

THE ENIGMA OF HOST-PARASITE RELATIONS IN AMEBIASIS

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*"The clear definition of a difficulty frequently hastens the advance
of science by indicating a pitfall or a wrong road of inquiry."*

CLIFFORD DOBELL. 1919.

IN A GENERAL way it may be said that the host-parasite relationship in any infection is concerned with the interaction between parasite and host, which are in constant conflict, the virulence of the former struggling to overcome the resistance of the latter. In the case of amebiasis, the progress in our knowledge of the actions and reactions of the two components of the host-parasite system has been unequal, and certain aspects of their association are still obscure.

The successful maintenance of *Entamoeba histolytica* in cultures and recent developments in the technique of animal experimentation have provided useful tools for the study of this problem, but, while notable advances have been made in our knowledge concerning the life history of the parasite and its effect upon the host, very little is known about the way the host reacts against the amebic infection. And indeed, most of the information regarding immunity and resistance in amebiasis is based upon circumstantial evidence rather than upon experimentally established facts.

ACTION OF PARASITE ON HOST

Unicium. For a critical appraisal of the role played by the human host in the course of amebiasis, it will be necessary to review the available data regarding the behavior of *E. histolytica* in the infection. One of the most important facts concerning the etiology of amebiasis is that, in the great majority of cases of infection throughout the world, *E. histolytica* lives in the lumen of the human gut as a commensal without causing any damage to its wall or detectable symptoms of disease (Hoare, 1952). Furthermore, among these symptomless infections a distinction should be made between those with the small and large races of *E. histolytica*. It is now generally admitted that the small race is inoffensive and—as far as its medical importance is concerned—should therefore be placed among the other non-pathogenic amebae of man, such as *E. coli*, *Endolimax* and *Iodamoeba* (Hoare, 1952, 1957; Ridley & Schofield, 1957). Moreover, the

small race is sufficiently characterized both morphologically and biologically to be regarded as a distinct species or subspecies, under the name *E. hartmanni* or *E. histolytica hartmanni*, respectively (Burrows, 1957; Hoare, 1949, 1952, 1957).

The exclusion of *E. hartmanni* leaves only the large race (*E. histolytica* proper) for consideration as a pathogen. In the past various hypotheses have been proposed to explain variations in the course of amebic infections. Thus, some authors maintain that, although in many cases the large race of *E. histolytica* lives in the lumen of the gut as a commensal ("minuta" form), feeding there on bacteria and saprozoically, it is potentially always pathogenic but its virulence (or invasiveness) remains dormant until activated by extraneous factors (such as harmful bacteria, functional disturbances, host's diet, which will be considered below) which affect the host's health, thereby lowering his resistance and impairing the structural integrity of the intestinal wall.

However, in discussing the possible factors affecting the pathogenicity of *E. histolytica* the characteristics of different strains have usually not been taken into consideration, though it is a well-known fact that the course of amebiasis varies considerably in different parts of the world. Thus, although the incidence of infection in countries with a temperate climate is comparable to that in hot countries, manifestations of disease (including dysentery) are common in the tropics but rare in temperate regions, where the infections are as a rule symptomless. Until quite recently it was generally assumed that *E. histolytica* is represented by one race of pathogenic amebae and that variations in the course of amebiasis observed under different climatic conditions were determined by various injurious factors mentioned above, which in the tropics tend to lower the host's resistance to invasion by the amebae. It is true that some observers suggested that the types of amebiasis prevalent in regions with different climates might be due to the existence of virulent strains in the tropics and of avirulent ones in temperate regions. However, it was thought that the invasive power was not an innate peculiarity of the amebae but depended on the conditions previously experienced by them in the host. Thus it was suggested that the virulence of a strain might be enhanced as the result of adaptation to life in the tissues (Reichenow, 1931), a view supported by experiments in which the virulence of *E. histolytica* was apparently increased by repeated passages through animals (Faust & Swartzwelder, 1935; Meleney & Frye, 1937). And, conversely, it was believed that prolonged sojourn in the lumen of the gut might reduce the invasiveness of this ameba (Westphal, 1950).

Dualism. This unicistic concept was opposed by Brumpt (1925, 1928, 1949) who propounded a dualistic hypothesis of the etiology of amebiasis.

According to his view, the large race of amebae with quadrinucleate cysts comprises two types, which he regarded as independent species, *E. dispar* and *E. dysenteriae* (= our *E. histolytica*). While *E. dispar* is a non-pathogenic ameba inhabiting the human intestine throughout the world, *E. dysenteriae* is the only pathogenic form responsible for amebic dysentery and other clinical manifestations in warm and hot countries to which this parasite is confined, though its virulence may be in abeyance in carriers. Brumpt thus believed that the only ameba indigenous to countries with a temperate climate was the harmless *E. dispar*, while the pathogenic *E. dysenteriae* is only occasionally introduced there by persons who contracted their infection in hot countries. Among the arguments in favor of the independence of these two species Brumpt (1928) pointed out that none of the factors which promote the development of clinical symptoms in infections with *E. dysenteriae* has any effect on carriers of *E. dispar* in temperate climates: thus, despite the presence of typhoid fever and bacillary dysentery in France, amebic dysentery does not occur there. On the other hand, in convalescent carriers, who had settled in France, the exotic *E. dysenteriae* does not lose its virulence. In other words, *E. dispar* and *E. dysenteriae* "breed true."

Strain variation. Brumpt's ideas, which were based on epidemiological data, were received with some scepticism during his life, but they have now been vindicated by experimental investigations on the virulence of strains of *E. histolytica* isolated from patients with different types of amebiasis. Although recent work on these lines fully supports Brumpt's main thesis, the conclusions derived from earlier experiments were contradictory (Dale & Dobell, 1917; Kessel, 1928; Meleney & Frye, 1933, 1935, 1937 and others), owing to misleading results obtained with kittens, whose susceptibility and vulnerability to infection with *E. histolytica* are exceptionally high. But with the introduction of rodents as experimental hosts this question could be studied under conditions more closely resembling those found in the human host. During the last few years this subject has been thoroughly investigated by Neal (1951a, b; 1954, 1956a; 1957; Neal & Vincent, 1955, 1956), who demonstrated that strains from symptomless human infections were invariably avirulent, producing no lesions in experimentally infected rats, whereas strains from clinical cases invaded the gut wall, causing typical ulceration. It was also shown that the invasiveness of these strains was a stable property which could not be modified by interchange of their floras, by diet, or by animal passages, for through all these manipulations the strains retained their original peculiarities. The avirulence of strains from symptomless cases and the virulence of strains from clinical cases were recently confirmed in rabbits by Hunninen & Boone (1957), while Beaver *et al.* (1956), who infected 46

volunteers with a carrier strain, found that it produced in all of them symptomless infections persisting in some cases up to 14 months.

Neo-dualism. Since the invasiveness of a strain cannot be altered experimentally, its characteristics appear to be fixed, indicating the existence of two types of *E. histolytica*: (1) the non-virulent one, corresponding to Brumpt's *E. dispar*, and (2) the virulent one, corresponding to his *E. dysenteriae*. In the light of these observations our concept of the host-parasite relations and etiology of amebiasis takes on a "new look": though corresponding to Brumpt's original views, it leaves the question regarding the validity of the specific independence of the two races open. This neo-dualistic hypothesis thus postulates that the human entamebae with 4-nucleate cysts are represented by the following categories: the small non-pathogenic *E. hartmanni* and the large *E. histolytica*, which in its turn comprises two types or races that are morphologically identical but differ in virulence, one being a non-pathogenic commensal, the other a pathogen capable of invading the host's tissues with the production of the well-known clinical manifestations of amebiasis. The invasive large race is restricted to warm and hot countries, where clinical amebiasis is endemic, whereas *E. hartmanni* and the avirulent large race of *E. histolytica* are apparently cosmopolitan and are the only indigenous parasites in countries with a temperate climate, though clinical cases of exotic origin are occasionally introduced there. In this connection it is significant that in a number of European countries (e.g. Britain, Germany, Holland, France) there is either direct proof or strong circumstantial evidence that local cases of amebic dysentery are due to importation of virulent strains of *E. histolytica* or have been in contact with extraneous sources of infection (cf. Hoare, 1950; Boyd, 1957).

It has been estimated (cf. Hoare, 1952) that the average prevalence of infection with *E. histolytica* (*sensu lato*) throughout the world is about 20% of the population, representing a global incidence of more than 400 million cases. Of this number roughly 80% have no signs or symptoms of disease, about one-third of them harboring the inoffensive small race (*E. hartmanni*) and the rest being infected with the large races. Among the symptomless infections with the large amebae, a high proportion are cases of non-pathogenic amebiasis harboring the avirulent race, whereas those parasitized with the virulent race are carriers in whom—under favorable conditions—clinical manifestations are liable to develop at any time. If this interpretation of the host-parasite relations in amebiasis is correct, the only parasite concerned with the etiology of the clinical disease is the virulent race of *E. histolytica*, but, at the present stage of our knowledge, in cases without clinical manifestations it can be differentiated from the avirulent race only by animal experimentation.

The changing views on the etiology of amebiasis are summarized in the following table:

ETIOLOGY OF AMEBIASIS

HYPOTHESES	UNICISTIC 1913-	DUALISTIC 1925-	NEO-DUALISTIC 1957
NON-PATHOGENIC AMEBIASIS	<i>E. histolytica</i> SMALL RACE	<i>E. hartmanni</i>	<i>E. hartmanni</i>
	LARGE RACE: Commensal phase ("minuta" form)	<i>E. dispar</i>	<i>E. histolytica</i> Avirulent large race
PATHOGENIC AMEBIASIS	↑ ----- ↓	<i>E. dysenteriae</i>	<i>E. histolytica</i> Virulent large race:
			Dormant in lumen of gut ↑ ----- ↓
Subclinical			
Clinical	Virulent (tissue) phase ("magna" form)		Invading gut wall

Pathogenesis. We now turn to a consideration of the factors which are held to be responsible for the activation of the dormant virulence of the invasive race of *E. histolytica*. One of the most important factors influencing the course of amebic infection is the intestinal flora. It is well known that the role played by bacteria in the life of *E. histolytica* is so essential that their association appears to be almost symbiotic. Thus, there is conclusive evidence that bacteria (or other suitable living cells) are indispensable for the maintenance and development of this amoeba *in vitro* (cf. Dobell & Neal, 1952), and it has been demonstrated *in vivo* that the amoebae are unable to establish an infection in "germ-free" animals (Phillips *et al.*, 1955). The intestinal flora not only serves as a source of food and of some unknown growth factors promoting the survival of *E. histolytica* in the lumen of the host's gut, but, according to many authors (Westphal, 1937, 1938; Deschiens, 1938; Phillips *et al.*, 1955, and others), it plays a major part in the pathogenesis of amebiasis, especially when virulent bacteria are present (Westphal, 1937, 1948). According to this view, the normal mucosa of the gut wall is resistant to penetration by the

amebae, but, when it is injured by virulent bacteria and their toxins, the way is paved for the amebae, which then invade the tissues and produce the characteristic lesions. On the other hand, there is some evidence at present that the amebae take a more active part in this process, and some authors even believe that penetration of the tissues can be effected by their own unaided efforts (Meleney *et al.*, 1939; Phillips & Bartgis, 1954; Neal, 1957). Nevertheless, the available facts strongly support the view that bacteria play an important contributory role in the production of lesions in amebiasis (Porter, 1953; Phillips *et al.*, 1955).

In addition to bacteria, it has been suggested that other causes of disturbed gastro-intestinal function may expose the intestine to invasion by *E. histolytica*, such as abnormal secretory function, temperature fluctuations and irritant foodstuffs (Westphal, 1938), as well as inflammatory and erosive processes manifested by colitis (Deschiens, 1950a). Some of these factors are held to be responsible for the prevalence of clinical amebic dysentery in the tropics, where inadequacies of diet and bacterial assault commonly provoke gastro-intestinal disorders and a lowering of the host's resistance. It has also been shown that variation in the host's diet and its vitamin content may be predisposing factors in amebiasis (this question has recently been fully reviewed by Porter, 1953; Frye, 1955; and Chandler, 1955, 1957). These are the main conditions which are thought to influence the course of amebic infection, but the data are inconclusive and it is obvious that the question of pathogenesis in this disease is in need of further investigation.

A good illustration of the potential pathogenicity of carrier strains from endemic areas of clinical amebiasis is provided by observations made during the First World War. On the one hand, it was shown (Woodcock, 1917) that 20% of the normal population of India were symptomless carriers of *E. histolytica*. On the other hand, it was found that both in Egypt (Woodcock, 1917) and in Mesopotamia (Ledingham, 1920) cases of amebic dysentery were far more frequent among the Indian troops than among British troops operating in these countries. In the light of our present knowledge of the etiology of amebiasis, it is conceivable that the Indians already harboured pathogenic strains, the dormant virulence of which was activated when amebic carriers were exposed to unfavorable conditions on the front. As regards the low incidence of disease among the British, it was probably due to the fact that local invasive strains had not yet succeeded in infecting many of them.

Attenuation of virulence. One of the puzzling phenomena in amebiasis is the failure of virulent exotic strains to establish themselves in temperate regions. Thus, after the First and Second World Wars, when troops which had been exposed to infection with virulent strains of *E. histolytica* in

tropical countries were returning to Europe and North America, medical authorities were much concerned about the possibility of the introduction and spread of clinical amebiasis among the local inhabitants: yet nothing happened. It is possible that convalescent carriers of exotic strains are present among us, but no relapses are occurring owing to the absence of predisposing factors. On the other hand, it is known (Deschiens, 1939, 1941; Chang, 1945; Neal, 1956a) that a virulent strain may become attenuated after prolonged cultivation, though its virulence can be restored by animal passages (Neal & Vincent, 1956). Since in a temperate climate the amebae usually live in the lumen of the gut in association with the conventional flora, it is conceivable that exotic strains of *E. histolytica* may gradually lose their virulence—as they do *in vitro*—and this might account for the scarcity of clinical cases in countries with a colder climate.

Invasion. There has been much speculation about the mechanism of tissue invasion by *E. histolytica*. The histological picture of early lesions indicates that the parasite elaborates a substance causing histolysis of the tissues, and various views have been advanced regarding its nature. While some authors (Rees, 1929; Meleney, 1944; Frye & Shaffer, 1948) thought that the secretion was a toxin, others found no signs of toxic effects (e.g. necrosis) in the lesions, nor were they able to reproduce symptoms of intoxication in rabbits inoculated with extracts of amebic cultures (Westphal, 1938). Those who held that penetration of the tissues by the amebae, and their pathological effect, are due to fermentative action sought to identify the enzymes responsible. Thus, over 30 years ago Craig (1927) discovered in *E. histolytica* a cytolytic substance (“cytolysin”); later Rees *et al.* (1953) demonstrated production of a gelatinase, and Deschiens (1950b) noted its ability to hydrolyse proteins *in vitro*. Recently Bradin (1953) discovered the presence of hyaluronidase in this ameba and suggested that it promotes tissue invasion, but DeLamater *et al.* (1954) failed to detect this enzyme. Finally, Neal (1956b) demonstrated the presence of proteolytic enzymes in this ameba.

It is thus seen that the evidence regarding the nature of the enzymes responsible for the invasion of the tissues by *E. histolytica* is still fragmentary and inconclusive. However most observers are agreed that this ameba produces some proteolytic enzyme, which liquefies the intestinal mucosa at the point of application, thereby enabling it to penetrate into the tissues, which continue to break down by histolysis as the amebae multiply and extend through the ulcers. In addition to the fermentative process, there can be no doubt that the progress of the amebae within the tissues is aided in a purely mechanical manner by their locomotion (Hoare & Neal, 1955).

REACTION OF HOST TO PARASITE

Having reviewed the present state of knowledge regarding the behavior of the parasite we are in a better position to discuss the question of immunity in amebiasis. Among the available facts the first in order are the well-known classical experiments of Walker & Sellards (1913), who had shown that human beings varied in their susceptibility to infection with *E. histolytica*, one and the same strain of which produced symptomless infection in some individuals and clinical manifestations in others. These results were interpreted as evidence for the existence of natural or innate immunity in certain persons (Dobell & Low, 1922; Craig, 1944; Craig & Faust, 1951; Frye, 1955). Similarly, the absence of clinical symptoms in carriers has also been attributed to natural resistance of the host (Craig, 1944). On the other hand, there is no clear evidence of acquired immunity to amebiasis in man, for persons who had recovered from or were cured of an infection are liable to be reinfected (Craig, 1944; Anderson *et al.*, 1953; Porter, 1953; Frye, 1955), and it is significant in this connection that amebic infections are remarkably persistent, lasting sometimes for years (Dobell & Low, 1922; Brumpt, 1928, 1949). That the human host does not acquire immunity to reinfection has recently been demonstrated experimentally (Beaver *et al.*, 1956), though dogs which had recovered from the disease appear to be resistant (Simić, 1935; Swartzwelder & Avant, 1952).

Among the barriers which an intestinal parasite like *E. histolytica* must overcome to gain access to the tissues is the normal mucosa of the gut wall. When its resistance is lowered by various factors mentioned above, this barrier may be broken at some points, exposing the tissues to attack by the amebae (Westphal, 1937, 1938; Fischer & Reichenow, 1952; Anderson *et al.*, 1953; Chandler, 1957). However, as noted already, it is not clear whether or not the amebae are capable of penetrating through this barrier without the aid of extraneous agents.

More tangible—though indirect—indications of some immunological response to amebic infection is provided by serological reactions, the most important of which is the complement fixation test. Though much work has been devoted to this test, the results are conflicting, some of the discrepancies probably being due to variations in the preparation of antigens and in technical procedure, or to antigenic differences between strains of *E. histolytica* used for the test (Hussey & Brown, 1950; Bozicevich, 1950). However, there seems to be a consensus that when the amebae are restricted to the lumen of the gut (as in symptomless infections) they do not stimulate antibody formation and the reaction is therefore negative, but when the parasite invades the tissues there is a positive

serological response, which is weak in the case of intestinal involvement and stronger in the case of amebic hepatitis (Hussey & Brown, 1950; Heinz *et al.*, 1956; Meleney, 1957).

Among other reactions for the demonstration of immune bodies in amebiasis, should be mentioned an intradermal test (Leal, 1953), and tests in which the presence of antibody in immune serum is revealed by immobilization of the amebae (Cole & Kent, 1953; Brown & Whitby, 1955; Valentino, 1956) or by their fluorescence when combined with fluorescein-tagged antibody (Goldman, 1953).

If the foregoing data on immunity in amebiasis are examined in the light of our present knowledge of the host-parasite relations, most of them will not stand the test of critical analysis. And, indeed, individual variation in susceptibility to infection with virulent strains of *E. histolytica* is probably due mainly to diversity in the intestinal flora, rather than to disparity in the innate immunity of different persons (as already suggested by Nauss & Rappaport, 1940, and Porter, 1953). It would seem, however, that in endemic areas the indigenous population might possess a degree of racial immunity. Thus, according to Boyd (1957), in 1930-1935 the incidence of amebic dysentery among British troops serving in India was higher than among Indian troops, in spite of the prevalence in that country of pathogenic strains of *E. histolytica*. Likewise, the absence of symptoms may be due either to infection with the avirulent race or to a lack of factors capable of activating the dormant virulent race. There is also fairly convincing evidence that the human host does not acquire immunity in the course of his infection. There remain the serological reactions, which do seem to point to some immunological response to amebic infection. Furthermore, the absence of inflammatory reactions to invasion by the amebae also indicates that the mechanism of defense is represented by humoral rather than cellular factors (Anderson *et al.*, 1953).

Since no complement-fixing reaction is produced in infections restricted to the lumen of the gut, it would seem that the great majority of cases of amebiasis develop no specific immunity against *E. histolytica*. As regards cases in which invasion of the tissues has taken place, only a slight immune response is detectable in them by serological reactions. From these facts it can be inferred that the amebic antigen is too weak to stimulate appreciable antibody formation in the human organism. In this respect *E. histolytica* is like many other pathogenic protozoa, the antigenicity of which is of a low grade, producing only transient immunity. The antigenic lability of protozoa has been attributed to the predominance in their body of lipid haptens, which are unable to induce lasting immunity, in contrast to carbohydrate haptens in bacteria, which provide powerful

antigens with a high antibody response (Kligler *et al.*, 1936, 1940). In this connection it may be significant that Ray & Sen Gupta (1954) have actually detected the presence of lipids in the cytoplasm of *E. histolytica*.

CONCLUSION

The views advanced by me in this survey may appear to be somewhat unorthodox, but they only represent deductions which are consistent with the mass of observational and experimental data on amebiasis that have accumulated up to date. Though a coherent pattern is emerging, the picture is by no means clear-cut, for many details are but faintly outlined, and much work remains to be done before the enigma of host-parasite relations in amebiasis is fully solved.

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