Reprinted from AMERICAN JOURNAL OF CLINICAL PATHOLOGY, Vol. 63, No. 4, April 1975

A Case of Hartmannellid Amebic Meningoencephalitis in Zambia

S. B. BHAGWANDEEN, CH.B., M.D., M.R.C. PATH., R. F. CARTER, M.D., F.R.C.P.A., M.R.C. PATH., K. G. NAIK, B.S., M.D. (PATH) AND D. LEVITT, M.B., CH.B., F.C.P. (S.A.)

Departments of Pathology and Microbiology and Medicine, University Teaching Hospital, Lusaka, Zambia, and Department of Pathology, Adelaide Children's Hospital, Adelaide, Australia

ABSTRACT

Bhagwandeen, S. B., Carter, R. F., Naik, K. G., and Levitt, D.: A case of hartmannellid amebic meningoencephalitis in Zambia. Am J Clin Pathol 63: 483–492, 1975. A case of amebic meningoencephalitis recognized in an adult Zambian is described. This is the first authenticated case from Africa. The morphologic features of the organism, its ability to form cysts in tissue, and the granulomatous tissue response denote that the ameba is an hartmannellid rather than a Naegleria. Free-living amebas of the family Hartmannellidae have not been incriminated before as a cause of primary amebic meningoencephalitis in man. To our knowledge this is the only case where such an ameba was responsible for fulminating meningoencephalitis. The presence of the amebas in a cellulocutaneous abdominal lesion suggests hematogenous dissemination. (Key words: Amebic meningoencephalitis; Naegleria meningoencephalitis; Hartmannellidae meningoencephalitis.)

AMEBIC MENINGOENCEPHALITIS due to free-living amebas, has been recognized as a clinicopathologic entity only since the report of Fowler and Carter in 1965.[16] Butt[1] proposed the name, "primary amebic meningoencephalitis" for the disease, and this has been adopted by most subsequent investigators.[2,21] The disease has now been recognized in many parts of the world.[3] However, only one doubtful case has been reported from Africa.[17]

Of the more than 60 cases reported, almost all have been due to Naegleria spp.[3] Infection of the brain by the hartmannellid group of free-living amebas has been recorded in only five cases,[18] but these are not regarded as typical cases of primary amebic meningoencephalitis. Indeed, it has been stated that only amebas of the genus Naegleria are responsible for primary meningoencephalitis in man.[3]

We here report a case which we believe to be the first authenticated case of primary amebic meningoencephalitis from Africa, in which the evidence suggests that a hartmannellid rather than a Naegleria sp. was responsible.

Received August 26, 1974; received revised manuscript October 15, 1974, accepted for publication October 15, 1974.

Address reprint requests to Professor S. B. Bhagwandeen, Department of Pathology, P. O. Box R.W. 110, Lusaka, Zambia.

Report of a Case

An African man, a market gardener, was admitted to the hospital on September 15, 1972. No coherent history could be obtained, and although the patient seemed

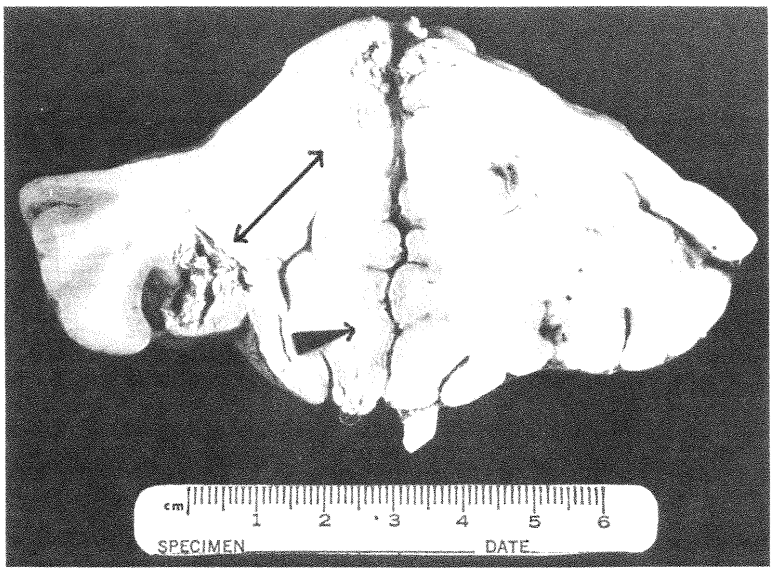


FIG. 1. Portion of temporal lobe of the brain, showing softening in the superficial cortical areas (*arrows*) in both hemispheres.

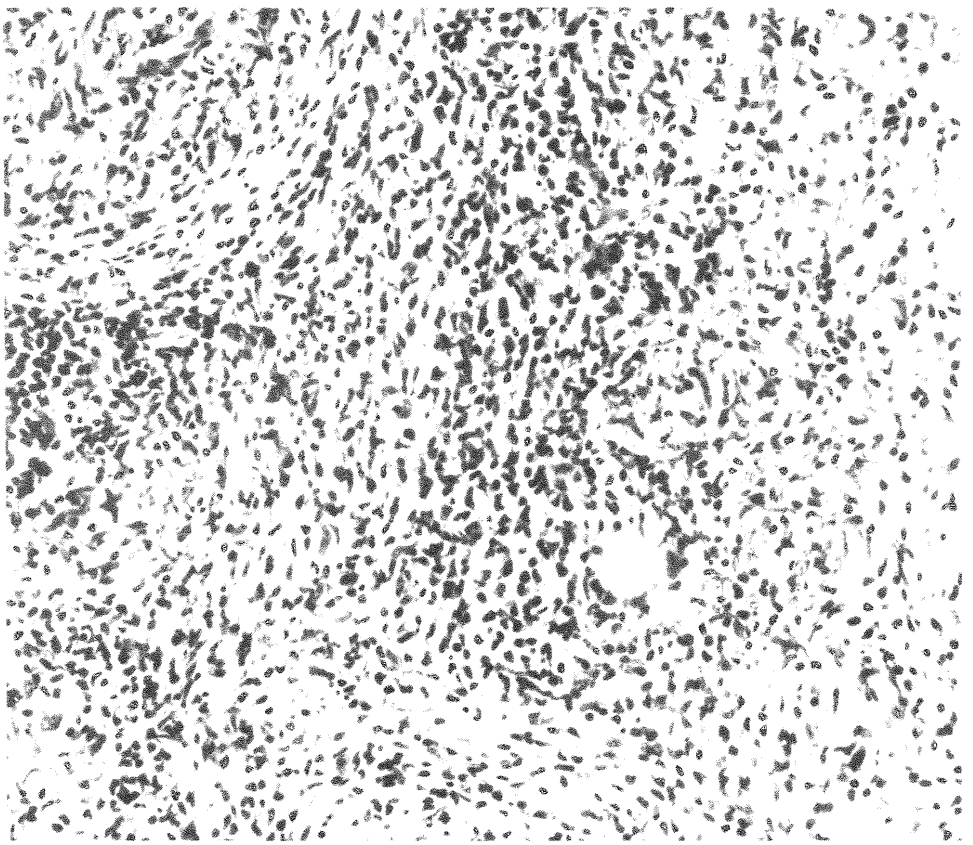


FIG. 2. Skin biopsy. The granulomatous, tuberculoid reaction is pronounced. There is marked narrowing of an arteriole (*upper left*). Hematoxylin and eosin. ×150.

to understand the questions, his replies were meaningless and rambling. A relative accompanying the patient explained that just prior to admission he had not been able to converse rationally with the patient. The patient had complained of frontal headache a day before admission.

On admission, he was conscious, but was described as having bizarre, confused behavior, with rambling speech. The temperature was 38 C. There was a slightly raised, tender, indurated patch, 6 cm in diameter, in the paraumbilical area.

The other positive findings were restricted to the central nervous system. In addition to his confused behavior, the patient had an ataxic gait. There was no paresis, but he had phasic, rotatory nystagmus towards the left. Tremor of both hands was pronounced. Moderate nuchal rigidity was present. The patient was markedly photophobic, but the fundi appeared normal.

A lumbar puncture was done. The pressure was not recorded. The cerebrospinal fluid was lightly opalescent, with 290 leukocytes per cu. mm., of which 62% were neutrophils and 38% lymphocytes; erythrocytes 600 per cu./mm.; protein 85 mg. per 100 ml.; sugar 16 mg. per 100 ml.; chloride 120 mEq. per l. No organisms were found on microscopy. (The culture was subsequently "sterile"). The patient was treated as for pyogenic meningitis.

During the next 24 hours the patient's condition deteriorated and nuchal rigidity became more pronounced. As tuberculous meningitis could not be entirely excluded, streptomycin and isonicotinic acid hydrazide (INH) were added to the therapy.

There was a suspicion that the lesion on the abdominal skin might be fungal in etiology, and in view of the bizarre presentation, the possibility of systemic spread and fungal meningitis was considered. Consequently, as the patient's condition was worsening, amphotericin B (12 mg. intravenously daily) was added to the therapy the third day after admission.

The following day the patient was afebrile, but his level of consciousness remained low and nuchal rigidity more pronounced. Examination of cerebrospinal fluid revealed leukocytes, 220 per cu./mm., 45% neutrophils and 55% lymphocytes; 1,200 erythrocytes per cu./mm.; protein 146 mg. per 100 ml.; sugar 30 mg. per 100 ml.; chloride 106 mEq. per l. Globulin was increased, but microscopy was again negative. Over the next 48 hours the patient's condition deteriorated; although afebrile, he became deeply comatose.

A biopsy of the cutaneous lesion on the fourth day after admission failed to reveal any fungal infection. There were, however, features very suggestive of erythema induratum (see details under Histology, below).

Over the next two days the patient's condition showed some improvement. Temperature remained normal, and the cutaneous lesions showed some improvement, which seemed to justify the diagnosis of deep mycosis with systemic dissemination. Consequently the antituberculous treatment was discontinued.

Over the next 48 hours the patient's condition gradually deteriorated. He had a temperature of 39 C., became deeply comatose, and died on the morning of the tenth day after admission.

Necropsy: (K.G.N.)

Necropsy was performed about 20 hours after death. The significant findings were limited to three sites; the skin, liver, and brain.

Skin: A large (5 × 4 cm), crusting, superficial ulcer was present in the paraumbilical area. It did not penetrate deeper than the subcutaneous tissue.

Liver: The liver weighed 1,000 Gm. and had a finely granular surface. The cut surface was typical of established early portal cirrhosis. The spleen weighed 250 Gm. and showed no abnormality.

Brain: The brain weighed 1,750 Gm.

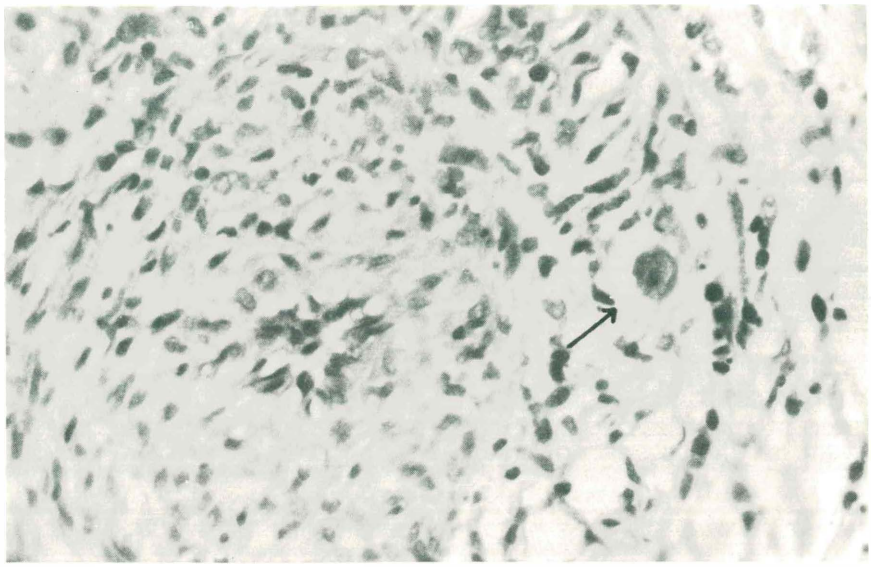


FIG. 3. High magnification of an almost totally obliterated arteriole with an ameba clearly identifiable. Hematoxylin and eosin. ×200.

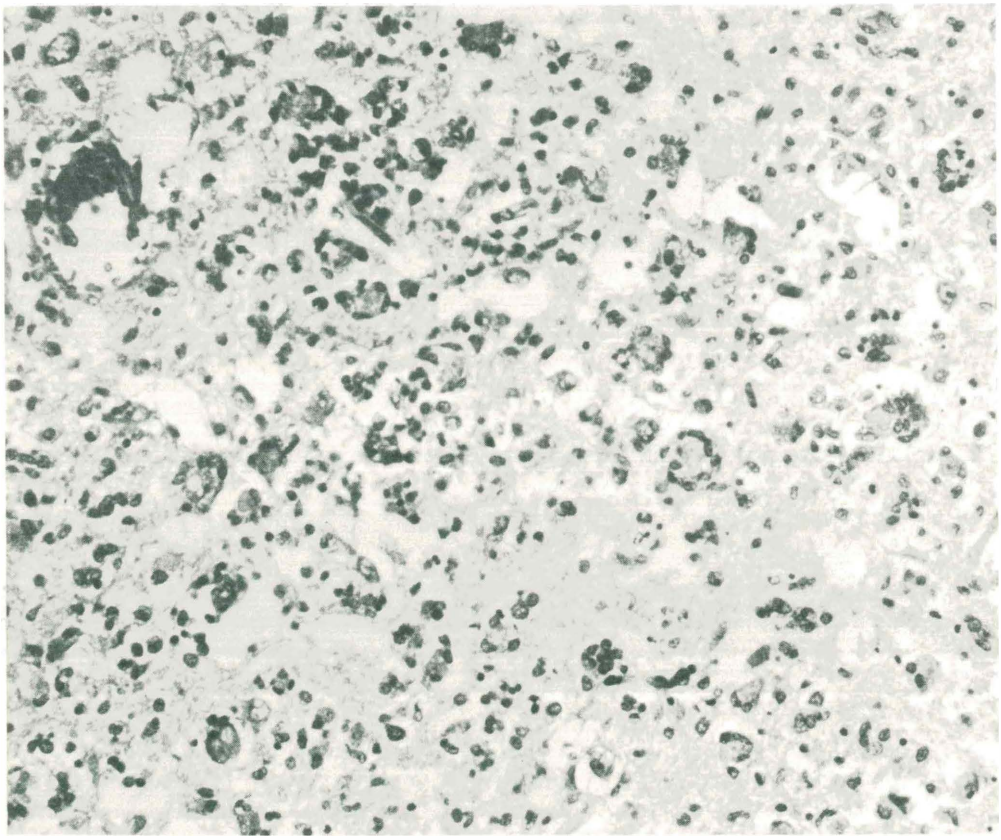


FIG. 4. Area of brain shows granulomatous reaction around amebas. Hematoxylin and eosin. ×400.

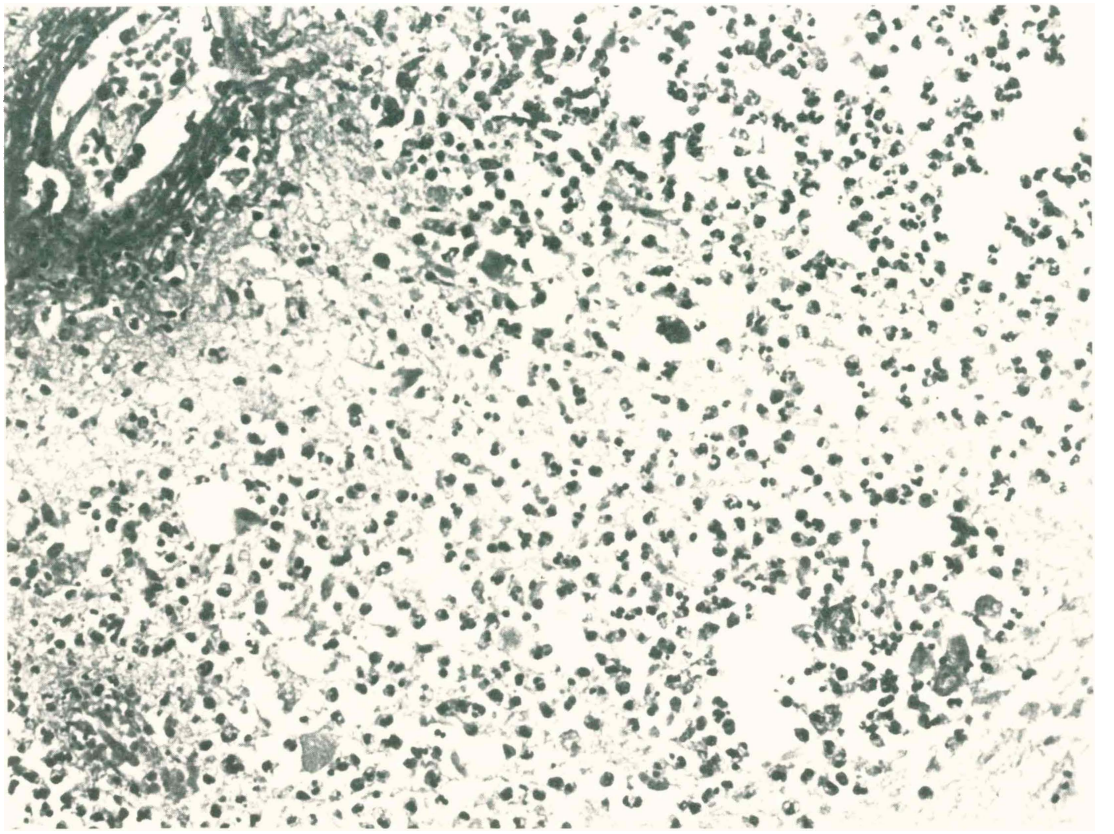


FIG. 5. Area of brain showing an acute inflammatory reaction with prominent allergic vasculities (*upper left*). A few amebas are recognizable (*lower right*). Hematoxylin and eosin. ×400.

The surface meninges were very congested, and at the base a patchy but impressive exudate was found. This involved the entire basal area, which appeared deeply congested and inflamed. The sectioned brain contained widely scattered focal hemorrhages, with softening throughout the superficial cortical areas, basal ganglia, and cerebellum (Fig. 1).

Skin Biopsy (original biopsy prior to diagnosis): The changes were confined mainly to the subcutaneous tissue but extended partly into the lower dermis. There was a pronounced granulomatous reaction with a tuberculoid type of response (Fig. 2). There was no definite evidence of caseation. The small vessels revealed impressive subendothelial proliferative changes, almost progressing to complete occlusion, with a variable amount of inflammatory change in the vessel wall. These changes were interpreted as reflecting allergic vasculitis. In retrospect, serial sections of the biopsy of the skin were cut, and in one of these amebas with limax nuclei were identified. One of these was in the wall of a thickened arteriole (Fig. 3), but others were found in the inflammatory exudate.

Histopathology of Brain

The outstanding feature was prominent meningoencephalitis. A nonspecific meningitis with plasma-cell and lymphocytic infiltration was present, but the encephalitis was the major feature. It involved mainly the superficial cortex and the basal regions.

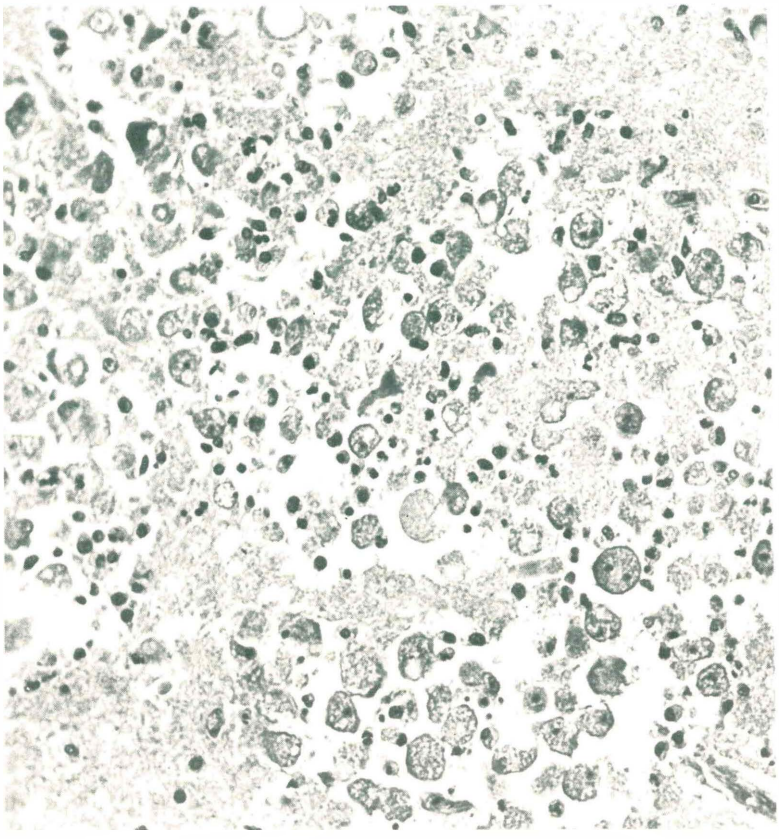


FIG. 6. Area of cortex, showing large number of trophozoites with minimal inflammatory reaction. Hematoxylin and eosin. ×400.

The character of the reaction varied in different regions. In some areas there was a predominantly granulomatous reaction with prominent giant-cell reaction (Fig. 4). In many of these giant cells amebas in various stages of degeneration could be recognized, and earlier stages where the amebas were being surrounded by microglia were also prominent. In these areas there was disruption of the normal brain tissue. Some gliosis, in addition to the chronic granulomatous reaction, was evident.

In some areas there was a predominantly subacute inflammation (Fig. 5). Amebas were again prominent in these areas, but with a predominantly acute inflammatory cellular infiltrate. Examination of small arteries and arterioles in these areas revealed marked allergic vasculitis with a fibrinoid type of necrosis.

Finally, in some areas the outstanding feature was the predominance of invasion by amebas with a minimum of an inflammatory reaction. The brain tissue in these areas was completely destroyed (Fig. 6).

Throughout all affected areas occasional amebas in stages of encystment could be recognized (Fig. 7). These were more numerous in areas of granulomatous reaction than in areas of acute reaction. Amebas could not be identified in the subarachnoid space, not even in the deeper Virchow-Robin spaces, However, the maximal changes in the brain tissue appeared to be around vessels.

The sequence of pathologic pregression of the lesions may be deduced from the observed changes. Invasion of the superficial cortex would indicate the earliest stage. This produced total disorganization of the cortical tissue but a minimal

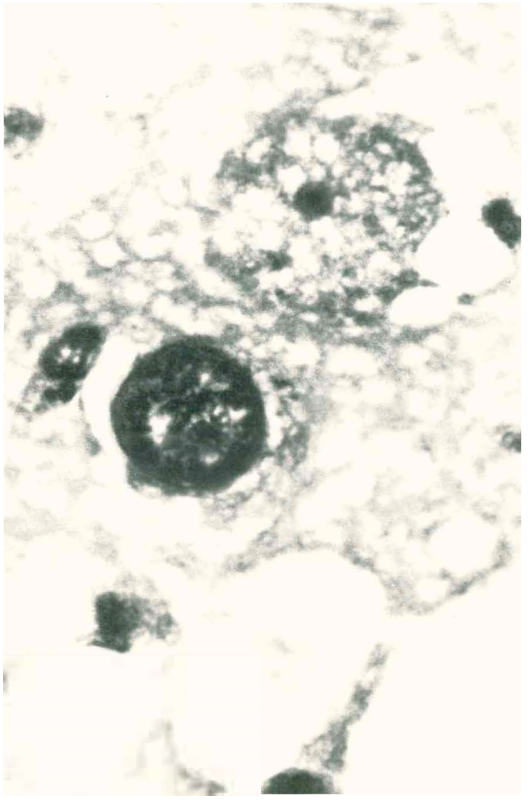
inflammatory reaction (Fig. 6). The presence of dead and dying amebas incited an acute inflammatory reaction in which allergic vasculitis was prominent (Fig. 5). This stage was succeeded by a granulomatous foreign-body giant-cell reaction in an attempt to clear the areas of dead and dying amebas (Fig. 4). Finally, the area healed by gliosis.

Identity of Ameba

The organisms in tissue sections are much larger than Naegleria spp., ranging in size from 30 to 80 μ. The typical limax nucleus can be recognized (Fig. 7) The organisms are diffusely scattered in the superficial cortex and are absent from the subarachnoid space. A similar distribution was found in experimental infections in mice with a mouse-pathogenic hartmannellid.[5]

There is evidence of encystment, and the cysts have a smooth circular double-walled outline (Fig. 7). The presence of cysts is indicative of a hartmannellid infection rather than Naegleria. Cyst formation in human tissue in cases of Naegleria has not been described.[12]

In one brain section, an ameba in the state of mitosis was identified (Fig. 8). This was clearly a mitotic type of division typical of hartmannellid amebas rather than the promitotic division with large polar masses and preservation of the nuclear membrane seen in Naegleria spp.



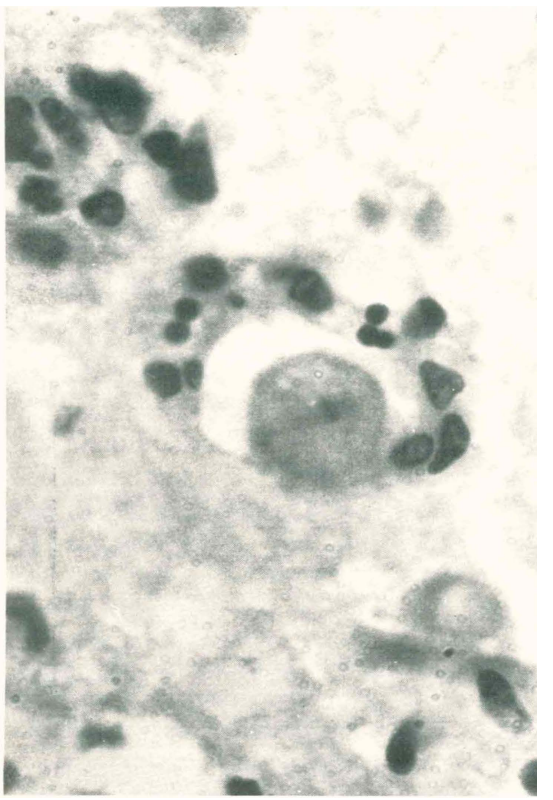


FIG. 7 (*left*). High magnification of a trophozoite (*upper*) and an encysted ameba (*lower*). Hematoxylin and eosin. ×1,200.

FIG. 8 (*right*). Section of brain, showing a trophozoite undergoing mitotic division. Hematoxylin and eosin. ×1,200.

This is the first time that it have been possible to identify the family of an invading free-living ameba by finding a mitotic figure of the organism in tissue section.

On the basis of the above criteria, the ameba can be positively identified as a hartmannellid. The chronic granulomatous reaction in the brain and the rather prolonged course of the disease also support the identity of the ameba as a hartmannellid.

Dr. Stamm kindly agreed to attempt identification of the ameba by the direct immunofluorescent technic. Paraffin-embedded sections of the brain were stained with antisera to Ryan, Neff, HN3, and AI strains of the genus Hartmannella and the HB-I strain of the genus Naegleria, with negative results. The failure to stain with HB-I antiserum makes it unlikely that the organism is a Naegleria; this supports the morphologic appearance.[21]

The failure to stain with the hartmannellid antisera probably means that the organism concerned is of a species other than those tested for, since it has been shown by Cerva and Kramer[8] that there are considerable antigenic differences between different species of hartmannellids.[21]

Discussion

Earlier reports of primary amebic meningoencephalitis in man suggested that the disease might be caused by free-living amebas of the family Hartmannellidae.[16] Subsequently, cultural isolation of the causative organisms showed that in most, if not all, cases, they belonged to a different family of free-living amebas and constituted a distinct new species, now known as *Naegleria fowleri* (*N. aerobia* and *N. invades* being junior synonyms).[5]

However, the possibility that both types of organisms might be involved continued to be entertained,[6,20,22,23] chiefly as a result of the work of Culbertson and coworkers, who showed that hartmannellid amebas could produce a variety of lesions, including meningoencephalitis, in experimental animals.[10,13,14]

Although the final proof of cultural isolation and precise identification are still to come, a recent report presented very strong morphologic evidence of hartmannellid etiology in a human brain lesion and reviewed four other cases in which similar amebas might have been involved.[18] However, in all these cases the disease had run a rather chronic and indolent course by comparison with primary amebic meningoencephalitis, which has been invariably a rapidly progressive disease, usually proceeding to a fatal outcome within a few days. Furthermore, in all the cases there was an underlying major disease process that might have predisposed to amebic infection, in contrast to primary amebic meningoencephalitis, where significant underlying disease has never been found.

We believe that the presenting symptoms, clinical features, and pathologic findings in our case justify the conclusion that we are dealing with a definite example of primary amebic meningoencephalitis caused by a hartmannellid ameba.

Clinical Features

The clinical presentation of our case conformed in many respects to the general descriptions by other authors of the Naeglerial disease.[4,15] The patient had frontal headache, fever, disorientation, and fluctuating coma, followed by death 10 days after admission. Although this course was rather more prolonged than that characteristic of Naeglerial infections, it was unquestionably still a rapidly progressive one, quite unlike that observed in previously reported cases of hartmannellid infections.[18]

Significant predisposing disease, recorded in all previously reported cases of hartmannellid infections,[18] does not ap-

pear to have been a factor in our case, as the only other disease process found was asymptomatic early portal cirrhosis.

The fortuitous use of amphotericin B, the only drug effective for treating primary Naeglerial meningoencephalitis,[3,15] might have modified the disease process in our case; pyrexia was relieved and the cerebrospinal fluid appeared to have been sterilized. However, amphotericin B has been shown to have no effect on hartmannellid amebas,[7] and the rather prolonged course of the disease compared with the Naeglerial variety therefore probably reflects the essentially less virulent nature of hartmannellid infections.[18]

Pattern of Pathology

The histopathology of the lesions in the brain conformed in some respects to the description of lesions produced by naegleria spp.[2,6,9] There were however, several significant differences, which can be explained on the basis of a hartmannellid infection. Amebas were absent from the subarachnoid space, even in the deeper Virchow-Robin spaces. Amebas were found only in the superficial cortical tissue of the brain. In addition, there were numerous areas with a granulomatous reaction around degenerating amebas. In other areas where there were large numbers of trophozoites the inflammatory reaction was minimal, but tissue necrosis was prominent.

The recent description of granulomatous tissue reactions to the presence of *N. aerobia* in guinea pig viscera shows that the nature of the reaction may depend on host–parasite relationships.[10] A further feature of similarity between our case and the experimental lesions produced by Culbertson and co-workers[10] was the vascular lesions. Vasculitis, in both the granulomatous lesions in the brain and the granulomatous cutaneous lesions, was quite pronounced in our case. In addition, like Culbertson and co-workers, we were able to demonstrate amebas in the vessel walls of the cutaneous lesion. Our case, therefore, had several features which up to now had been demonstrated only experimentally with *N. aerobia* infection in guinea pigs.[14] However, morphologically there can be no doubt that our case was caused by a hartmannellid.

Chronic granulomatous lesions in association with primary amebic meningoencephalitis due to Naegleria spp. have not been described. Even in cases of localized lesions resulting from hartmannellid infection such lesions have not been found.[18]

Portal of Entry

In primary meningoencephalitis caused by Naegleria spp., the route of entry of the amebas has been shown beyond doubt to be through the nasal mucosa, via the cribriform plate.[4,12] The portal of entry in hartmannellid infections is less certain.

In each of the five cases reported previously there had been some predisposing factor favoring secondary invasion by hartmannellid amebas, but the route of entry of the amebas was not suggested.

Culbertson and co-workers[10] have demonstrated widespread visceral lesions after subcutaneous inoculation of guinea pigs with *N. aerobia.* Also, there may be widespread dissemination without demonstrable disease.[15] However, hematogenous spread is thought to be unlikely in primarily amebic meningoencephalitis because of the presence in man of a factor amebicidal to Naegleria spp.[3] An amebicidal factor against hartmannellids in man has not been demonstrated.

There was no doubt about the amebic cellulitis of the abdominal wall, where amebas could be demonstrated, in our case. It is tempting to postulate that this was the portal of entry, with hematogenous dissemination to the brain.

The disease was, however, predominantly basal meningoencephalitis, and the olfactory bulbs were not unlike those described to occur in Naeglerial menin-

goencephalitis, where entry of organisms has been via the cribriform plate. The localization of the major changes in the brain around the basal cortex suggests that the route of entry may have been similar to that of Naeglerial meningoencephalitis.

Epidemiology

Primary amebic meningoencephalitis has been reported from widely scattered areas of the world, and has been reviewed critically by Carter.[3] There has been one (doubtful) report from Central Africa, by Grundy and Blowers.[17] For several reasons it is unlikely that this was a case of primary amebic meningoencephalitis.[3] If we exclude the case of Grundy and Blowers, except for our case, no proven case has been reported from Africa. We find this surprising in view of the distribution of the free-living amebas in nature.[3,11,15,23] Free-living amebas have been discovered in South Africa.[19] The causative organisms thus are present, but the disease has not been found. That all the cases are being overlooked throughout Africa is too simplistic an explanation, and we believe that, for a rare disease, it is even rarer in Africa. We can offer no other explanation.

Acknowledgments. Dr. W. P. Stamm offered encouragement in this case and attempted to identify the ameba by immunofluorescent staining. Mr. Ansary assisted with the illustrations.

References

1. Butt CG: Primary amebic meningoencephalitis. N Engl J Med 274:1473–1476, 1966
2. Callicott JH Jr: Amoebic meningoencephalitis due to free-living amoebas of the Hartmannella (Acanthamoeba) Naegleria group. Am J Clin Pathol 49:84–91, 1968
3. Carter RF: Acute pyogenic meningitis probably due to Acanthamoeba sp. Br Med J 2:740–742, 1972
4. Carter RF: Description of a *Naegleria* sp isolated from two cases of primary amoebic meningoencephalitis, and of the experimental pathological changes induced by it. J Pathol 100:217–244, 1970
5. Carter RF: Primary amoebic meningoencephalitis. Unpublished Thesis, University of Adelaide, 1971
6. Carter RF: Primary amoebic meningoencephalitis: clinical, pathological and epidemiological features of six fatal cases. J Pathol Bacteriol 96:1–25, 1968
7. Casemore DP: "Sensitivity of Hartmannella (Acanthamoeba) to 5-fluorocytosine, hydroxy stilbimadine, and other substances. J Clin Pathol 23:649–652, 1970
8. Cerva L, Kramer J: Folia Parasitol (Praha) 20:113–118, 1973
9. Cerva L, Novak K: Amoebic meningoencephalitis: Sixteen fatalities. Science 160:92, 1968
10. Culbertson CG: Amebic cellutocutaneous invasion by *Naegleria aerobia* with generalized visceral lesions after subcutaneous inoculation: An experimental study in guinea pigs. Am J Clin Pathol 57:375–386, 1972
11. Culbertson CG: Pathogenic acanthamoeba (Hartmannella). Am J Clin Pathol 35:195–202, 1961
12. Culbertson CG: Pathogenic naegleria and Hartmannella (Acanthaemoeba). Ann NY Acad Sci 174:1018–1022, 1970
13. Culbertson CG, Ensminger PW, Overton WM: Hartmannella (acanthamoeba). Experimental chronic, granulomatous brain infections produced by new isolates of low virulence. Am J. Clin Pathol 46:305–314, 1966
14. Culbertson CG, Smith JW, Cohen HK, et al: Experimental infection of mice and monkeys by Acanthamoeba. Am J Pathol 35:185–197, 1959
15. Duma RJ, Rosenblum WI, McGehee RF, et al: Primary amebic meningoencephalitis caused by Naegleria. Two new cases, response to Amphotericin B and a review. Ann Intern Med 74:861–869, 1971
16. Fowler M, Carter RF: Acute pyogenic meningitis probably due to Acanthamoeba sp. Br Med J 2:740–742, 1965
17. Grundy R, Blowers R: A case of primary amoebic meningo-encephalitis treated with chloroquine. E Afr Med J 47:153–158, 1970
18. Jager BV, Stamm WP: Brain abscesses caused by free-living amoebae probably of the genus Hartmannella in a patient with Hodgkin's disease. Lancet ii:1343–1345, 1972
19. Lastovica AJ, Elsdon-Dew R: Primary amoebic meningo-encephalitis caused by Naegleria sp. S Afr J Sci 67:464–465, 1971
20. Skocil V, Cerva L, Serbus C, et al: Epidemiological study of amoebas of the Limax group in military communities. J Hyg Epidemiol Microbiol Immunol (Praha) 14:61–66, 1970
21. Stamm WP: Personal communication, 1973
22. Stamm WP: Primary amoebic meningoencephalitis (discussion). Trans R Soc Trop Med Hyg 66:210–211, 1972
23. Symmers W StC: Primary amoebic meningoencephalitis in Britain. Br Med J 4:449–454, 1969

*Reference # 3 should be
Carter, RF: "Primary amoebic
meningoencephalitis an appraisal
of pressent knowledge"
Trans.Roy.Soc.Trop.Med.Hyg.
66: 193-213, 1972