A HISTORICAL OVERVIEW OF AMEBIASIS*

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THE claim that amebiasis is an ancient disease of mankind can be cogently argued, but not convincingly proved. Although detailed descriptions of dysenteries were recorded by the Chinese, Hebrews, Greeks, and others, they do not represent, given the protean nature of many of the symptoms and signs of the disease, unequivocal reports of amebiasis, nor can they. No doubt, some of these writings do indeed describe amebiasis; others describe a variety of dysenteries.

It is clear from a review of British writers of the early 19th century that physicians in India were aware that there were different types of dysentery. Ballingall,[1] in 1818, wrote about "acute colonitis" and "hepatic flux," and described a surgical technique to drain liver abscesses.

In 1828 James Annesley[2] published two monumental folio volumes in which he gave detailed descriptions of cases of both liver abscess and dysentery, but he was unsure of the relationship between the two. In his discussion of what he termed "hepatic dysentery or dysentery complicated with disease of the liver," he was not sure whether liver abscess came first and caused dysentery or vice versa. But his recorded clinical observations leave little doubt that he was describing amebiasis.

In 1832 William Twining[3] published a book in which he confirmed many of Annesley's observations, but added little that was new. Twelve years later, Parkes,[4] a physician in the Indian Army Medical Service, published a book containing detailed descriptions of clinical cases and postmortem examinations of patients with amebiasis. Parkes clearly associated dysentery and liver abscess. He wrote: "The causes of primary hepatitis, meaning by that term the low insidious suppurative form, generally in an advanced stage, compli-

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cated with dysentery, are much more obscure.'' He comments on the use of ipecacuanha in treating the disease. It should be noted that ipecac bark had been a widely used empirical agent for intestinal diseases and specifically dysentery since its introduction into Europe in 1658 by Piso. Parkes states that large doses of ipecacuanha are far more effective than smaller ones. This was later confirmed by Docker[5] in 1858, who treated cases of amebiasis in Mauritius. Parkes treated 50 cases with ipecacuanha over a period of four years with only one death. He wrote: ''... ninety grains of ipecacuanha have been given, and forthwith the character of the disease, or I should rather say, the character of the symptoms has been entirely changed; for the disease itself is literally cured, put a summary stop to, driven out.''

In 1859 Wilhelm Lambl published a report on the character of the stools in disease in general. Although he described some intestinal parasites, he did not apparently describe amebae.[6] In 1860 he published another paper and, while discussing the existence of organisms in the stool, did not mention amebae.[7] We shall probably never know whether or not Lambl actually did see amebae. His descriptions and drawings are difficult to interpret in the light of subsequent knowledge. Dobell[8] suggested that Lambl may actually have seen degenerating forms of the flagellate *Trichomonas hominis*.

Lewis[9] in 1870 and Cunningham[10] in 1871 described amebae in the stools of cholera patients in India. Whether these amebae were *Entamoeba histolytica* or some other species such as *Entamoeba coli* is unknown.

Fedor Losch (Lesh),[11] a Russian physician, published what many considered the first detailed description of a case of amebic dysentery in 1875. What places Losch's publication in a superior category is its detailed clinical descriptions of recurrent intestinal amebiasis, the author's meticulous studies of autopsy material, and, perhaps most important, his careful analyses and descriptions of the content of the patient's stools. Losch gives a splendid description of amebae, including structure, size, motility, nucleus, vacuoles, and such intracytoplasmic elements as red blood cells. Losch also provided drawings of amebae to accompany the article. In addition, he performed experiments in which he administered one to two ounces of the patient's stool to four dogs, orally and rectally. Only one of the animals developed dysentery, and its stool, both pre and postmortem, contained large numbers of amebae.

Losch named the amebae from his patient *Amoeba coli*. He concluded that his patient's dysentery was sustained by the amebae, but not caused by it, because of the different clinical courses observed in his patient and in the one

experimentally infected dog which became ill. The former presented with an acute disease characterized by fever and "disturbances of his general well being." The dog, however, developed mild symptoms slowly without fever or generalized debilitation. Had Losch entertained the notion that the disease could have a broad clinical spectrum, he might have reached another conclusion.

In 1883 Koch,[12] working in Egypt, carefully studied five cases of dysentery, two complicated by hepatic abscess. He not only observed numerous amebae in colonic ulcers, but also noted on microscopic examination of tissue sections that the amebae were situated deep in the tissues and in capillaries close to the walls of hepatic abscesses. Koch thought that the amebae might have a role in the pathogenesis of the disease and encouraged his associate Kartulis to study this. Koch was the first to describe amebae in stained tissue sections, but did not publish his 1883 observations until four years later, after Kartulis had published his.

Kartulis[13] published his observations on 150 cases of dysentery among Egyptians in 1886. He described the organisms he found in both stool specimens and in tissue sections, and conducted a number of experiments on their survival time in sugar solutions, salt water, and in hanging drop preparations. He not only provided splendid descriptions of the changes produced by amebae in the bowel but also reported on the results of colonic introduction of infected stool into two guinea pigs and a rabbit. None of the animals developed the disease. Kartulis' conclusion that amebae were present in every case of dysentery was probably erroneous in light of current knowledge, but he clearly attributed a casual role for dysentery to amebae and coined the term "tropical dysentery" for the disease. The following year, 1887, Koch published his 1883 observations and Kartulis[14] published a second paper, in which he described his studies of amebic liver abscess, a complication of tropical dysentery.

The same year, 1887, in which Koch and Kartulis published their observations, a Czech, Jaroslav Hlava,[15] working in Prague, reported that he had found amebae similar to those described by Losch in all of his 60 cases of dysentery. He injected stools containing amebae into the rectums of 17 dogs, six cats, and a number of other animals. He obtained positive results in two dogs and four cats.

Hlava's publication appeared in Czech and was abstracted for the German literature by Kartulis the year it appeared, 1887. Unfortunately, his name was not included, and the first two words of the title in Kartulis' abstract "*O*

Uplavaci,'' which means ''On Dysentery,'' was thought to be the author's name for almost 50 years and was listed as such in numerous bibliographies until Clifford Dobell, a British protozoologist, clarified the error in 1938.[16]

The historical importance of Hlava's observations lies in the fact that he experimentally infected kittens with *Entamoeba histolytica*. Cats and kittens were extensively used by subsequent researchers for the experimental study of amebic dysentery.

William Osler was the first to describe ameba in a case of dysentery and liver abscess in the United States. His patient, a 29-year-old physician, had resided in Panama for almost six years, and Osler saw him at the Johns Hopkins Hospital. In his 1890 publication[17] Osler concluded: ''It is impossible to speak as yet with any certainty as to the relation of these organisms to the disease.'' His observations, however, did ignite a great deal of interest among his colleagues at Johns Hopkins and elsewhere. The same year, Henri Lafleur, a Canadian resident at Johns Hopkins, reported a case of dysentery in a sailor from whom *Amoeba coli* was isolated in the stool. Simon, who was also at Johns Hopkins at the time, described a case of amebic hepatic abscess with perforation into the lung and *Amoeba coli* in the sputum.[18]

A number of other excellent observations on the disease were published in 1890, including those of Stengel[19] and Muser.[20] These were followed by Dock's[21] report in 1891.

In 1891 William Councilman, associate professor of pathology at Johns Hopkins, working under Welch in the Pathological Laboratories, and Henri Lafleur published a landmark monograph.[22] The three previously published cases were presented and a total of 15 cases reviewed. Councilman and Lafleur first recognized amebiasis as a distinct clinical disease due to a specific pathogen, which they called *Amoeba dysenteriae*. They were the first to use the now common terms ''amebic dysentery'' and ''amebic abscess of the liver.'' Their descriptions of the pathological lesions present in the disease and of the parasite are superb. They underscored the significance of hepatic abscess, pointing out its occurrence in individuals not suffering from dysentery. The detailed pathological and clinical descriptions and accompanying drawings have made Councilman's and Lafleur's monograph a classic still current today.

In 1892 Kovacs[23] successfully produced dysentery in five kittens inoculated with fecal material from patients with human amebic dysentery. And in 1894 Kruse and Pasquale[24] produced a monumental work of 149 pages describing experiments with kittens and confirming previous clinical reports.

Employing pus from hepatic abscesses, they conducted three experiments. In two of these they established that the hepatic pus was free from bacterial contamination. When their kittens developed amebic dysentery, it finally proved that the disease was directly initiated by amebae. Until that time, the general view was that amebae were opportunistic organisms which simply invaded and aggravated existing lesions.

In 1893, a year prior to the publication of Kruse and Pasquale, Quinck and Roos[25] published an extremely important paper, long overlooked. Working in Kiel, they first demonstrated the cyst form of amebae and reported that vegetative forms caused dysentery when injected into the rectum of kittens but were harmless when given by mouth. They also demonstrated that cysts could survive up to 20 days in a moist chamber, and cause dysentery when given by mouth. They also distinguished between *Entamoeba histolytica* and *Entamoeba coli,* and opened the way to the elucidation of the mode of transmission of amebic dysentery.

At the time, however, *Entamoeba histolytica* was called *Amoeba dysenteriae* by the Americans and *Amoeba coli Losch* or *Amoeba coli felis* by a number of Europeans, including Quincke and Roos. The latter two researchers proposed the name *Amoeba intestini vulgaris* for an ameba which was not pathogenic for man,[26] probably *Entamoeba coli.* In addition, they described another ameba, which they named *Amoeba coli mitis,* and which they claimed was pathologic for man but not cats.

In 1893 Schulberg[27] tried to put some order into the conflicting reports and claims regarding the pathogenicity of various amebae, but met with little success. Casagrandi and Barbagallo[28] described *Entamoeba hominis* (called *Entamoeba coli* today) in 1897.

The discovery of the dysentery bacillus by Shiga in 1898 and its confirmation by Flexner in 1900 went a long way toward demonstrating that dysentery could be independently caused by different pathogens.

In 1903 Huber[29] detailed the number of nuclei found in cysts of *Entamoeba histolytica*: "On occasion one finds two to four nuclei." Huber published further on this matter in 1909.[30] The decade following Huber's work was characterized by great confusion. Some investigators ignored or disregarded earlier work and Fritz Schaudinn, an otherwise brilliant zoologist, came to some conclusions about the reproduction of amebae that in retrospect Dobell[31] found "so incredible that it is difficult to believe that they were not sheer inventions." Added to this was the perplexing observation of amebae in patients to which no causation of

disease could be established. This had been pointed out not only by Casagrandi and Barbagallo, but by Grassi in 1879, Calandruccio in 1890, and Cellini and Fiocca in 1894. Although Huber personally communicated with Schaudinn about finding multiple nuclei in amebic cysts, the latter proposed that *Entamoeba histolytica* reproduced by spore formation and *Entamoeba coli* by schizogony.[32] Schaudinn claimed that Huber's quadrinucleated cysts belonged to a distinct species which he called *Entamoeba tetragena*. Schaudinn's eminence intimidated Huber, who went along with the former's interpretation, abandoning his own.[33]

Schaudinn, however, did differentiate between the harmless *Entamoeba coli* and the pathogenic *Entamoeba histolytica*. He coined the name *Entamoeba histolytica* because of its tissue-destroying capacity and *Entamoeba coli Losch* for the nonpathogenic ameba. Schaudinn himself died at the age of 35 of complications of self-inflicted amebiasis.[34] Schaudinn's views confused matters for a decade, and a number of scientists significantly erred in trying to fit their findings into his concepts: Viereck,[35] who described cysts of the supposed *Entamoeba tetragena* as a variant of *Entamoeba coli*, Hartmann,[36] who considered *Entamoeba tetragena* a distinct species, and Elmassian,[37] who coined the term *Entamoeba minuta* for the nonhematophagous trophozoites found with quadrinucleate cysts.

Craig,[38] an American parasitologist, reported his confirmation of Schaudinn's and his own misinterpretations. Later he demonstrated that *Entamoeba histolytica* reproduced in cyst form by formation of four nuclei.[39] Such errors are understandable because these researchers were attempting to unravel what we now know to be a life cycle ranging from trophozoite to cyst forms and a spectrum of clinical behavior ranging from harmless commensalism to lethal pathogenicity.

In 1900 Strong,[40] working in the Philippine Islands, differentiated pathogenic amebae (*Entamoeba histolytica*) from nonpathogenic ones (*Entamoeba coli*), but his work was generally overlooked. A year later, in 1901, Harris produced amebic liver abscesses by intrarectal infection of puppies with *Entamoeba histolytica*. Other workers later duplicated this experiment in cats, including Craig (1905), Huber (1909), Wenyon (1912), Baetjer and Sellards (1914), and Dale and Dobell (1917).[41]

The next major breakthrough came with the work of Walker and Sellards[42] in 1913, who conclusively demonstrated what Strong had demonstrated 13 years before, that *Entamoeba coli* was nonpathogenic and *Entamoeba histolytica* pathogenic. They also demonstrated that *Enta-*

moeba histolytica did not always give rise to clinical disease. Their experiments fed material containing the two types of cysts to human volunteers in a Manila prison. Their classic experiment not only differentiated the pathogenicity of the two amebae, but also showed that man could be infected by the cysts of *Entamoeba histolytica*. Walker and Sellards suggested that *Entamoeba histolytica* could act as a commensal, a view strongly denounced by a number of eminent authorities including Craig, Dobell, D'Antoni, and Faust.[43]

In 1912 Leonard Rogers,[44] professor of pathology at the Medical College Hospital in Calcutta, reported the successful treatment of both intestinal and hepatic amebiasis by injectable salts of emetine. Rogers administered emetine to three patients who were unable to take ipecacuahana by mouth. Emetine, the principal alkaloid of ipecacuahana, had been shown the previous year by Vedder[45] to be effective in killing amebae *in vitro*. This was a major breakthrough in the treatment of the disease. Two years previously, in 1910, Rogers had reported the prevention and treatment of amebic abscess of the liver with ipecac.

Musgrave and Clegg[46] were the first to cultivate *Entamoeba histolytica in vitro* in 1904, using blood agar plates containing a single species of bacteria. They also introduced the term "amebiasis." It was not until 1925 that Boeck and Drbohlav[47] cultivated the organism on an artificial medium.

During the first quarter of the present century, great controversy stormed among parasitologists over the species splitting of amebae and the issue of pathogenicity. Dobell,[48] an eminent British protozoologist, and others maintained that *Entamoeba histolytica* can only exist by living on the tissues of its host. They rejected the concept of commensalism which finally won acceptance in the early 1950s with Hoare's work.[49,50] Hoare's work was built upon the earlier observations of Walker and Sellards and on those of Kuenen and Swellengrebel[51] who in 1913 reported that *Entamoeba histolytica* had three phases: an invasive *histolytica* phase, a commensal *minuta* phase, and a cystic *tetragena* phase.

As battles over taxonomy and commensalism were being fought, progress was made in laboratory diagnosis and treatment. It was early observed that routine stool examinations did not always result in positive identification of amebae. Higher yields were obtained with fresh and purged specimens. In 1938 Faust and his colleagues[52] developed the important zinc sulfate technique to concentrate parasitic organisms in the stool. This

was followed in 1948 by Ritchie's[53] formalin ether concentration technique and in 1953 by the merthiolate-iodine formalin (MIF) procedure of Sapero and Lawless.[54] A fixative procedure known as the polyvinyl alcohol fixative technique, developed by Brooke and Goldman[55] in 1949, preserved the trophozoites for subsequent staining. In 1967 Burrows[56] improved the polyvinyl alcohol fixative and over the years a variety of stains were developed to facilitate identification of amebae.

A number of methods were developed to culture *Entamoeba histolytica in vitro*. Among the first of these was the medium developed in 1925 by Boeck and Drbohlav.[57] Other workers developed various media, but none ever proved critical in identification of the organism. For, even if cultures are positive, the basic problem of differentiating *Entamoeba histolytica* from other amebae remains.[58]

As early as 1914 Izar[59] developed a complement fixation test for amebiasis, using an antigen composed of the watery extract of stool containing the parasite. Craig[60] worked many years to develop a complement fixation test using alcohol extracts of amebae-rich dog feces, culture material obtained from the Boeck and Drbohlav technique, and Stone's antigen consisting of washed cysts. In 1942 he was optimistic about its usefulness, but others found that it gave inconsistent results and was not particularly useful in the diagnosis of the disease. One problem with the test was the presence of bacteria in cultures used for antigen production.

Rees[61] and his co-workers made a significant advance in 1942 with the development of microisolated amebae which were cultured and from which antigen was then obtained. This test subsequently proved of considerable value in the diagnosis of amebic liver abscess. Elsdon-Dew and Maddison[62] showed in 1952 that antigen produced from monobacterial cultures gave high positive results in amebic liver abscess but was not especially useful in cases of intestinal amebiasis.

Kessel and co-workers[63] first reported results using the indirect hemagglutination test. Maddison, Powell, and Elsdon-Dew[64] compared results from the indirect hemagglutination test and a gel diffusion test in 1965. Goldman[65] reported on the results of immunofluorescence in 1966. In 1970 Tupasi and Healy[66] reported on the bentonite flocculation test and Morris, Powell, and Elsdon-Dew[67] on the latex agglutination test.

These efforts at developing serologic techniques for the diagnosis of amebiasis have demonstrated that antibodies arise only as a result of parental contact with amebae. Negative results occur when amebae are

confined to the lumen of the bowel. Antibodies persist for a long period of time and produce positive reactions even when active infection is no longer present.[68] Elsdon-Dew[69] points out that positive results imply past or present invasion with *Entamoeba histolytica*.

The history of the development of therapies for amebiasis is indeed a long one. Rogers' 1912 report on the use of injectable emetine was a major turning point in the treatment of the disease. However, it is well to remember that ipecacuanha, which had been introduced into Europe in 1658 from Brazil, was widely used in India in the mid-19th century for certain types of dysentery. It is the dried root of *Psychotria ipecacuanha*, which has been grown in India and Malaysia for a long time. Craig[70] reports that emetine, one of the three alkaloids found in ipecacuanha, was first isolated in 1817 by Pelletier. Until 1909 the powdered root was used widely, especially in India. But in that year both Simon[71] and Dock[72] urged the use of salol-coated pills of ipecacuanha in amebiasis. Vedder's demonstration of the powerful amebicidal effects of emetine *in vitro* paved the way for Rogers' clinical use of injectable salts.

In 1908 Deeks reported his experience with bismuth subnitrate in the Panama Canal Zone. It gave excellent results, but in 1914 he combined it with emetine with even better results[73] because of the intestinal action of bismuth and the extraintestinal action of emetine. This combination, however, never became popular, especially after Du Mez[74] reported excellent results from the Philippines using emetine-bismuth-iodide, a combination that gained wide acceptance among the British for many years.

In 1923 a French worker, Marchoux,[75] introduced an arsenical, acetarzone (Stovosal), for treating syphilis, which was also used for treating amebiasis. It is extremely toxic and never won wide acceptance for treatment of amebiasis. In 1921 Muhlens and Menk[76] introduced chinofon, an iodo-hydroxyquinoline relatively free of toxic side effects, but this drug, like bismuth and the arsenicals, acted on the intestinal phase of amebiasis, and had no efficacy in treating extraintestinal amebiasis.

Much excitement was created in 1932 when Reed and his co-workers[77] reported on their carefully controlled study of another arsenical, carbarsone. Because of its cysticidal and trophozoiticidal potency and because it was relatively nontoxic in therapeutic doses, it was a major advance in the treatment of amebiasis.

The 1930s witnessed the introduction of two important halogenated hydroxyquinolines. Important among these were iodochlorohydroxyquine

(vioform), introduced by Anderson and Koch[78] in 1931 and diodoquin, introduced in the early and mid-1930s by a number of workers. Following World War II, other agents were introduced. Berberian[79] published his experience with bismuth glycolylarsanilate (milibis) in 1948, and Anderson and co-workers[80] reported on the efficacy of thiocarbarsone in 1947. Conan[81] reported on the effectiveness of chloroquine in extraintestinal amebiasis in 1948. Two years previously, Jones[82] had shown that atabrine was more effective in extraintestinal amebiasis than emetine.

With the advent of antibiotics, it was to be expected that a number of them would be tested for their effectiveness as amebicides. Hargreaves[83] reported in 1946 that penicillin and sulfonamide were nonspecific for amebiasis, but useful adjuvants. Aureomycin had no lasting therapeutic effect, and, although terramycin had some efficacy in acute amebic infections, significant numbers of relapses occurred. Anderson[84] reported in 1952 on the efficacy of fumagillin.

Paromycin, an antibiotic produced from cultures of *Streptomyces ramosos* and marketed under the trade name of Humatin, was first shown by Elias and Gonzalez[85] in 1959 to be effective in human amebiasis. Its *in vitro* effectiveness had been previously demonstrated by Thompson and co-workers.

Dehydroemetine, synthesized by Brossi and his co-workers in 1959, has been widely used because of its similarity to emetine in mode of action and lower levels of toxicity.

Metronidazole (commercially known as Flagyl) was first used as a trichomonicidal agent, and in 1966 Powell and his colleagues[86] demonstrated its effectiveness as an amebicidal agent in both intestinal and extraintestinal amebiasis. A number of other therapeutic agents have been developed over the past 50 years, some of them still in use today.

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