G.P. Review Article

AMOEBIASIS: ITS MEANING AND DIAGNOSIS

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There are few other conditions about which there have been so many misconceptions or as much misunderstanding as amoebiasis. However, had later workers taken the trouble to study early writings, much of this could have been avoided. The original description of the amoeba by Lösch[35] in 1875 is a masterpiece, not only of observation, but also of experiment and deduction. Similarly, in 1891, Councilman and Lafleur[7] published a description of the clinical and necropsy findings which has never been equalled.

HISTORY

It was about the beginning of the century that the position became confused, and it has taken over half the century for some semblance of reason to reappear. The full story has appeared in a recent publication,[21] but the salient points are worthy of repetition.

The Cysts of Entamoeba histolytica

The original description distinguishing *Entamoeba histolytica* from *Entamoeba coli* by Schaudinn[54] contains such flights of imagination as budding and spore-formation, and the author claimed to have infected cats by means of dried spores. It must be recalled that it was Schaudinn's description of the entry of a malaria sporozoite into a red blood cell which delayed for so long the discovery of the exo-erythrocytic phase of these parasites.

Schaudinn insisted that the quadrinucleate cysts were those of another parasite, which he named *Entamoeba tetragena*, a name which persisted until Walker[56,57] showed that these and the *Entamoeba minuta* of Elmassian[17] were phases in the life-cycle of *E. histolytica*.

Commensal and Promethean

The infection experiments of Walker and Sellards[58] are often cited, but one wonders whether the quoters ever read the original, for few seem to have appreciated the nuances of the report. Kuenen and Swellengrebel[33] also suggested that the 'histolytica-stadium' played no part in the normally commensal life-cycle of the parasite, a view also held by Mathis and Mercier.[39] Their concept that the 'minuta' non-haematophagous trophozoites found associated with cysts constituted the normal relationship with man, was derided by Dobell[12] in his book *The Amoebae Living in Man*, which was to become the vade-mecum of so many medical parasitologists. At that time (1919), Dobell believed that the equilibrium between man and amoeba could be likened to that between Prometheus and the eagle, an analogy which has led to the use of the word Promethean to describe that school of thought which believed that, as the amoeba nibbled the mucosa, this was more or less continuously replaced. The school following Swellengrebel was called Commensalist.

Reichenow, himself a carrier, pointed out[48,49] that the enormous number of cysts passed by apparently healthy people was incompatible with the Promethean concept that their progenitors were attacking the mucosa. Brumpt,[4] struck by the differing manifestations in tropical and temperate climates, postulated that 2 morphologically identical species were involved—the potentially pathogenic *E. dysenteriae* of the tropics, and the harmless *E. dispar* of temperate zones.

Unfortunately, few people seem to have read a later paper by Dobell,[13] in which he reversed his earlier view stating that the amoeba 'sometimes lives in man as a harmless commensal'. It was Hoare[30] who consolidated the commensalist view, concluding: 'Since they are the only amoebae capable of producing cysts, the *minuta* forms represent the essential stage of *E. histolytica*, whereas the haematophagous amoebae have no place in its normal life-cycle'.

Clinical Interpretation

Iatrogenic amoebiasis. Unluckily, the Promethean view was given enormous publicity, particularly in the USA, and in textbooks which were regarded as authoritative.[9,10] The gamut of symptomatology attributed to the amoeba became enormous, covering practically every clinical presentation except pregnancy.[38] Obviously 'Amoebiasis became the regular resort of the diagnostically destitute',[19] and such iatrogenic amoebiasis[20] became a fashionable disease and a social status symbol in some communities. Many a patient found it a convenient excuse for his inability to cope with his environment. Unscrupulous physicians found the diagnosis lucrative—Sir Philip Manson-Bahr's 'Emetine Joe' was famous.

Chronic amoebic hepatitis. Rogers must have been horrified by the misinterpretation of his famous Lettsomian Lectures.[52] In endemic areas, the use of a systemic amoebicide in cases of acute painful hepatomegaly associated with fever and a leucocytosis may well avert the formation of an amoebic liver abscess, but there seems little justification for the concept of a non-suppurative, diffuse amoebic infiltration of the liver postulated to account for 'chronic amoebic hepatitis'. A number of workers[31,32,45,46,51] have deprecated the concept, having been unable to find pathological justification. Though the idea has been revived, somewhat vociferously, by Doxiadis,[15,16] his findings have not proved acceptable.

Morphological Confusion

Entamoeba hartmanni. Wenyon and O'Connor[59] were struck by the variation in cyst size in their cases in Egypt, and Dobell and Jepps[14] felt that there were races of *E. histolytica* with cysts of different sizes. Though Von Prowazek[55] had described a species with quadrinucleate cysts from 6 to 8 μ in diameter, acceptable to Kuenen and Swellengrebel[34] and to Brug,[3] Dobell, in his classic book,[12] was scathing of their views, insisting that there was but one species. This view was perpetuated by Craig and Faust[10] whose *Clinical Parasitology* became a standard text.

In 1942, Sapero *et al.*[53] published a statistical study which showed that there were 2 significantly different races —a large one and a small one. They would not go so far

as to say that these were different species, and the terms 'large' and 'small' were used. Compound names such as *E. histolytica* var. *histolytica* and *E. histolytica* var. *hartmanni* were suggested but, ultimately, other evidence was forthcoming—morphological,[5,6] cultural[24,25] and serological[28]—and *Entamoeba hartmanni* is now regarded as a distinct species.

Laredo. In 1956, Dr Connell of Houston, Texas, cultured an amoeba from a patient with a 3-year history of diarrhoea. Morphologically, it was apparently *E. histolytica,* but it multiplied in culture at room temperature as well as at 37°C and it became known as the Laredo strain of *E. histolytica.* However, in 1962, Goldman *et al.*,[29] using micro-immuno-fluorimetry, showed that there were antigenic differences, and Bragg and Reeves[2] showed differences in enzyme systems. Entner and Most[23] found that Laredo and 2 other temperature-tolerant strains—AG from a case of 'mild chronic amoebiasis' and JA from a mental patient —were much more resistant to drugs than was a regular strain of *E. histolytica* (K9 from the Korean epidemic).

Richards *et al.*[50] isolated yet another strain (403, from an asymptomatic carrier) and found that it and the classical Huff strain used by Beaver *et al.*[1] in their prison experiment were also insensitive to temperature. They went on to show that all 5 strains—Laredo, AG, JA, 403 and Huff—could tolerate considerable dilution of the culture overlay, developing contractile vacuoles similar to those seen in free-living amoebae. Goldman and Cannon[27] later showed that, in addition to antigenic differences, these temperature-tolerant strains were not infective to guinea-pigs. Neal and Johnson[41] showed that they were less virulent to rats.

It is thus apparent that there are, in the human gut, species of amoebae morphologically indistinguishable from *E. histolytica,* but whose other characteristics are very different. At the Teheran Conferences on Tropical Medicine and Malaria it was decided that, as a working name, the term 'amoebae of Laredo-type' be used.

The implications of these findings are indeed far-reaching. These amoebae have deceived the experts, and many conclusions reached in the past will have to be reviewed in the light of the new knowledge. Survey results, even if they did take *E. hartmanni* into account, now have even less value. Many experiments will have to be repeated to ensure that the '*E. histolytica*' used were not in fact of Laredo type. It is possible that Brumpt's[4] *E. dispar* was temperature tolerant, particularly as all isolations of this latter group have been in temperate climates.

THE PRESENT

Morphology

Entamoeba histolytica is but one of a number of species of amoebae found in the human gut. Many of these can be recognized by experienced microscopists, the most reliable feature being the nature and distribution of the nuclear chromatin. This is but seldom examined in routine laboratories as it implies staining and a more prolonged search for the amoebae.

Most commonly encountered are the cysts, those of histolytica-type having 4 nuclei with fine peripheral chromatin and a centrally placed karyosome. The cysts of *E. hartmanni* can be distinguished by their size, but, as there is overlap, the distinction must be based on the average diameter of a number of cysts; mixed populations may cause difficulty. Morphological criteria are inadequate for the recognition of the Laredo-type amoebae ; cultivation, always an erratic procedure, is necessary. In any case, the finding of cysts of *E. histolytica* would merely be evidence of a commensal infection, and no direct indication of invasion of the tissues.

The trophozoites of *E. histolytica* occur in 2 forms, both having fine peripheral nuclear chromatin and a central karyosome. The smaller 'minuta' form, with purposeful movement and clear pseudopodia, contains bacteria and other faecal debris indicative of its commensal character. Staining may be necessary to exclude *Endolimax nana* and *E. hartmanni.* The other form is larger and even more active, and often contains red blood cells. Such haematophagy is evidence of the invasive nature of the infection and, as it has not been reported in Laredo-type, is probably an absolute criterion of species, as well as of pathogenicity. Had the old-fashioned idea that no non-haematophagous amoeba be called *E. histolytica* been sustained, much misdiagnosis and no little grief would have been averted. Its presence or absence should always be reported by an examining laboratory.

Thus, there is only the one morphological criterion on which the crucial diagnosis of 'invasive amoebiasis' can be based. The finding of cysts or of non-haematophagous trophozoites, even were their identity certain, is no evidence of disease. It must also be remembered that, as not infrequently happens in liver abscess, invasive amoebiasis may occur in the apparent absence of the parasite in the gut.

Serology

For proper appreciation, it is necessary to follow the evolution of the methods in use. The tedium of stool examination prompted many early workers to attempt a serological approach, but there were 2 main difficulties which delayed the development of a comprehensible test. The production of antigenic material is no easy affair, and indeed, the first antigen, used by Craig[8] in a complement-fixation reaction, consisted of an alcoholic extract of mucoid faeces from dogs infected with *E. histolytica.* Cultivation is not easy and until recently[31] it has not proved possible to grow the amoeba in the absence of other living organisms, which of course contribute to any extract used as antigen. Such extraneous material might, in such 'blind' tests as complement fixation, give rise to fallacious results.

The second difficulty, that of evaluating any procedure devised, proved a major stumbling-block for many years. Any attempt to correlate the finding of antibody with the presence of parasites in the stool was, as can be appreciated from preceding paragraphs, doomed at the outset. Similarly, correlation with the peculiar clinical diagnoses current was unlikely to prove profitable. It is small wonder that the tests fell into disrepute. Only complete integration of laboratory and clinical aspects would allow a reliable evaluation to be made.

A preliminary trial in Durban,[22] using a complement-fixation reaction with an antigen derived from micro-isolated amoebae grown with a single bacterial concomi-

tant, convinced us that, though there was something in the concept, an *ad hoc* approach would be futile and more exploration was necessary.

It was at this time that Ouchterlony[42] published the technique of double diffusion-in-gel which was to have such an impact on serology. In this test antigen and antibody are allowed to diffuse towards one another from wells in an inert gel such as agar. A number of bands of precipitate may appear, each corresponding to an individual antigen-antibody system. By use of appropriate patterns of wells, with differing contents, it is possible to gain some idea of the identity of the systems, and the technique forms an elegant and powerful tool.

The micro-isolation used in the original attempt was tedious, exacting and not very efficient—a 2% success being gratifying—and other methods of obtaining monoxenic (single concomitant) cultures of amoebae were sought. Bacteria-free amoebae could be obtained from human liver abscess[28] or from liver abscesses established in the hamster,[40] and these were used in the assessment of various organisms as concomitants in culture. The most consistent growth of amoebae was given by *Clostridium welchii*, and this organism was used as concomitant in the bulk production of amoebae for the preparation of antigen extracts.

The welchii-factor[36,37] in the antigen preparation is heat stable; is probably a glycoprotein; can be obtained from other sources such as Jack-Bean meal; is non-immunogenic; and is probably related to, though not identical with, the C-reactive group of proteins.

This finding illustrates the value of the Ouchterlony technique, for, despite this confusing element in the antigen preparation, specific amoebic bands are recognizable. This would not be possible with such blind techniques as complement fixation and indirect haemagglutination, as these reveal a sum of the antibodies—specific or not—present, whence the need for a diagnostic titre. With gel-diffusion, extraneous systems can be recognized.

Application of the amoebic gel-diffusion test to a large series of well-documented sera led to appreciation of the significance of the precipitins. In a review[44] of patients in whom a diagnosis of amoebic liver abscess was entertained, the test was positive in 96% of the 360 cases in which the diagnosis was confirmed by the aspiration of typical pus, and in 90% of 168 cases in which the diagnosis was purely clinical, pus not having been obtained. In a similar review[43] of dysenteric conditions, 92% of 400 cases of acute amoebic dysentery gave positive results, whereas 16% of those with bacillary dysentery had precipitins. Over-all, 16% of the control group of patients with 'miscellaneous non-dysenteric disease' had antibodies. These and other results are illustrated in Fig. 1.

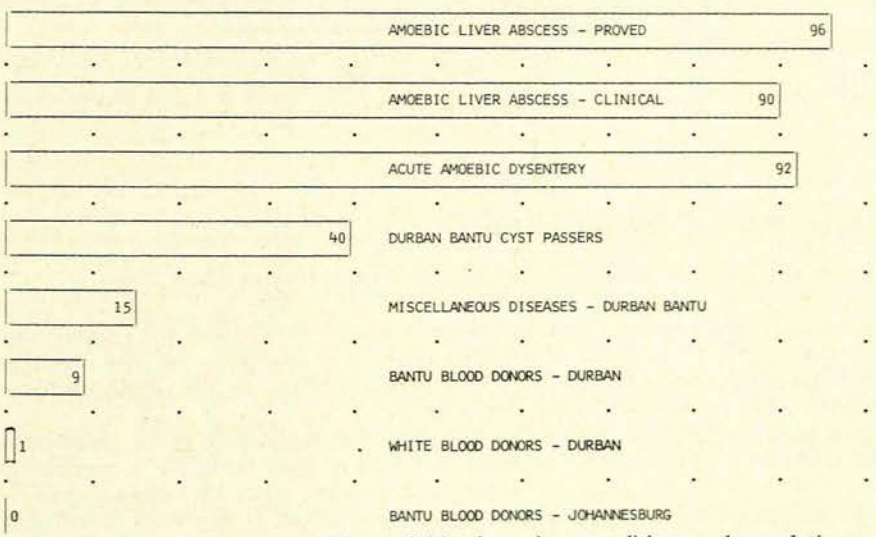

Fig. 1. Prevalence of anti-amoebic precipitins in various conditions and populations.

The antibodies apparently persist for some time after the amoebae have disappeared, a fact which can be used for epidemiological judgement,[38] but which must be taken into consideration in the interpretation of a positive result. Durban Bantu have a 'background noise' of some 15%, whereas in the White this is less than 1%, and a positive result in one of the latter is thus more significant than in one of the former.

The use of the test in epidemiology is not only free of the subjectivity and fallacies of coprological survey, but, as it indicates the prevalence of invasion, is a direct measure of the impact of *E. histolytica* on a community. Persons harbouring the commensal form of the amoeba do not show antibodies unless there has, at some time, been invasion of the tissues. Use of the test should determine the aetiological role, if any, of the amoeba in such controversial conditions as non-suppurative amoebic hepatitis.

A positive result is indicative of past or present tissue invasion by *E. histolytica*, but must be interpreted in the light of the background prevalence of amoebic antibodies in the community. A negative result gives a high degree of probability that the subject is not being invaded by amoebae, whether or not these have been found in the faeces.

THE FUTURE

Identification

Present morphological methods do not allow adequate identification of *E. histolytica* in any form other than the haematophagous trophozoite encountered in acute dysentery or in liver abscess. Culture methods, though slow and uncertain, are necessary. Improved, more reliable techniques must be developed.

It might prove possible to identify individual amoebae by the immuno-fluorescence technique developed by Goldman *et al.*,[29] but at present this is quantitative, as the immunoglobulins used contain 'genus' as well as 'species' antibodies.

The enzyme analysis used by Bragg and Reeves[2] requires amoebae in bulk, but their studies might lead to some histochemical micro-method.

Clinical

Clinicians should be more critical in interpretation of laboratory findings. The term 'invasive amoebiasis' should be used to indicate the pathological state. Safe, effective therapy[45,47] is available.

Serology

Though the gel-diffusion technique permits identification of specific amoebic antibodies, even with a crude mixture of antigens, it is desirable that the extraneous material be removed from antigenic preparations before such are released for general use. It should prove possible to apply the techniques developed in cysticercosis[40] to amoebic material.

Should isolation of 'species' antigens be practicable, corresponding fluorescent antibodies could be used for the identification of amoebae in microscopical preparations.

SUMMARY

Failure to appreciate the fact that *Entamoeba histolytica* is normally a commensal restricted to the bowel contents led to its erroneous association with clinical conditions with which it had no aetiological relationship. When it changes its habits and invades the tissues, the manifestations are usually of the classical type.

Dependence on morphological criteria has led to laboratory misdiagnosis. There are species of intestinal amoebae which cannot readily be distinguished from *E. histolytica*, and the only unassailable morphological criterion of species is haematophagy, which is fortunately also indicative of invasion.

Serology provides information as to whether the subject has at some time been invaded by amoebae, and is thus useful in excluding *E. histolytica* as an aetiological agent and in the epidemiological assessment of its impact on a community.

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