Papers and Originals

Primary Amoebic Meningoencephalitis in Britain

W. ST. C. SYMMERS,* SENIOR, M.D.

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Summary: Primary amoebic meningoencephalitis is caused by amoebae of the genera Naegleria and Hartmannella (Acanthamoeba), which ordinarily are free-living saprophytes. The infection may be acquired from fresh water—for example, while bathing—the amoebae invading the nasal mucosa and reaching the meninges and brain along the olfactory nerve filaments. The disease is designated "primary" to distinguish it from meningo-cerebral infection caused by the parasitic amoebae, particularly Entamoeba histolytica, which invade the central nervous system only as a result of dissemination in the blood stream from lesions in other parts of the body.

During histological reappraisal of old specimens in a medical museum in London an instance of amoebic meningoencephalitis histologically indistinguishable from the published cases has been found. The specimen dates from 1909. The patient was said to be from Essex. What may have been another case, seen in Northern Ireland in 1937, is also described briefly. These observations may indicate that this disease occurs in the British Isles.

Primary amoebic meningoencephalitis should be considered in the differential diagnosis of every case of acute meningitis.

Introduction

When we refer to amoebiasis without qualification we mean infection by *Entamoeba histolytica*. Although several other amoebic species have been identified as causing disease, *E. histolytica* is the only one that ranks as a major human pathogen—infections by the others are so rarely recognized that they rate little more than curiosity value, apart from their potentially fatal significance to the individual patients.

The amoebae that have been identified as causes of disease fall into two categories, parasitic and free-living.

The parasitic amoebae multiply only within the animal body, where they live as commensals or, occasionally, pathogens. The species parasitic in man include *E. histolytica, Entamoeba coli* and *Entamoeba gingivalis, Endolimax nana, Iodamoeba buetschlii* and *Dientamoeba fragilis*. *E. histolytica* has already been mentioned as the major pathogenic amoeba. *Entamoeba coli* can cause disease, but usually, if not always, as an "opportunist"—that is, when the patient's resistance to its invasion is lowered by other disease or by side-effects of treatment. More rarely, others among the amoebae named have been known to cause "opportunistic" infections.

The free-living amoebae, as this description indicates, are ordinarily found free in nature; they may also occur in the animal body—for example, in faeces. Organisms of two of the free-living genera, *Hartmannella (Acanthamoeba)* and *Naegleria*, have now been recognized as occasional human pathogens, causing primary meningoencephalitis and possibly upper respiratory infection.

* Pathologist, Histopathology Laboratory, Charing Cross Hospital and Medical School, London W.C.2.

The first report of naturally occurring infection by free-living amoebae was published in the *B.M.J.* on 25 September 1965 by Fowler and Carter from the Adelaide Children's Hospital in South Australia (Fowler and Carter, 1965). Their recognition of these organisms as the cause of an acute fatal meningoencephalitis was the climax of a series of observations that had begun 35 years earlier, when Castellani (1930) described an amoeba that he had found growing in a mixed culture of "*Cryptococcus pararoseus*" and bacteria. This amoeba was named *Hartmannella castellanii* by Douglas (1930). Similar observations of amoebic contamination of bacterial cultures followed (Hewitt, 1937). The next important development was in 1956, when Jahnes and his associates found an organism, identified as an acanthamoeba, contaminating tissue cultures of monkey kidney that were being used in virological research (Jahnes, Fullmer, and Li, 1957). Since then there have been numerous similar observations of the contamination of cultures, particularly tissue cultures, by free-living amoebae. It was from such a finding that the first evidence that these organisms could be pathogenic stemmed: in 1958 Culbertson published the first of a notable series of collaborative studies on the experimental pathogenicity of *Hartmannella (Acanthamoeba)* (Culbertson, Smith, and Minner, 1958).

The initial observation of Culbertson and his team was crucial. They discovered a hartmannella in lesions in the brain and spinal cord of monkeys and mice that had died after inoculation with tissue-culture fluid. The tissue cultures were originally thought to have become contaminated by an unknown virus—in fact, the contaminant proved to be no virus but the hartmannella (Culbertson, Smith, Cohen, and Minner, 1959). Further experiments confirmed the pathogenicity of this organism for laboratory animals (Culbertson, Overton, and Reveal, 1961; Culbertson, Ensminger, and Overton, 1966). These observations led Culbertson and his colleagues to postulate the existence of naturally occurring infections caused by free-living amoebae (Culbertson *et al.*, 1961). It must have been wholly unexpected that the first naturally occurring disease attributable to these organisms should prove to be a rapidly fatal meningitis in man (Fowler and Carter, 1965).

Because of the continuing taxonomical controversy that makes classification of the amoebae, particularly the free-living amoebae, so difficult, there is some confusion about how best to refer to the disease with which this paper is concerned. It is clear that at least two genera of amoebae may be responsible for such infections, *Hartmannella* and *Naegleria*, and that they cannot be distinguished with certainty in histological preparations of infected tissues. For the present, then, such possible names as hartmannellosis and naegleriosis are generally inadmissible; so, too, is acanthamoebiasis, being derived from a rejected synonym of *Hartmannella*. Carter (1968) has followed the example of Butt (1966), adopting his term for the disease, primary amoebic meningoencephalitis. This avoids the taxonomical difficulties and also, through inclusion of the qualification "primary," sufficiently distinguishes these infections from disease of the central nervous system caused by

E. histolytica, which is always an outcome of blood-borne dissemination from the lesions of intestinal amoebiasis or its complications. The precedent established by Butt (1966) and Carter (1968) will be followed in this paper.

Published Cases of Primary Amoebic Meningoencephalitis

In their now classic paper Fowler and Carter (1965) reported four cases of primary amoebic meningoencephalitis. Three of the patients were children, the fourth an adult. All came from the same small South Australian seaport. The first case had been recognized in 1961; the others occurred in 1965. In each, symptoms of acute upper respiratory infection led to the development of suppurative meningitis and death a few days later. The post-mortem findings suggested that the amoebae had entered the body through the nasal mucosa, reaching the central nervous system along the olfactory nerve filaments, a possibility supported by the particularly heavy infection and resulting destruction in the olfactory bulbs (Carter, 1968). Culbertson and his associates (1961) had already shown experimentally that fatal meningitis quickly follows intranasal instillation of cultures of *Hartmannella.*

The article by Fowler and Carter (1965) has been followed by a series of reports of further cases that, so far as I have been able to cover the literature, have brought the number on record up to 36 (excluding the cases in this paper, both of them ill-documented and of historical rather than practical interest).† Butt (1966)‡ described three cases, all from a single small locality in central Florida, two of them recognized in 1962 and the third in 1965. Patras and Andujar (1966) reported a case that they had seen in Texas in September 1964.‡ Callicott (1968) reported seven cases from Richmond, Virginia—the first of these to be diagnosed correctly occurred in 1966, and it was recognition of this case that led to review of histological material from two small outbreaks of meningitis, totalling nine cases, that had been observed in 1951 and 1952: the amoebae were demonstrated in sections of brain in six of these nine cases. In 1968 a series of 16 cases was recorded by Červa, Novák, and Culbertson (Červa and Novák, 1968; Červa, Novák, and Culbertson, 1968): these cases accounted for a succession of small outbreaks of meningitis originating in the same district of northern Bohemia from 1962 to 1965. Carter (1968) added two more South Australian cases, dating from 1963 and 1966, to the series that Fowler and he had originally described (Fowler and Carter, 1965)—one of the additional cases came from the same town as the first four. Butt *et al.* (1968) reported a fourth case from the same locality in Florida as Butt's (1966) first three cases. Most recently Callicott *et al.* (1968) reported two further cases, seen in 1967, from the same locality as Callicott's (1968) first seven cases.

Aetiology

Geographical Distribution.—Czechoslovakia, the United States of America, and Australia, in that order of the numbers of published cases, have monopolized the recognition of this disease up to now. The two possible cases reported in this paper are, so far as I know, the first in which the diagnosis of meningoencephalitis caused by free-living amoebae has been suggested in the British Isles: in one case this diagnosis is probably justified, in the other it is based on evidence so circumstantial that but for the opportunity to note it here it would not have been thought worth the mention.

Age and Sex.—The age of the 36 patients ranged from 8 to 59 years, with preponderance of children and young adults. The four oldest patients were 27, 28, 37, and 59. Twenty of the 36 patients were male.

Previous Health.—The patient whose case was reported by Patras and Andujar (1966) was unique among the 36 published cases in that he was already seriously ill when he developed amoebic meningoencephalitis. It may be relevant that at 59 he was the oldest patient in the series by more than 20 years. He had advanced cirrhosis of the liver with various manifestations of serious liver failure, and there was a background of drug addiction. It may be that these conditions had so lowered his resistance that the amoeba was able to establish what was in essence a form of "opportunistic" infection (Symmers, 1965). All the other patients appear to have been fit and healthy, enjoying an active life until struck down by the amoebic invasion.

Causative Organisms.—The amoeba has been cultured in only four of the 36 published cases (Carter, 1968; Butt *et al.,* 1968—see also Culbertson, Ensminger, and Overton, 1968; Callicott *et al.,* 1968—2 cases. In the cases of Carter (1968) and Butt *et al.* (1968) it was grown from cerebrospinal fluid collected post mortem and identified as a species of *Naegleria.* In both cases of Callicott *et al.* (1968) the organism was isolated from cerebrospinal fluid in life, a result of an intensive search for such cases that had been instituted following Callicott's (1968) earlier experiences with amoebic meningoencephalitis: in the fatal case of Callicott *et al.* the organism was identified as *Naegleria gruberi* and in the other as *Acanthamoeba astronyxis.* Carter (1969) mentioned another case, not yet published, in which he isolated *Naegleria* sp. before the patient's death. In most of the earlier reports the amoebae had been tentatively identified by their appearances in histological preparations as belonging to the genus *Hartmannella* (synonym, *Acanthamoeba*). Červa *et al.* (1968) suggested that the amoebae in the cases from Bohemia were naegleriae: they based this on the smaller size of the organisms in the sections in comparison with those in cases of experimental infection with known hartmannellae. *Naegleria* and *Hartmannella* are genera of free-living amoebae differing in that the former has appreciably smaller trophozoites and has a flagellate phase when exposed to unfavourable environmental conditions—for example, distilled water.

Summing up present knowledge—(1) it has been proved by culture that *Naegleria* is a cause of primary meningoencephalitis; (2) the suggestion that other cases are caused by *Hartmannella* (*Acanthamoeba*) is based, so far, only on interpretation of the appearances of the trophozoites in histological preparations, except in a mild and non-fatal case reported by Callicott *et al.* (1968—Case 2) in which the organism was cultured from cerebrospinal fluid.

Habitat of the Amoebae.—The free-living amoebae are found in moist soil, where they feed on bacteria, fungi, and probably other organic matter. They also live in water, including slow-moving streams, ponds, and even garden puddles; other haunts include sewage and manure, bacteria again being their main nutriment. Many free-living amoebae, probably including the potentially pathogenic strains of *Naegleria* and *Hartmannella,* grow most actively within the temperature range of 25 to 30° C. As the temperature of the environment falls the motility of the amoebae decreases until ultimately they encyst. Encystment also occurs with drying. The cysts are very resistant both to temperatures below freezing point and to desiccation. It is possible that the cysts may be lifted and carried by currents of air (Hewitt, 1937).

Source of Infection.—Of the 36 published cases, all but that of Patras and Andujar (1966)—the patient with possibly predisposing cirrhosis (see above)—were associated with a history suggesting that "nasopharyngeal inoculation during swimming

† This total of 36 cases also excludes two proved cases that so far have had only a passing mention in the literature—the fifth case in Florida, recognized by Poppiti (personal communication to Butt, Baro, and Knorr, 1968) and the seventh South Australian case (Carter, 1969).

‡ An account of two of Butt's first three cases had been presented at a meeting of the American Society of Clinical Pathologists in October 1964 (Butt, 1966). Patras and Andujar (1966) presented their case at the same Society's meeting in October 1965, the month after publication of the paper by Fowler and Carter (1965) (Butt, 1966).

or other close contact with contaminated water was the likely means of infection" (Carter, 1968). For instance, all seven patients in Callicott's (1968) series had a recent history of swimming in local ponds and lakes. Again, Červa and Novák (1968) noted that "the only possible source of the infectious agent was a modern indoor swimming pool in which the water was kept at about 24° C.": all the 16 patients in their series had bathed in this pool, the interval between doing so and the onset of symptoms never being more than seven days (Červa *et al.*, 1968). This Czechoslovakian experience suggests that some modern swimming baths may not be free from this hazard; how far it might be eliminated by antimicrobial treatment of the water remains to be determined.

Three pieces of evidence support the aetiological significance of bathing and other close contact with infected water—(1) free-living amoebae, indentical with those causing primary meningoencephalitis, have been found at bathing places and have been shown to be pathogenic to laboratory animals (Butt, 1966); (2) there is histological evidence confirming the direct pathway of infection through the nasal mucosa and along the olfactory nerve filaments (Carter, 1968); (3) recent bathing in particular localities has been a feature of reported outbreaks of the disease (Butt, 1966; Butt *et al.*, 1968; Callicott, 1968; Červa *et al.*, 1968).

Seasonal Incidence.—The 16 cases in Bohemia all occurred in summer or autumn (1962 to 1965) (Červa *et al.*, 1968). The six South Australian cases also occurred in summer or autumn—that is, December to March—one in 1961, one in 1963, three in 1965, and one in 1966, and all in one of the hottest populated areas of Australia (Carter, 1968). The nine Virginian cases occurred in summer—two in July 1951, four in July 1952, one in July 1966, one in June 1967, and one in July 1967 (Callicott, 1968; Callicott *et al.*, 1968). Butt's (1966) first three cases occurred in Florida in August 1962, September 1962, and August 1965; his fourth case was in July 1966 (Butt *et al.*, 1968). The Texan case of Patras and Andujar (1966), in which cirrhosis of the liver was a possible predisposing factor, presented in September 1964.

Thus all the 36 fully reported cases occurred in the hottest season of the year, an observation possibly related to the relatively high range of temperature (25° to 30° centigrade) that best suits the free-living amoebae. It seems clear that warm weather predisposes to the development of the disease: it is speculative whether this is because the amoebae are more numerous and more active then, a time when people are likelier to bathe in order to keep cool.

A Note on Infections of the Central Nervous System Caused by Parasitic Amoebae

The parasitic amoebae that have been named as causes of infection of the central nervous system include *Entamoeba histolytica* and *Iodamoeba buetschlii* (*Endolimax williamsii*).

(*a*) Infection of the central nervous system by *E. histolytica* is totally distinct in aetiology and pathogenesis, pathology, and parasitology from meningoencephalitis caused by free-living amoebae (*Naegleria* and *Hartmannella*). It is always a result of extension of the disease from some focus of amoebiasis elsewhere in the body, particularly spread through the blood from lesions in the bowel or in organs like the liver and lungs. The frequency of involvement of the central nervous system has been variously stated—in a series of 210 necropsies in Mexican cases of amoebiasis caused by *Entamoeba histolytica* the brain was involved in 17 (8·1%) (Lombardo, Alonso, Arroyo, Brandt, and Mateos, 1964). It should not be possible to confuse these two quite different diseases.

(*b*) The trophozoites of *Iodamoeba buetschlii* in the brain in the two recorded cases (Derrick and Wenyon, 1948; Kernohan, Magath, and Schloss, 1960) were found by Butt *et al.* (1968) to be identical in appearances with the trophozoites of *Naegleria* in their material from cases of primary amoebic meningoencephalitis. This may throw doubt on the identification of the supposed *Iodamoeba* in the two cases mentioned. However, neither case in any way resembled primary amoebic meningoencephalitis caused by free-living amoebae. The involvement of the meninges and brain in the case of Derrick and Wenyon (1948), though comparable in the histological appearances to primary amoebic meningoencephalitis, was merely part of a very widespread invasion of many organs and tissues by the amoebae, which were considered to have invaded the body through the extensive ulceration of the gastrointestinal tract that they had in part caused. The patient, a Japanese prisoner of war in Australia, was in poor general health and in addition had what seemed to be bacillary dysentery, which may have further lowered his resistance to the amoeba and, through ulceration, have facilitated its invasion. In the case reported by Kernohan, Magath, and Schloss (1960) the lesion was a localized cerebral granuloma, a lesion pathologically altogether unlike anything so far recorded in infections by the free-living amoebae. The relation between these two cases and primary amoebic meningoencephalitis should be kept under review.

Pathology

The pathology of primary amoebic meningoencephalitis has been comprehensively described by Carter (1968). Both neutrophils and macrophages contribute conspicuously to the acute inflammatory exudate in the subarachnoid space. The superficial encephalitis results from direct invasion by amoebae in the meninges, the organisms first passing along the perivascular spaces and then entering the cortical substance. In the more superficial areas the amoebae attract inflammatory cells; but in many parts there is little or no sign of cellular reaction, a finding that may indicate continued migration and proliferation of the amoebae after the patient's death and give a somewhat exaggerated impression of the real extent of the disease. However, the accumulation of inflammatory cells, thrombosis and necrosis of blood vessels, focal haemorrhages, and other changes indicate the severity of the antemortem process.

The inferior aspect of the olfactory bulbs is particularly heavily involved, in conformity with the demonstrable pathway of infection from the nose along the olfactory nerve filaments. It is probably from the necrotic and disrupted olfactory bulbs that the organisms gain access to the subarachnoid space and so become widely disseminated (Carter, 1968).

Diagnosis

The first lead to the diagnosis of primary amoebic meningoencephalitis is awareness of the occurrence of the disease. It *must* be considered in the diagnosis of every case of acute meningitis.

The amoebae can be recognized microscopically in fresh specimens of cerebrospinal fluid—for example, in a cell-counting chamber. However, delay between collection of a specimen and its examination may be accompanied by sufficient cooling to make the organisms sluggish or even virtually immobile. When sluggish their pseudopodial activity may be mistaken for that of macrophages. Again, in wet preparations amoebae can be overlooked unless the microscope's substage condenser diaphragm is stopped down—the phase-contrast microscope and, though less satisfactory, dark-ground examination have decided advantages over conventional microscopy.

It is important to note that these amoebae are virtually undetectable in stained films, in which they appear at best as nondescript smudges. Fresh, unstained, wet preparations are essential: preferably they should be kept at a temperature between 25° and 37° C., and a microscope with a warm stage

is an advantage. If a specially built warm stage is not available a very old-time procedure (that in the context of primary amoebic meningoencephalitis has come to be known as "Phelan's penn'orth") may be used with great advantage—the simple expedient of warming the microscope slide with a hot penny (T/Sgt. M. Phelan, acknowledged by Butt, 1966—Case 2).

Serological methods of studying infections by the free-living amoebae have not yet been developed, and in any circumstances would be unlikely to be of diagnostic importance in relation to the acute clinical problem. Similarly, cultivation of the amoebae from pathological material has, at present, no practical diagnostic significance in the management of a disease so rapidly fatal that hours and even minutes count if appropriate treatment is to be acceptably effective. Methods of culture are available (for example, as described by Band, 1959), and are of obvious importance from the point of view of long-term studies of this disease, its causes, and its treatment.

Treatment

Culbertson, Holmes, and Overton (1965) found that, among the otherwise unspecified groups of drugs that they tested, only sulphonamides—particularly sulphadiazine—protected mice from the effects of experimental inoculation with hartmannellae that regularly caused fatal meningitis in untreated control animals. In contrast to this experience with *Hartmannella*, Carter (1969) found sulphonamides to have no effect in therapeutically attainable concentrations on a species of *Naegleria* isolated from one of his cases of primary meningoencephalitis; the same was true of antibacterial antibiotics, methotrexate, emetine, quinine, and metronidazole. However, he found the naegleria to be highly sensitive in vitro to the antifungal antibiotic amphotericin B, which proved to protect mice effectively from experimental infection by this organism (Carter, 1969).

So far, no case of primary amoebic meningoencephalitis has responded to any sort of treatment (Callicott *et al.*, 1968, did not attribute recovery in their Case 2 to treatment (ampicillin)). Amphotericin B has been used only once, in the case of a moribund child whose illness was too advanced for a response to be hoped for (Carter, 1969—Addendum); the naegleria isolated in this case was sensitive in vitro to the drug. Carter (1969) emphasized the urgency of getting the drug to the infected sites in sufficient concentration, and he recommended its intraventricular administration in the highest permissible dosage, supplemented by intravenous administration. His paper should be consulted (Carter, 1969), but it is clear that the treatment of this disease has not yet passed from a theoretical estimate of what may be appropriate into the stage of experience gained from practical therapeutics.

For some time to come each patient with this disease will be an urgent and individual therapeutic problem, unpredictable as to outcome and most demanding of clinical and therapeutic acumen. Not least of the problems will be the choice of drugs. At the moment Carter's (1969) work makes amphotericin B an essential agent: he has shown the high level of amoebicidal activity that it has against the species of *Naegleria* that he isolated from two cases. No one seems yet to have investigated the activity of amphotericin against *Hartmannella*, and until this is known it would seem essential to give large doses of sulphadiazine concurrently with amphotericin B because of the known efficacy of that sulphonamide against experimental hartmannella infections (Culbertson *et al.*, 1965).

Prognosis

All but one of the 36 published cases of primary amoebic meningoencephalitis was fatal. In the fatal cases the average duration of the illness from the onset of symptoms was 4½ days; 34 of the patients died within seven days of the onset—one was kept alive for 11 days by artificial respiration. Epidemiological evidence provided by some of the cases in the Bohemian outbreaks indicates that in at least some cases the interval between exposure to infection and the onset of symptoms is not more than seven days (Červa *et al.*, 1968). This means that the time between exposure and death may be under two weeks, sometimes by a considerable margin. It is clear that this is a disease of exceptional gravity and of exceptionally rapid course.

The extent of the damage done by the invading amoebae to the surface of the brain in so short a time indicates a possibility that success in overcoming the infection by treatment may not be accompanied by as satisfactory an outcome in terms of cerebral function. For this reason it seems wise to be guarded in considering the prognosis for mental and physical health in cases in which anti-amoebic therapy may save life.

Other Manifestations of Infection by Free-Living Amoebae

The fact that free-living amoebae can cause disease has been recognized for the first time so recently that we have no idea of the real range and variety of their pathogenic effects or even of the frequency of the infections that they cause. The only defined disease attributable to them is primary amoebic meningoencephalitis, our knowledge of which is almost confined to the 36 published cases, all but one of them fatal, and shockingly brief in their course. The impression apt to be gained by the doctor reading about these organisms is that they cause a rare and fatal disease, and nothing besides.

It seems inconceivable that organisms that are so numerous in nature, that have been found to be capable of causing disease, and that may be understood to have so many opportunities of infecting people should cause solely a disease that is both very rare and almost invariably fatal. But what, then, are the less extensive, mild infections, the *formes frustes* of this disease that at present we recognize only in an extreme presentation? It is strange that up to now they are represented only by the non-fatal case of meningitis described by Callicott *et al.* (1968—Case 2).

There is some evidence that these free-living amoebae do in fact also cause lesser disease. This is suggested by, for instance, their identification with the cytopathic agent that was formerly described as "Ryan virus" and has since been shown by Armstrong and Pereira (1967) to be an amoeba of the genus *Hartmannella*. This agent was found in cell cultures that had been inoculated with material "from the respiratory tract in certain patients, mostly young children, with illnesses of varying severity but with fever and respiratory symptoms as common features" (Armstrong and Pereira, 1967).

It is by no means impossible, therefore, that these organisms—species of *Hartmannella*, and possibly other genera—cause trivial self-limiting upper respiratory infections with unsuspected frequency. They may well prove to have other pathogenic roles also.

Do Infections by Free-Living Amoebae Occur in the British Isles?

Free-living amoebae of the genera that have already been identified in cases of primary meningoencephalitis are part of the microscopical fauna of the British Isles. It will not be surprising if they turn out to cause infections here. The two cases that follow do not prove this possibility, for one is insufficiently documented to confirm that the histologically typical infection was acquired in Britain, while in the other there is not even proof that the amoebae were the cause of the disease.

Two Cases of Primary Amoebic Meningoencephalitis in the British Isles?

Case 1: Primary Amoebic Meningoencephalitis in England in 1909?

In the course of thinning out the shelves of a pathological museum in London that had been condemned by Authority as having outlasted its usefulness, certain specimens were set aside by the pathologists for re-examination before they were discarded to make room for activities more in keeping with a contemporarily scientific approach to medical studies. Among the unsuspected wealth of that discredited collection several remarkable examples of rare diseases turned up, masquerading under commonplace diagnoses. Three of these were infections—the first recognized case of cryptococcal mastitis in the human (Symmers, 1966), a case of chromomycotic meningoencephalitis ("cladosporiosis" of the central nervous system), and a case of amoebic meningoencephalitis histologically indistinguishable from those described by Carter (1969) as probably caused by *Naegleria* species.

The case of amoebic infection was labelled "Meningitis Carcinomatosa." The only record in the files of the museum read—

> "Cancerous dissemination in the leptomeninges. The primary growth was not discovered during *Sec. Cad.** April 1909. A lad of Essex. Microscopy shows spheroidal-celled carcinoma simplex throughout subarachnoid spaces and invading the superficial grey matter."

* *Sectio cadaveris*—the post-mortem examination.

No other information about the patient or the specimen can be traced.

The olfactory bulbs and most of the olfactory tracts were missing, probably from some carelessness in removing the brain from the skull: this would be all the likelier to result if these parts were already damaged through a necrotizing inflammatory reaction such as has been characteristic of other instances of this type of infection. Otherwise the only external abnormality of the brain was a moderate and patchy accumulation of creamy-looking matter in the subarachnoid space, mainly at the base of the brain and in some sulci on the convexity.

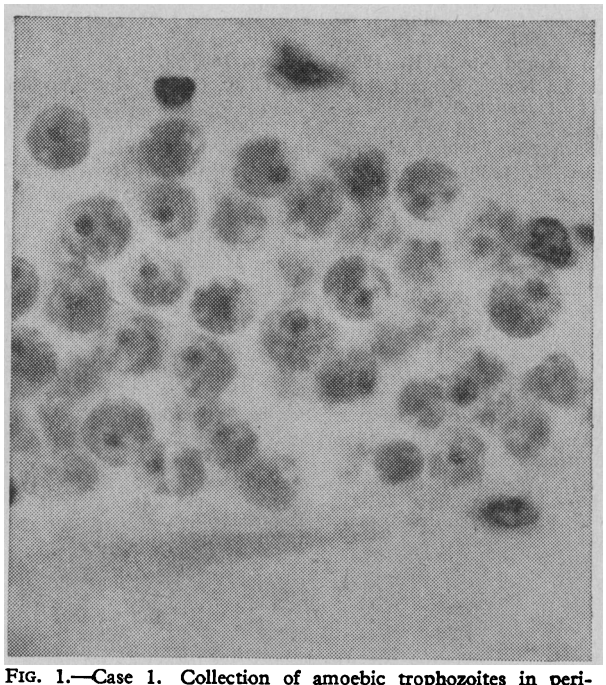

FIG. 1.—Case 1. Collection of amoebic trophozoites in perivascular space in cerebral cortex. The small nucleus and its conspicuously stained karyosome are well seen in many of the organisms. The rounded shape of the trophozoites is a fixation artefact—in the fresh state they are generally shaped like a slug, with a rounded and a pointed end. [Note.—The section illustrated is of tissue taken for histological processing after the specimen had been sealed for almost 60 years in preserving fluid of unknown composition: the tissues and the amoebae have retained their affinities for histological stains remarkably well.] (Haematoxylin-eosin. ×1,100.)

Histological examination showed dense infiltration of the subarachnoid space by neutrophils, macrophages, and amoebae. The organisms appeared as distinctive, palely stained, rounded cells with rather granular cytoplasm and a small, usually eccentric nucleus that contained a conspicuous karyosome (Fig. 1). They had invaded the superficial part of the cerebral cortex, filling the perivascular spaces and migrating into the substance of the brain, often with little or no evidence of any inflammatory reaction (Fig. 2).

The pathological findings were identical with those in the cases studied by Carter (1969) and others.

FIG. 2.—Case 1. Low-power field of cerebral cortex showing large numbers of amoebae in the perivascular spaces and within the substance of the brain. Inflammatory cells are conspicuous only in a few areas, particularly close to the blood vessel walls. It is possible that the amoebae continued to multiply and migrate in the tissues after the patient's death (see text, "Pathology)". (Haematoxylin-eosin. ×100.)

Comment.—Presumably the pathologist who originally examined the brain mistook the amoebae for spheroidal carcinoma cells. It has not been possible to trace any post-mortem report corresponding to the specimen or any record of such a case in the hospitals associated with the medical college housing the museum. Therefore it cannot be said that the patient became infected in the British Isles, though this may be a reasonable surmise.

Case 2: Primary Amoebic Meningoencephalitis in Northern Ireland in 1937?

While a resident pupil in the Mater Infirmorum Hospital in Belfast in 1937, I had the good fortune to be attached to the late Dr. Eileen Hickey's firm. Dr. Hickey's appointment as physician to the hospital carried with it charge of the pathology laboratory, a post in which she had succeeded my father when he retired from the chair of pathology and bacteriology in Queen's University in 1929. The pupilship gave an opportunity, now rarely available to students, of participating actively in the laboratory side of the hospital's work for the patients whom it was the pupil's responsibility to clerk. Dr. Hickey, like some of her contemporaries in the Belfast medical school, where the apprenticeship tradition continued longer than in most others in the British Isles, expected her pupils to accompany her in her private practice as well as on hospital rounds.

A girl, about 10 years of age, was admitted to a private nursing home in Belfast, under Dr. Hickey's care, early one Sunday morning. She had a 24-hours history of acute meningitis. Lumbar puncture produced cloudy cerebrospinal fluid which we brought to the hospital for examination. The fluid contained many pus cells, yet no

bacteria were found in stained films. Examination of the fresh unstained fluid in a cell-counting chamber showed that many of the cells were putting out pseudopodia and moved actively across the field. In my ignorance I took these cells to be peculiarly active leucocytes: Dr. Hickey recognized them at once as amoebae. She pointed out that they were quite different from *E. histolytica*—their movements were much more active, their shape was basically like that of a slug (one end pointed and one blunt), and when stained their nucleus contained a large karyosome: from these characteristics she suggested that they were possibly a species of *Endolimax*.

The child died within a few hours of admission, having been ill less than two days. A necropsy was not permitted. All that I know of her history is in this short note. She came, I believe, from the Antrim Road district of Belfast; about 10 days before her death she had been rescued from drowning at the public swimming place in the Belfast City Waterworks.

Comment.—The identity of the organism responsible for the illness will never be known. Perhaps it was a species of *Hartmannella* or of *Naegleria*. It probably was not a laboratory contaminant, for the fluid was examined within half an hour or so of the lumbar puncture. The history of near-drowning in fresh water may be irrelevant; on the other hand, in view of the possibility of infection through bathing in fresh water that has been remarked on in such a large proportion of the recorded cases of primary amoebic meningoencephalitis, this child's experience may have been causally related to her infection.

It is a pleasure to thank Dr. Rodney Carter and Dr. Malcolm Fowler for allowing me to study material from their cases of primary amoebic meningoencephalitis during visits to Dr. Fowler's department in the Adelaide Children's Hospital in 1966 and 1969. I must particularly acknowledge my debt to the late Dr. W. E. D. Evans, curator of the Pathology Museum in Charing Cross Hospital Medical School, for teaching me to heed the old rule never to throw out a specimen until its nature has been confirmed by microscopy. Mr. R. S. Barnett helped in the preparation of the photomicrographs.

Addendum

On the day after this paper was completed the national press in Britain carried a news report of a case of amoebic meningitis in Bristol (*Times*, 13 August 1969—Late London edition, page 1). I am indebted to Dr. A. P. C. H. Roome and his colleagues in the Bristol Royal Hospital for Sick Children and the Liverpool School for Tropical Medicine for confirmation that a naegleria was found in the cerebrospinal fluid of this child. Respiratory failure necessitated artificial respiration throughout 16 days of deep coma; during this period, treatment with amphotericin B was accompanied by a very striking reduction of the number of the organisms in the cerebrospinal fluid. The child, along with two others, had played by a garden puddle in Bristol. These two developed signs of an upper respiratory infection and incipient meningitis; they were treated with amphotericin B and recovered completely; a naegleria was isolated from the cerebrospinal fluid of one of these children, the brother of the patient who died.

Thus, by coincidence, the occurrence of infection by free-living amoebae has been confirmed in Britain. The two proved cases in Bristol can be added to the series of only six cases referred to in the literature in which primary amoebic meningoencephalitis was diagnosed during life (Butt, 1966; Carter, 1968; Callicott *et al.*, 1968; Carter, 1969); it is only the second to be treated with amphotericin B (Carter, 1969). It is notable that the Bristol cases, in common with others in the literature, may perhaps be attributable to infection from surface water.

We may look forward to early publication of the account of the Bristol cases by Dr. John Apley and his colleagues.

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