Primary Amoebic Meningoencephalitis in Britain

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Summary: Meningoencephalitis proved to be due to an amoeba (*Naegleria*) has been diagnosed in Great Britain for the first time. The first patient (a boy of 2) survived longer than any previously recorded cases, but in spite of early diagnosis and treatment he died 15 days after the onset of meningeal symptoms.

Two other children who were exposed to the same possible source of infection (a warm, muddy puddle) had similar symptoms and developed mild meningitis. A *naegleria* was isolated from the cerebrospinal fluid of one of them. Both recovered after treatment with amphotericin.

Introduction

Free-living amoebae can be found in almost any sample of soil taken anywhere in the world, and are commonly present in the air (Kingston and Warhurst, 1969) and in contaminated water. Among many different genera two common ones are *Hartmannella* (*Acanthamoeba*) and *Naegleria*. Until the pioneering work of Culbertson, in 1959, free-living amoebae were not thought to be pathogenic. In a series of experiments Culbertson showed conclusively that *Acanthamoeba* could produce an acute and fatal meningoencephalitis in laboratory animals if introduced by the intracerebral or nasal route (Culbertson *et al.*, 1959; Culbertson, 1961).

It is surprising that infections with such amoebae had not been reported before in man, but Fowler and Carter (1965) described four cases of acute pyogenic meningitis in South Australia. The patient died quickly in spite of the usual antibiotic treatment, and in all cases brain histology after death showed large numbers of amoebae. Morphologically these were similar to the amoebae in the brains of Culbertson's experimental animals. Since then similar cases have been described in Florida (Butt, 1966; Butt *et al.*, 1968; R. J. Poppiti, personal communication, 1969), Virginia (Callicott, 1968; Callicott *et al.*, 1968; Wagner *et al.*, 1969), Texas (Patras and Anduiar, 1966), Czechoslovakia (Červa *et al.*, 1968, 1969), South Australia (Carter, 1968, 1969), and New Zealand (*New Zealand Medical Journal*, 1969). Of the 44 cases reported, only nine were diagnosed during life. Two further possible cases have been described recently in Great Britain (Symmers, 1969).

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The causative agent has been cultured from cerebrospinal fluid (C.S.F.) or post-mortem material in six cases (Butt *et al.*, 1968; Callicott *et al.*, 1968; Carter, 1968, 1969; Červa *et al.*, 1969; *New Zealand Medical Journal*, 1969). In all these cases the amoeba proved to be not a *hartmannella* but a *naegleria*. All 44 cases were fatal; but a further patient, mentioned by Callicott *et al.* (1968), recovered without treatment though an *acanthamoeba* was isolated from the C.S.F. This may well have been a contaminant (R. J. Duma, personal communication, 1969).

We report here a fatal acute case of meningoencephalitis in which a *naegleria* was isolated. In addition the illnesses of two children who were exposed to the same possible source of infection are described.

Case 1

A boy aged 2 years 9 months was admitted in early August 1969 with *one week's history* of anorexia, mild irritability, and slight sore throat. On the day before admission he complained of headache and more severe sore throat, and the family doctor prescribed oral penicillin. On the day of admission he became progressively more ill and vomited all food or liquid taken. Attacks of intermittent pallor and flushing with peripheral cyanosis were noticed by the parents.

On admission he was pale and conscious but disorientated. There was pronounced head retraction and opisthotonus with obvious neck rigidity and positive Kernig's sign, temperature 102.4° F. (39.1° C.). Examination of the nervous system showed no localizing signs. The optic fundi were normal. The throat was inflamed, with purulent exudate over both tonsils. The clinical diagnosis made was pyogenic meningitis.

Investigations.—C.S.F.: clear, colourless, pressure not raised; 850 red cells (R.B.C.), 6 lymphocytes, 6 polymorphs per cu.mm.; protein 180 mg./100 ml.; sugar 50 mg./100 ml.; sterile bacteriologically. E.S.R. 24 mm. in one hour. Blood sugar 60 mg./100 ml. Serum bicarbonate 13 mEq/l. Other serum electrolytes normal. Blood urea normal. Bacteriological examination of throat swab and urine normal. Heaf test negative.

Treatment was started with intramuscular sulphadiazine, penicillin, and ampicillin.

Six hours after admission the child had stopped breathing. His pupils became dilated. His heart rate remained normal. Resuscitation was carried out, at first with mouth-to-mouth respiration and then with positive-pressure respiration through an endotracheal tube. Spontaneous respiration did not begin and after 60 minutes the positive-pressure respiration was transferred to a Cape ventilator and a Jackson Reece nasotracheal tube. The blood pH was 7.6, the PCO₂ and bicarbonate levels being consistent with poorly compensated respiratory alkalosis. Carbon dioxide was therefore added to the input of the Cape ventilator and corrected the alkalosis.

The patient remained comatose, with fixed dilated pupils and complete lack of response to stimuli. There was no papilloedema. Antibiotics were given in an intravenous infusion of 0.18% sodium chloride with 4.3% dextrose.

FIG. 1.—Case 1. C.S.F. collected two days after admission. To show characteristic viscid white deposits scattered over inside of bottle.

Wet preparation of C.S.F. two days after admission showing an amoeba. Phase contrast. (X 7,635).

Two days after admission lumbar puncture yielded a fluid which looked remarkably different. It was reddish brown and thick with a creamy deposit (Fig. 1). It contained much amorphous debris and numerous red cells and about 3,000 "white cells" per cu.mm., of which half were leucocytes (90% polymorphs, 10% lymphocytes) and half were amoebae (Fig. 2). Direct phase contrast microscopy of the C.S.F. showed that these amoebae were slightly larger than polymorphs, with a clear ectoplasm and a granular endoplasm containing a contractile vacuole. They had three or four spiky pseudopodia and moved very slowly, alteration in shape being perceptible over a period of 30 to 45 seconds. No cysts were seen.

Treatment was changed to amphotericin B (0.25 mg./kg. in one daily dose given over three to four hours intravenously, increasing over a week to 1 mg./kg.). This treatment was continued until the death of the patient. Sulphadiazine was continued intravenously (750 mg. six-hourly). Other antibiotics were stopped.

C.S.F. was sent to Mr. Belton at the Microbiological Research Establishment, Porton, and the presence of amoebae was confirmed. C.S.F. was also sent to the Liverpool School of Tropical Medicine, where the amoebae were cultured. They were identified as *Naegleria* sp., from the morphology of the trophozoite and cystic stages and the mitotic figures, and the organism's ability to produce a characteristic flagellate stage in distilled water (Singh, 1952; Culbertson *et al.*, 1968; Kingston and Warhurst, 1969).

On the sixth day the patient's temperature fell to 95° F. (35° C.). Subsequently increased environmental heat was required to maintain the temperature at 97° F. (36.1° C.).

On the seventh day a few spontaneous movements were noticed in the legs, but otherwise there was no change clinically. Lumbar puncture yielded a xanthochromic fluid containing 14,000 R.B.C., 1,400 polymorphs, 150 lymphocytes, and 650 amoebae per cu.mm. No pseudopodia were seen and the amoebae were moving only very slowly. Many appeared to be dead. No cysts were seen. The level of amphotericin in the C.S.F. was 0.184 µg./ml. No virus was isolated from the nose, throat, or C.S.F.

On the eleventh day the C.S.F. was still xanthochromic and heavily blood-stained, but no polymorphs or amoebae were seen. The level of amphotericin in the C.S.F. was 0.224 µg./ml.

On the fourteenth day the child attempted a few spontaneous

respiratory movements when the respirator was stopped for adjustment. Some response to pain was elicited. Oedema of the face, limbs, and sacrum was observed. At this stage the haemoglobin was 5.5 g./100 ml.; blood urea 42 mg./100 ml. Serum electrolytes were normal.

On the fifteenth day two attacks of cyanosis and rigidity occurred. Treatment with phenobarbitone (15 mg. twice daily) was started. Spontaneous voiding of urine was not taking place and intermittent carbachol injections were given.

On the sixteenth day oedema was more pronounced and the liver and spleen were palpable. There were repeated episodes of cyanosis and generalized increased extensor tone. Convulsions occurred which responded to intramuscular paraldehyde. In view of the clinical evidence of congestive cardiac failure, digitalis was given. In the evening the patient developed episodes of bradycardia followed by cardiac arrest, responding to adrenaline given intravenously. After five of these attacks he died.

Post-mortem Findings

The brain showed extensive softening of its whole substance (so-called "pump brain") such as is found in patients who have been kept alive with a respirator. In addition there was a severe haemorrhagic meningitis with fibrous thickening of the meninges. This extended over the base of the brain from the orbital surfaces of both frontal lobes, up both Sylvian fissures, over the base of both temporal lobes, back on to the midbrain, pons, and medulla, on to the left cerebellar hemisphere, and down the spinal cord. Microscopically there was extensive chronic inflammatory infiltration of the meninges accompanied by fibrosis and early calcification. The most severely affected area was the brain stem, where there was also haemorrhage and inflammation within the brain substance. Three or four cells which might have been *naegleria* were seen in this area.

Pieces of brain (the inferior surfaces of the frontal lobe and both temporal lobes) were sent to Liverpool, where amoebae were cultured from all three pieces. The trophozoites and cysts were very much smaller than those isolated from the C.S.F. Identification of this isolate is under way.

Case 2

A boy aged 6 years, the brother of Case 1, was admitted two days after his brother. On the morning of admission he complained of headache and feeling cold. He became anorexic and in the evening developed a sore throat and neck pains.

On admission he was flushed and had an oral temperature of 103° F. (39·4° C.). He was conscious and well orientated. He had no neck stiffness at this time and Kernig's sign was negative. The central nervous system and optic fundi were clinically normal. The throat was inflamed but not purulent.

Investigations.—C.S.F.: clear, no cells or amoebae seen, protein 32 mg./100 ml., no amoebae isolated on culture of specimen six days later. Blood: white cells 8,400/cu.mm. (84% polymorphs, left shift, many metamyelocytes), haemoglobin 11·6 g./100 ml. Throat swab, urine, and blood culture normal.

The clinical picture was that of an upper respiratory infection, but in view of his brother's serious illness it was decided to treat with amphotericin B and sulphadiazine while awaiting the results of further investigations. The amphotericin was given in doses of 0·25 mg./kg. intravenously over three to four hours daily and continued for eight days. During this period he improved, the signs of upper respiratory infection subsided, and he became symptom-free.

On the eighth day he complained again of sore throat, with head and neck pains. The temperature was 101° F. (38·3° C.). A maculopapular rash was seen on the buttocks and thighs. The throat appeared inflamed and the cervical glands were palpable and tender. There was obvious neck stiffness and a positive Kernig's sign was elicited. On lumbar puncture the C.S.F. was clear and colourless and contained 30 R.B.C. and 2 polymorphs per cu.mm. and less than 20 mg. of protein per 100 ml. No amoebae were seen on direct microscopy, but some were grown (see below). No virus was isolated from the nose, throat, C.S.F., or faeces. Since the C.S.F. appeared normal, and he was showing signs of sulphadiazine toxicity, all treatment was stopped.

On the ninth day the rash had become morbilliform and was symmetrical over the trunk, limbs, and face. He felt much better, the neck was less stiff, and the temperature was 102° F. (38·9° C.).

By the twelfth day he had become afebrile, had no meningism, and felt very well. Though he had appeared to have recovered, the isolation of amoebae from C.S.F. taken four days previously was now reported. These were similar morphologically to those isolated from the C.S.F. in Case 1. Treatment was therefore started again with amphotericin B (0·25 mg./kg. given daily intravenously over four hours, increasing to 0·75 mg./kg. after four days for a total of 10 days; total dose 6·5 g./kg.). Specimens of C.S.F. taken on the twelfth and eighteenth days were normal, and no amoebae were grown from them. The patient was discharged home symptom-free. Ten days later a further lumbar puncture yielded a normal C.S.F. from which no amoebae were isolated.

Case 3

A boy aged 4 years 5 months, a neighbour of Cases 1 and 2, was admitted six days after Case 1, at first under another physician. Two days before admission he had had a booster dose of diphtheria, pertussis, and tetanus vaccine. On the morning of admission he complained of sore throat and headache. He vomited three times and complained of abdominal pain.

On admission he was conscious, well orientated, but irritable. His temperature was 103° F. (39·4° C.). The tonsils were inflamed. There was no neck stiffness, Kernig's sign was negative, and the central nervous system and fundi were normal. C.S.F.: clear, colourless, no cells, protein 60 mg./100 ml., sugar 85 mg./100 ml., no bacterial growth. Blood: white cells 12,000/cu.mm., haemoglobin 13·6 g./100 ml. Urine normal. Throat swab normal. Heat test negative. No treatment was given and the patient was kept under observation.

By the third day the temperature had become normal, but the headache continued and slight neck stiffness was elicited, though Kernig's sign remained negative. The C.S.F. on this day contained 1,280 R.B.C., 25 lymphocytes, and 3 polymorphs per cu.mm. No amoebae were seen. No virus was isolated from the nose, throat, C.S.F., or faeces. In view of the relative lymphocytosis in the C.S.F., treatment was started with sulphadiazine and amphotericin B (0·25

mg./kg. given daily in one dose over four hours intravenously). The next day the patient complained of abdominal pain and there was albuminuria and macroscopic haematuria. The treatment with both drugs was therefore stopped, the haematuria rapidly disappeared, and he became quite well.

On the eighth day growth of amoebae from the C.S.F. of Case 2 was reported. Consequently, though the present patient seemed quite well, daily amphotericin (0·25 mg./kg. increasing to 0·75 mg./kg.) was given intravenously for 10 days. C.S.F. specimens taken on the eighth, fourteenth, and twenty-fourth days were all normal and no amoebae were isolated. He was discharged home on the fourteenth day symptom-free.

Possible Source of Infection

Case 3 lived three doors away from Cases 1 and 2 in a block of terraced houses on the outskirts of Bristol. There was no history of recent bathing by these children, but all three had splashed about in a muddy puddle. This puddle had appeared the night before, during a heavy thunderstorm following a long period of exceptionally hot, dry weather. The puddle, a few inches deep, was in a low-lying part of a garden near the houses of the patients and covered part of a flower bed. By the next day the puddle had dried up. The first respiratory symptom of Case 1 appeared within two days of playing in the puddle. The onset of respiratory symptoms in Cases 2 and 3 occurred 9 and 13 days after splashing in the puddle. Soil from the bottom of the dried up puddle was taken for investigation and has yielded amoebae of many different species which are still being studied.

Discussion

Causative Organism.—In its presentation and course the illness in Case 1 was typical of the syndrome of amoebic meningoencephalitis, first described by Fowler and Carter (1965). All organisms isolated from similar cases have proved to be *Naegleria* sp., and an amoeba of this species was isolated from the C.S.F. in Case 1 two days after the onset of meningitis. At necropsy 13 days later most of the naegleria had disappeared. None were cultured from the brain and only a few were seen. Instead, very much smaller amoebae of a different species, which are still being identified, were cultured from all parts of the brain, though they could not be seen in the sections. Unlike the naegleria, which were seen in the C.S.F. as well as being cultured, these small amoebae may have been post-mortem room contaminants.

How Infection Occurred.—Most of the reported cases of amoebic meningoencephalitis occurred in areas with a warm climate, and there was usually a strong association with swimming, and particularly diving, in inland lakes or polluted pools. An exception to this is that some of the Czechoslovak cases (Červa *et al.*, 1968) occurred in October and November. This outbreak was associated with swimming in a heated indoor pool. The New Zealand cases also occurred in the winter and were associated with swimming in a hot mineral pool (*New Zealand Medical Journal*, 1969). Though the weather in the western part of Britain is usually temperate and much cooler than areas from which amoebic meningoencephalitis has been reported, the summer of 1969 was exceptionally warm. From the middle of July to the beginning of August daily temperatures in the area where the present cases occurred were usually more than 20°C. and twice exceeded 28°C. The muddy puddle in which these children played was formed during a thunderstorm after a particularly hot day. It was shallow and was situated partly on a flower bed. It seems probable that the puddle contained amoebae in a rapidly growing trophozoite form. Mice have been shown to be infected intranasally by trophozoites of acanthamoebae, espe-

cially when they are rapidly growing (Červa, 1967). There is no indication that cysts are infective.

How Common is this Condition?—The diagnosis of amoebic meningoencephalitis has been shown to be easily overlooked. In Czechoslovakia (Červa *et al.*, 1968) and the United States (Callicott, 1968) 23 cases have been found retrospectively by examining post-mortem material from cases originally diagnosed as purulent meningitis. It is possible that in Britain the climate is such that the disease is extremely rare. We have looked through post-mortem material from deaths due to meningitis, with no organism identified, from several local hospitals and found no cases. So far as we know the only person to have found such amoeba in post-mortem material in this country is Symmers (1969), who found them in a brain from a necropsy done 60 years ago.

Diagnosis.—The diagnosis of amoebic meningoencephalitis is not difficult if the condition is suspected. The amoebae are the same size as the polymorphs also present in the C.S.F. If examined in a counting chamber, or in fixed and stained preparations, the amoebae can readily be mistaken for polymorphs or lymphocytes. If the C.S.F. is examined under high-power phase contrast, and each cell is studied for several seconds, the movement of the pseudopodia and the contraction of the contractile vacuole will be seen. Culture of the amoebae can be difficult and will be discussed in a later paper. The condition should be suspected in all cases of meningitis with a high cell count if no bacteria are seen.

Do these Patients ever Recover?—To our knowledge only one previous patient ever recovered (Callicott *et al.*, 1968), but the amoeba in that case was not a naegleria (see above). In our Case 2 we have considered whether the isolation of amoebae from the C.S.F. was due to laboratory cross-infection, as amoebae were not seen on direct examination. From a study of the laboratory manipulations taking place at the time the specimen was examined this appears to be unlikely. Case 3 must at present be considered to have been only doubtfully infected with amoebae. Unfortunately insufficient C.S.F. was taken at the height of the meningitis for any to be sent to Liverpool for culture for amoebae. Thus it seems likely that the illness was due to naegleria in Case 2, but possibly not in Case 3. If these children did recover from amoebic meningoencephalitis, amphotericin treatment may have been a deciding factor.

Treatment.—Though the diagnosis in Case 1 was made relatively early, and treatment with amphotericin B started promptly, the patient died after being treated for 13 days. Soon after admission, however, he stopped breathing and thereafter was kept alive by a respirator. The severe cerebral changes found at necropsy suggested anoxic damage as well as continued amoebic invasion. In other described cases amoebae were seen in all areas of meningitis. In this treated case amoebae were found only in the brain stem. Carter (1969), using a naegleria isolated from a patient with meningo-

encephalitis, found that amphotericin B was the only effective agent, being highly amoebicidal both in vitro and in mouse experiments. On the basis of this work he advised rapid treatment with a high dose of amphotericin given both intravenously and intraventricularly. Amphotericin at a concentration of 0.22 µg./ml. was found in the C.S.F. of our Case 1 though he was receiving amphotericin only intravenously. No detectable amphotericin was found when the C.S.F.s of our Cases 2 and 3 were examined during intravenous amphotericin treatment. Wagner *et al.* (1969) treated two patients with chloroquine and intravenous amphotericin and in addition gave amphotericin intracisternally to one patient and intraventricularly to the other. Both succumbed within three days of the start of treatment. The search for effective treatment will receive an impetus as more cases are recognized.

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