the opinion that Loesch had observed *E. coli.* The demonstration of a practicable method of growing the amoeba by Boeck and Drbohlav (9) in 1925, has led to the gradual emergence of a third epoch which, as already indicated, is characterized by wide discrepancies among the viewpoints of present day investigators.

After having made the above-mentioned analysis of the history of amoebiasis the writer noted an analysis previously made by Calkins (10). Calkins divided the history into four periods: *"Early Taxonomic Observations," "Early Etiological Observations," "Period of Taxonomic Chaos,"* and *"The Modern Period."*

His analysis, as well as the earlier one by Dobell (4), and a somewhat later one by Craig (11) give the significant events in considerable detail so that further elaboration here is unnecessary. It is of interest, however, that all three of these authors accepted the viewpoint that *E. histolytica* is an obligate pathogen.

The present epoch is characterized by conflicting viewpoints of those who adhere, with certain reservations, to the above-mentioned opinions concerning pathogenicity, and others who follow an opinion expressed as early as 1909 by Elmassian (12) and advocated most clearly by Reichenow (13, 14, 15,) in 1929 and later that *E. histolytica* may be a commensal. In the first category such authors as Craig (16), Faust (17), and more recently Anderson *et al.* (18) may be considered, while Hoare (19) is a most recent advocate of Reichenow's views. The occurrence of "carrier" cases is explained by those who believe that *E. histolytica* is always pathogenic on the basis of continuous healing of small lesions produced by the amoeba so that well-balanced host-parasite relationships became established. Reichenow's principal argument against this viewpoint is the occurrence of numbers of cysts, and/or trophozoites in the stools of "carrier" cases beyond those that may be calculated on the basis of amoebae escaping from small lesions. To account for this situation he supported the viewpoint that *E. histolytica* may multiply solely within the lumen of the intestinal tract. Carrier cases are also characterized as "cyst passers," sometimes without apparent recognition of the fact that the cysts are derived from amoebae that proliferate at certain levels of the digestive tract. As pointed out by Reichenow, trophic amoebae are passed following administration of cathartics. Examination of such trophozoites shows ingested bacteria and particles of the intestinal content. Seldom, if ever, have ingested erythrocytes in the amoebae, or other evidences of intestinal hemorrhage, been observed in these cases. A principal obstacle in the way of acceptance of Reichenow's view is its failure to account for the admitted pathogenicity of the amoeba. If, as advocated by Craig (20) and others, *E. histolytica* produces a cytolysin or a toxin some damage to the

intestinal mucosa must always occur. French investigators, Brumpt (21), Deschiens (22, 23), and others, have advanced a theory that there are two species having the cytological characteristics of *E. histolytica* except as to size, one being nonpathogenic and the other pathogenic. A considerable number of investigators, including Meleney and Frye (24), and Anderson *et al.* (18), have produced and cited evidence for the occurrence of strains of *E. histolytica* differing in virulence. In discussing pathogenicity, Anderson and associates concede that many factors such as changes in the physiological state of the parasite and of the host may be operative. In this connection, the studies of Alexander and Meleney (25) are of particular interest. One community in Tennessee had a high incidence of infection with *E. histolytica* with little evidence of pathogenicity. Another community having a high incidence had many cases of dysentery. Persons in the community with little evidence of pathogenicity had better diets, with a greater variety of foods, with richer vitamin content than those in the community with comparatively many cases of dysentery. There was greater use of milk and other foods containing vitamin D in the first community than in the second.

A most attractive theory involves the participation (symbiosis) of the amoeba with certain species of bacteria in the development of pathogenicity. This theory has many advocates dating back even to the time of Loesch, and has been emphasized by other prominent students of the problem such as Councilman and Lafleur (26), Walker and Sellards (3), Westphal (27), and Cleveland and Sanders (28). Westphal infected himself with *E. histolytica* by swallowing material from a convalescent case of amoebic dysentery, becoming positive for the amoeba within several days but developing no symptoms of dysentery during a subsequent period of eight months. He then drank fluid from the stool of another case of amoebic dysentery from which the amoebae had been removed by passage through filter paper. Probably attributable to the bacteria occurring in this fluid he developed amoebic dysentery after twenty-three days. A friend without infection with *E. histolytica* also drank some of the