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THE SUSCEPTIBILITY OF PATHOGENIC FREE-LIVING AMEBAE TO CHEMOTHERAPEUTIC AGENTS

A thesis presented in partial fulfilment of the requirements for the degree of

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ABSTRACT

The treatment of infections caused by pathogenic free-living amebae (PFLA) has, until only recently, been far from successful. The continued screening of chemotherapeutic agents against amebae of the genera Naegleria and Acanthamoeba is therefore of the utmost importance.

Seven chemotherapeutic agents, amphotericin B, rifampicin, tetracycline, polymyxin B sulphate, 5-fluorocytosine, miconazole and R41,400 were screened for activity against a non-pathogenic and pathogenic species of Naegleria and a non-pathogenic and pathogenic species of Acanthamoeba in axenic culture. For the Naegleria spp. amphotericin B, miconazole and R41,400 were found to be active. Acanthamoebae spp. were found to be susceptible only to 5-fluorocytosine and R41,400.

The possible use of combinations of drugs against the amebae was also investigated in axenic culture. For Naegleria fowleri (MsT) amphotericin B with either tetracycline or rifampicin showed a synergistic effect. Polymyxin B sulphate and 5-fluorocytosine showed synergistic activity against Acanthamoeba culbertsoni (A-1) but when polymyxin B was combined with tetracycline or rifampicin no significant additive effect was seen.

After axenic culture testing the susceptibility of the pathogenic species, N. fowleri (MsT) and A. culbertsoni (A-1), to the agents which showed activity, was investigated in a Vero cell culture system. For N. fowleri (MsT) the results of axenic testing were confirmed with amphotericin B, miconazole and R41,400 protecting the monolayer from the destructive effects of the amebae. 5-Fluorocytosine inhibited the formation of cytopathic effect (CPE) when the cell cultures were inoculated with A. culbertsoni (A-1) but viable amebae were still present. R41,400 had no effect on A. culbertsoni (A-1) at concentrations at or above those which were cytotoxic to the Vero cells.

The use of combinations of drugs was also investigated in Vero cell culture. Amphotericin B and rifampicin showed an antagonistic rather than a synergistic effect when used against N. fowleri (MsT) in cell culture but amphotericin B and tetracycline showed synergistic activity.

For A. culbertsoni (A-1) the synergistic activity of polymyxin B and 5-fluorocytosine was confirmed. The lack of an additive effect

between polymyxin B and either tetracycline or rifampicin was also shown in cell culture.

The new imidazole derivative R41,400, which showed promise against N. fowleri (MsT) in in vitro tests was then tested in the in vivo situation. Mice experimentally infected with N. fowleri (MsT) were treated once or twice daily intraperitoneally with different doses of R41,400. At the higher dosage levels tested the drug appeared to have a deleterious effect, the average time for death being less than that for the controls.

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CHAPTER ONE: INTRODUCTION

1.1. The History of Free-Living Amebae as Disease Agents

The history of pathogenic free-living amebae (PFLA) of the genera Acanthamoeba and Naegleria has been extensively reviewed elsewhere (Culbertson, 1971; Duma et al., 1971; Chang, 1971, 1974a; Carter, 1972; Cursons, 1974; Cursons & Brown, 1976).

The commonest disease caused by PFLA is known as Primary Amebic Meningo-encephalitis (PAM) and in an extensive worldwide survey, Willaert (1974) tabulated 84 cases from all continents with the exception of Antarctica. Since then at least ten additional cases have been reported.

Prior to 1968, most cases were attributed to Acanthamoeba spp.. This is probably a reflection of the pioneering work of Culbertson et al. (1958, 1959, 1965) whom, whilst working on the production of polio vaccine, found an ameba which contaminated the cultures of monkey kidney cells. When the cultures were inoculated intracerebrally into mice and primates, a necrotizing, hemorrhagic meningo-encephalitis was produced that killed the animals in four to seven days. The responsible ameba was identified as an Acanthamoeba and they predicted, on the basis of this finding, that this ameba could be capable of producing disease in humans. This ameba was previously considered to be a harmless, free-living ameba.

However, in 1968, Butt et al., Carter and Culbertson et al., showed that the incriminating species of most reported human cases belonged to the related genus Naegleria. In 1970, on the basis of morphological, cultural and pathogenicity differences, Carter named the pathogenic species Naegleria fowleri distinguishing it from the non-pathogenic Naegleria gruberi.

The disease caused by PFLA can be divided into two types:

i) a swimming - associated acute meningo-encephalitis (Primary Amebic Meningo-encephalitis (PAM) - Martinez et al.(1977) caused by N. fowleri.

and ii) a non-swimming - associated chronic meningo-encephalitis (Amebic Meningo-encephalitis (AM) - being regarded as a secondary invasion of the central nervous system (CNS) having spread

from other foci of infection (Martinez et al., 1977)) caused by a variety of pathogenic Acanthamoebae, notably Acanthamoeba culbertsoni, Acanthamoeba castellanii and Acanthamoeba polyphaga (Chang, 1974a).

Subsequently, Acanthamoeba spp. have also been indicated in a number of chronic illnesses such as respiratory infections (Martinez et al., 1975), corneal ulceration of the eye resulting in blindness (Nagington et al., 1974; Visvesvara et al., 1975) and together with Naegleria spp. in humidifier fever (M.R.C. Symposium, 1977).

Henceforth in the text the nomenclature of Martinez et al.(1977), PAM for Naegleria infections and AM for Acanthamoeba meningo-encephalitis, will be adopted.

The controversy regarding the classification of PFLA (Cursons & Brown, 1976) appears to be settled with the majority of authors preferring Chang's (1971) classification scheme. The identification of isolates involves the exploitation of specific cytological, morphological, physiological, immunological, growth and pathogenicity characteristics in an ordered sequence readily usable by hospital and public health laboratory staff (Cursons & Brown, 1976).

1.2 Occurence and Distribution

The summary of recorded isolations from a wide variety of environmental sources provided by Cursons (1978) emphasizes the truly ubiquitous distribution of PFLA. The ability of PFLA to form resistant cysts undoubtedly enables them not only to withstand unfavourable conditions, e.g., the isolation of pathogenic Acanthamoebae from $2^{o}C$ (Brown & Cursons, 1977), but also to take advantage of the intermittent occurrence of favourable conditions.

The distribution of the pathogenic species in relation to non-pathogenic ones is still unknown (Cursons, 1978). In general, non-pathogenic species are more prevalent at ambient temperatures in temperate zones. The repeated isolations of PFLA from water above ambient temperature, i.e., $\geqslant 30^{o}C$ (De Jonckheere et al., 1977; De Jonckheere & Van De Voorde, 1977a; Stevens et al., 1977; Wellings et al., 1977; Cursons et al., 1978b), combined with their higher optimum temperature of growth (Griffin, 1972) suggests that pathogenic amebae are environmentally selected over non-pathogenic amebae in

waters above ambient temperature. The source of pathogenic amebae in these waters is unknown but as Cursons et al. (1978b) and Wellings et al. (1977) have succeeded in isolating PFLA from soil it is possible that soil acts as a reservoir of pathogens and contamination occurs via run-off after rain (Cursons, 1978).

1.3. Pathogenicity

The invasion of organs and tissues by PFLA is now well documented (Culbertson et al., 1959, 1968, 1972; Carter, 1968, 1970, 1972; Callicott et al., 1968; Chang, 1971, 1974a & b, 1976; Culbertson, 1971; Martinez et al., 1973, 1975, 1977; Visvesvara & Balamuth, 1975; Wong et al., 1975a & b; Hoffman et al., 1978). It has been established experimentally that the portal of entry into the CNS in N. fowleri infection is via disruption of the olfactory mucosa, penetration of the organisms into the submucosal nervous plexus, probably by phagocytosis of the amebas by the sustentacular cells of the olfactory neuroepithelium and passage through the cribiform plate to the subarachnoid space (Martinez et al., 1973).

However, in cases of Acanthamoeba meningo-encephalitis the involvement of the CNS appears to be a secondary phenomenon representing metastatic spread from a primary focus in the skin, genitourinary or respiratory tract (Martinez et al., 1977). Cutaneous ulceration as a possible point of entrance with hematogenous spread to the CNS was reported by Bhagwandeen et al. (1975) and Martinez et al. (1977) report involvement of the genitourinary tract. Lower respiratory tract infection in experimental animals have been reported (Martinez et al., 1975).

AM due to Acanthamoeba spp. appears to be an opportunistic infection of the CNS. AM occurs in patients who are chronically ill, debilitated or those whose cell-mediated immune responses have been impaired as a result of either underlying systemic disease or its treatment by immunosuppresive methods (Kernohan et al., 1960; Jager & Stamm, 1972; Robert & Rorke, 1973; Sotelo-Avila et al., 1974; Bhagwandeen et al., 1975).

Acanthamoeba infections of sites with reduced accessibility to the immune system e.g., the eye also demonstrate the opportunistic nature of these infections. Isolates from the cases reported by Nagington et al. (1974) were shown by Visvesvara et al. (1975) to be

of low virulence and infection resulted after damage to the cornea.

Once invasion of the brain has been established in PAM and AM, destruction of surrounding brain tissue is thought to be brought about by a combination of phagocytosis and pinocytosis of host tissue by N. fowleri and solely by pinocytosis in the case of A. culbertsoni (Visvesvara & Callaway, 1974; Maitra et al., 1974, 1976).

The examination of sections from brains infected with either N. fowleri or A. culbertsoni reveals areas of extensive demyelination leaving the trophozoites surrounded by a clear halo (Martinez et al., 1975; Chang, 1976; Maitra et al., 1976). Many authors have speculated on the possibility that enzymes secreted by the amebae are responsible for this. Experiments have shown that both cytotoxic enzymes and phospholipases are produced by PFLA (Elson et al., 1970; Chang, 1971, 1974a, 1976; Hax et al., 1974; Visvesvara & Callaway, 1974; Victoria & Korn, 1975; Visvesvara & Ballamuth, 1975; Cursons & Brown, 1976, 1978; Cursons et al., 1978c; Maitra et al., 1976). The level of production of such cytopathic enzymes may explain the differences in virulence reported amongst Acanthamoeba and N. fowleri isolates (Culbertson, 1971; De Jonckheere & Van De Voorde, 1977b).

1.4. Immunity

The low incidence of PAM and AM in the human population has puzzled many authors in view of the ease and frequency of isolation of virulent PFLA from the environment (Anderson & Jamieson, 1972; Cursons et al., 1976b, 1977; John et al., 1977; Wellings et al., 1977; Haggerty & John, 1978). This has led to speculation on the existence of host related susceptibility factors and the demonstration of specific antibodies to free-living amebae in human sera has been reported (Chang & Owens, 1964; Edwards et al., 1976; Cursons et al., 1977; M.R.C. Symposium, 1977).

Observations that previous exposure of mice to live N. gruberi significantly protected them against a subsequent lethal challenge with N. fowleri (John et al., 1977) supports the idea that unwitting exposure to the ubiquitous N. gruberi may immunize against N. fowleri. A similar immunization may also occur with Acanthamoeba. Cell-mediated immunity (CMI) also appears to play an important part in protection against PFLA (Diffley et al., 1976; Cursons et al., 1977).

1.5. Diagnosis

Successful treatment of this rapidly progressive disease is wholly dependent on prompt and definitive diagnosis. The survival of a nine year old female in Torrance, California (Seidel et al., pers. comm., 1978) and a fourteen year old male in Australia (Anderson & Jamieson, 1972) is attributed to early diagnosis and prompt treatment.

A Naegleria brain infection should be suspected when there is a history of swimming about seven days prior to the abrupt onset of fever, headache, sore-throat, nausea and vomiting (Carter, 1972; Chang, 1974a). The most important laboratory procedure for the diagnosis of PAM is the microscopic examination of cerebro-spinal fluid (CSF). Overall, the CSF is indistinguishable from that obtained from patients with bacterial meningitis and diagnosis relies upon amebae being seen in the fluid and the culture of these for complete diagnosis. Species identification can then be achieved by the method outlined by Cursons & Brown (1976).

In post-mortem diagnosis, a degree of encephalitis is invariably present. The brain shows swelling and redness with the purulent exudate more extensive on the ventral surface of the cerebrum or cerebellum and over the brain stem (Carter, 1972). The grey matter of the cerebral hemispheres and cerebellum shows variable sized lesions which tend to be hemorrhagic and quite soft when they are large (Culbertson, 1971). The existence of redness and destruction of the olfactory nerve occurs only in PAM and could serve to distinguish it from bacterial meningitis (Carter, 1972).

The immunofluorescent antibody (IFAB) technique applied to histologic brain sections taken post-mortem, is a valuable tool in identification of amebae in brains of patients who died from meningo-encephalitis. Antisera can be produced in rabbits and can be made species-specific by suitable absorption. In addition to their value in clinical diagnosis IFAB provide rapid screening methods for the detection of PFLA in swimming pool, tap and other domestic and recreational water supplies.

Immunoperoxidase methods have been used by Culbertson (1975) and Cursons et al. (1976) to demonstrate both Naegleria and Acanthamoeba in the brain sections of patients who died from PAM and AM respectively. This method may be shown in the future to be more valuable than the immunofluorescent technique. It has certain advantages over IFAB, e.g.,

permanent preparations can be made, no specialized equipment is necessary and clear, definitive staining of the tissue elements results (Culbertson, 1975).

Acanthamoeba brain infections are difficult to diagnose even in advanced cases due to lack of specific symptoms and signs and the absence of amebae in the CSF (Chang, 1974a). Nasal and throat swabs may provide more information.

Post-mortem diagnosis relies on the presence of confined, superficial lesions in the grey matter with a minimum inflammatory reaction, and the finding of double-walled wrinkled cysts in apparently normal tissue bordering the lesion (Chang, 1974a). In all the reported cases, except that reported by Bhagwandeen et al. (1975), there was lack of evidence of the involvement of the olfactory bulb and the absence of inflammatory reactions in the surrounding grey and white matter. These observations may help in distinguishing between PAM and AM.

Positive diagnosis was possible in the cases of eye infections reported by Nagington et al. (1974) and Jones et al. (1975) by isolation, and subsequent identification of Acanthamoebae spp. taken from corneal scrapings.

1.6. Control Measures

The necessity for an effective disinfectant can be judged by the increasing number of isolations of free-living amebae from potable and treated and untreated recreational waters (Cerva, 1971a; Chang, 1971; Anderson & Jamieson, 1972; Cerva & Huldt, 1974; Molet et al., 1976; Lyons & Kapur, 1977). The majority of amebae isolated in these studies belonged to the genus Acanthamoeba indicating its greater resistance to chlorine than Naegleria spp..

In reviewing sixteen fatal cases of PAM from an indoor chlorinated swimming pool Cerva (1971a) stated that, "it appears that the constant presence of numerous populations of amebae of the limax group cannot be prevented even under the strictest observations of all routine safety measures applied to potable waters." However, Lyons & Kapur (1977) in a survey of 30 halogenated public swimming pools concluded that the low amebic densities ($< 1.1^{-1}$) in the majority of the pools illustrated that these organisms could be adequately controlled by proper pool maintainence. The possession of resistant cysts, however, constantly compli-

cates the disinfection process.

In a study of alternative disinfectants, Cursons et al. (1978b) found that deciquam 222, chlorine, chlorine dioxide and ozone all possessed potential disinfecting properties for PFLA, but at higher levels than those for disinfecting bacteria. Of the four disinfectants examined, deciquam 222 proved to be the most effective amebicide followed by chlorine, chlorine dioxide and ozone. The final choice of a particular disinfectant must however, remain tied to the physical and chemical properties of the water to be disinfected.

1.7. PAM Cases and Their Treatment

In 1974, Willaert provided an extensive worldwide survey of cases of PAM. Since then at least ten additional cases have been reported (Table I). Conceivably, the actual number of cases may be higher since the symptoms of PAM parallel those of aseptic meningitis. Retrospective studies have disclosed a possible case dating back to 1909 (Symmers, 1969) and fluorescent antibody staining has confirmed that the 1948 case reported by Derrick, originally thought to be due to Iodamoeba butschlii was in fact caused by N. fowleri (McMillan, 1977). The reidentification of the etiological agents of the 1968 cases of PAM in New Zealand as N. fowleri (Cursons & Brown, 1975; Cursons et al., 1976a) has dismissed the notion of slime moulds being involved in the etiology of PAM (Mandal et al., 1970).

The results of treatment of PAM have been far from encouraging. Willaert's summary (1974) provides information on ten possible survivors of PAM and the Californian case of Seidel et al. (pers. comm., 1978) makes the world total eleven survivors (Table II). Such a result is hardly surprising in the earlier cases, where the amebic nature of the disease had not been suspected, and treatment consisted only of antibacterial agents such as sulpha-drugs, penicillin, streptomycin, tetracyclines and chloromphenicol (Fowler & Carter, 1965; Butt et al., 1968; Cerva & Novak, 1968; Dos Santos, 1970; Van den Driessche et al., 1973). However, even in later cases where the antiprotozoal drugs emetine, chloroquine and metronidazole were often used, the course of the disease was not affected in the slightest (Carter, 1968, 1970, 1972; Duma et al., 1971), except in the unproved case of Grundy and Blowers (1970) in which survival was attributed to chloroquine. In this case, amebae, believed to be Naegleria, were

COUNTRY	YEAR	NUMBER OF CASES	CAUSATIVE ORGANISM	DIAGNOSIS	TREATMENT	OUTCOME	REFERENCE
NEW ZEALAND	1974	1	N. fowleri (MsT)	isolation from CSF	Penicillin Ampicillin Amphotericin B	died	Cursons et al., 1976b
	1978	1	N. fowleri (MsM)	isolation from CSF	Amphotericin B	died	Cursons et al., pers. comm., 1978
U.S.A.	1974	1	N. fowleri (Lovell)	isolation from CSF	Unknown	died	De Jonckheere, 1977
	1974	1	Acanthamoeba sp.	IFAB	Steroids Penicillin	died	Martinez et al., 1977
	1975	1	Acanthamoeba sp.	IFAB post-mortem	Unknown	died	Hoffman et al., 1978
	1978	1	Naegleria sp.	isolation from CSF	Amphotericin B Miconazole Rifampin	survived	Seidel et al., pers. comm., 1978
VENEZUELA		1	A. culbertsoni	IFAB	Steroids	died	Martinez et al., 1977
PERU		1	A. castellanii	IFAB	Steroids Antibiotics	died	Martinez et al., 1977
ZAMBIA	1972	1	Acanthamoeba sp.	post-mortem	Antibiotics Amphotericin B	died	Bhagwandeen et al., 1975
KOREA	1958	1	Acanthamoeba sp.	post-mortem	Penicillin Streptomycin Chloramphenicol	died	Ringsted et al., 1975

Table I: Cases of Primary Amebic Meningo-encephalitis Reported After 1974 (modified from Cursons, 1978)

COUNTRY	YEAR	NUMBER OF CASES	CAUSATIVE ORGANISM	TREATMENT	REFERENCE
UGANDA	1968	1	Naegleria	Metronidazole - Emetine - Penicillin Chloroquine.	Grundy & Blowers, 1970
U.S.A.	1967	1	A. astronyxis	Ampicillin Penicillin - G	Callicott et al., 1968
	1978	1	Naegleria	Amphotericin B Miconazole Rifampin	Seidel et al., pers. comm. 1978
INDIA	1970	2	Naegleria	Streptomycin Isonicoteinhydrosine Sulphadexanathosone Amphotericin B	Pan & Ghosh, 1971
	1973	3	N. fowleri	Unknown	S.R. Das, pers. comm. to Willaert(1974)
ENGLAND	1969	2	Naegleria	Antibiotics Sulphadiazine Amphotericin B	Apley et al., 1970
AUSTRALIA	1971	1	N. fowleri	Amphotericin B Sulphadiazine	Anderson & Jamieson, 1972

Table II: Probable and Definite Survivors of Primary Amebic Meningo-encephalitis

seen in the CSF and survived for awhile in culture but were not positively identified. The patient also presented atypical clinical features. Treatment consisted of metronidazole, emetine, penicillin, sulphane and chloroquine.

The ineffectiveness of antibacterial agents against pathogenic Naegleria has been confirmed in vitro by Carter (1969) and Mandal et al. (1970). Of the antiprotozoal agents, emetine HCl is effective against N. fowleri in vitro. Carter (1969) reported a minimum immobilizing level of 12.5 $\mu g.cm^{-3}$ and Krishna Prasad (1972) and Das (1975) report minimum amebicidal concentrations of 16 and 15 $\mu g.cm^{-3}$ respectively, but, it does not protect animals from the disease (Culbertson et al., 1968) probably because it is unable to pass the blood-brain barrier (Parmer & Cottrill, 1949). The ineffectiveness of chloroquine and metronidazole has also been confirmed by in vitro tests and animal protection studies (Carter, 1969; Mandal et al., 1970; Duma et al., 1971).

The only drug to appear promising in the early 1970's was the antifungal agent amphotericin B and as can be seen from Table II it was used in the treatment of all survivors (except the unproved case of Grundy & Blowers, 1970 and Callicott et al., 1968). Amphotericin B is a polyene antibiotic and has been shown to be highly amebicidal to pathogenic Naegleria in vitro (Carter, 1969; Mandal et al., 1970; Duma et al., 1971; Schuster & Rechthand, 1975; Visvesvara & Balamuth, 1975; Duma & Finley, 1976; De Jonckheere & Van De Voorde, 1977; Donald et al., 1979) and to protect mice from the disease (Culbertson et al., 1968; Carter, 1969; Das, 1971).

In 1969, Carter suggested that amphotericin B be tried in the treatment of PAM by simultaneous intravenous (IV) and intraventricular (IVent) administration; the doses recommended were - 0.25 $mg.kg^{-1}$ IV and 1.0 mg into the cerebral ventricles in the first 24 hours. Carter (1972) also suggested sulphadiazine should always be used as well as amphotericin B in case the amebae should occasionally prove to be Acanthamoebae. These amebae have been shown to be resistant to both drugs in vitro (Casemore, 1970; Chang, 1971; Visvesvara & Balamuth, 1975; Duma & Finley, 1976; Nagington & Richards, 1976; Donald et al., 1979), but there is good evidence that they are affected by sulphadiazine in vivo (Culbertson et al., 1965).

Subsequently, such treatment was tried on two patients in the U.S.A. (Duma et al., 1971) who were in the early stages of the disease

and should have responded. In the first of these, (Patient 3, Duma et al., 1971) a right ventricular tap was performed and after removal of fluid (containing many motile amebae) 1.5 mg of amphotericin B dissolved in 5 cm^3 of 5% dextrose injection solution (D_5W) was slowly injected into the ventricle. 10 mg of amphotericin B and 10 mg dexamethasone were administered IV. A nasogastric tube was inserted, through which 400 mg of metronidazole was given four times daily. The patient also received chloroquine base, 200 mg and dexamethasone, 4 mg, intramuscularly (IM) every six hours. Sixteen hours after admission the ventricular tap was repeated and 1.5 mg of amphotericin B was again injected into the ventricle. Seventy-two hours after admission he became shock-like, respirations ceased and he died.

The second case (Patient 4, Duma et al., 1971) received, within two hours of admission, 1.5 mg amphotericin B diluted in 5 cm^3 D_5W intracisternally (ICist) and 10 mg amphotericin B and 50 mg hydrocortisone IV over the next four hours. In addition, chloroquine sulphate, 200 mg IM; metronidazole, 500 mg by nasogastric tube every six hours; and diphenylhydantoin (Dilantin), 100 mg IM every eight hours, was given. Eighteen hours after admission, the patient again received 1.5 mg amphotericin B ICist and the IV amphotericin B increased to 20 mg. The patient died 66 hours after admission.

Carter (1972) reports similar findings to Duma et al.(1971) in two patients (7th and 9th, Table III, Carter, 1972) who had been treated in the same way.

Apley et al.(1970) described three cases of PAM in Great Britain, two of which were diagnosed presumptively because of association with the fatal proven case. They had the same early symptoms but neither actually developed convincing signs of meningitis. Naegleria was cultured from the CSF of the child who died and from one of the others but the amebae proved to be N. gruberi.

In the fatal infection, amphotericin B treatment was begun two days after admission, when amebae had been seen in the CSF. Amphotericin B, 0.25 $mg.kg^{-1}$ in one daily dose over three to four hours IV increasing over a week to 1 $mg.kg^{-1}$, was administered till the patient died on the sixteenth day after admission. Sulphadiazine, 160 mg IV every six hours was also given. On the seventh day after admission, 650 $amebae.mm^{-3}$ were seen in the CSF, but many appeared to be dead. The concentration of amphotericin B in the CSF was 0.184 $\mu g.cm^{-3}$. On the eleventh day after admission, the CSF contained no amebae and the

amphotericin B concentration was 0.224 $\mu g.cm^{-3}$. Although the diagnosis in this case was made relatively early and treatment with amphotericin B started promptly the patient died after being treated for thirteen days.

The second case reported by Apley et al. (1970) was the brother of case one and was admitted to hospital two days later. On the morning of admission he complained of headache and in the evening developed a sore throat and neck pains. A CSF sample was taken and it was clear and no amebae were seen. The clinical picture was that of an upper respiratory tract infection but in view of case one treatment with amphotericin B and sulphadiazine was begun. By the seventh day he was symptom-free. On the eighth day he again complained of sore throat with head and neck pains. No amebae were seen in the CSF but some were grown and appeared similar in morphology to those isolated from the CSF of case one. By the twelfth day he was afebrile and had no signs of meningitis but in view of growth of amebae from the CSF taken four days earlier, amphotericin B treatment was started again. 0.25 mg amphotericin $B.kg^{-1}$ IV daily over four hours increasing to 0.75 $mg.kg^{-1}$ after four days for a total of ten days was given. CSF taken on the twelfth and eighteenth days appeared normal and no amebae were grown. The patient was discharged, symptom-free. Ten days later a CSF sample was taken and again no amebae were grown.

The third case was admitted to hospital six days after case one. On the morning of admission he complained of sore throat and headache, vomiting and abdominal pain. The CSF was normal and no treatment was given. On the third day, the temperature had become normal but the headache continued and there was slight neck stiffness. No amebae were seen in the CSF but in view of slight lymphocytosis treatment was started with sulphadiazine and amphotericin B (0.25 $mg.kg^{-1}$ daily in one dose over four hours IV). On the fourth day signs of drug toxicity were noted and treatment was stopped. On the eighth day, growth of amebae from case two was reported and although the patient was well, daily amphotericin B, 0.25 $mg.kg^{-1}$ increasing to 0.75 $mg.kg^{-1}$ was given IV for ten days. CSF specimens on the 8th, 14th and 24th days were normal. No amebae were isolated at any time from this case. He was discharged on the fourteenth day, symptom-free (Apley et al., 1970).

Apley et al. (1970) do not believe that isolation of amebae from the CSF of case two was due to laboratory cross-infection "but case three must be considered to have been only doubtfully infected with

amebae." Griffin (1976), has disputed the diagnosis of Naegleria meningo-encephalitis in cases one and two. He contends that an Acanthamoeba was involved and that sulphadiazine, rather than amphotericin B, which was given on admission, was responsible for the prolonged survival in the first case and survival in the second.

The two cases reported by Pan and Ghosh (1971) were similarly inconclusive in the nature of the etiological agent involved and the effective agent in treatment. Their report deals with two Indian children, aged six months and three years, with CNS infections of slow onset (3-5 months). CSF samples showed "motile amebae with thin pseudopods". No strains were isolated and both patients survived. They were treated with amphotericin B, sulphadiazine and intrathecal steroids.

Anderson and Jamieson (1972) reported the case of a fourteen year old boy from Queensland who had typical acute symptoms and was already in the fourth day of illness and comatose by the time amphotericin B treatment was begun. The diagnosis was confirmed by finding 12,000 white cells.mm^{-3} and numerous amebae in the CSF; the amebae were cultured and shown to be N. fowleri. Amphotericin B was given in a dose of 1 $mg.kg^{-1}$ per day IV and penicillin, ampicillin and sulphadiazine, he had been having for three days previously, were continued. Within two days he became afebrile and was talking rationally. After five days the CSF white cell count had fallen to 15.mm^{-3} but many atypical amebae were still present. Amphotericin B was therefore given IT and later IVent in small doses (0.1 mg on alternate days) and the fluid gradually cleared. He was discharged from hospital without any neurological deficit. This case represents the first survival where there is definite proof that N. fowleri was involved and survival can be attributed to amphotericin B.

Seidel et al. (pers. comm., 1978) report a more recent case in Torrance, California. The patient, a nine year old female, presented with typical symptoms of meningo-encephalitis three days before admission to hospital. Lumbar puncture revealed a purulent CSF and motile amebae were seen. Amphotericin B (1 $mg.kg^{-1}$), sulphadiazine (50 $mg.kg^{-1}$), chloramphenicol (25 $mg.kg^{-1}$) and penicillin G (3.4×10^5 units) were all administered IV immediately on admission. 1.5 mg amphotericin B was also given IT.

The patient was then transferred to Harbour General Hospital and was in a coma on admission but responsive to pain and tactile

stimulation. On arrival the treatment outlined in Table III was instituted.

Table III: Treatment Protocol Used in a Case of PAM
(Seidel et al., pers. comm., 1978)

DRUG	ROUTE	DOSAGE
Amphotericin B	IV	1.5 $mg.kg^{-1}$ per day ÷ bid x 3 days → 1.0 $mg.kg^{-1}$ per day qd x 6 days
Amphotericin B	IT	1.5 mg per day x 2 days → 1mg QOD x 8 days
Miconazole	IV	350 $mg.m^{-2}$ per day ÷ tid x 9 days
Miconazole	IT	10 mg x 2 days → 10 mg QOD x 8 days
Rifampin	Oral	10 $mg.kg^{-1}$ per day ÷ tid x 9 days

bid = twice daily
tid = three times daily
qd = every day
QOD = every other day

Sulphadiazine (4 g per day IV) was continued for three days until studies confirmed the diagnosis of Naegleria meningo-encephalitis. Penicillin and chloramphenicol were continued for three days until bacterial CSF cultures were negative. Decadron (dexamethasone) and Dilantin (diphenylhydantoin) were given for increased intracranial pressure and seizure activity, respectively.

The patient stabilized clinically over the first 48 hours. Gradually over the next month of hospitalization her mental status improved. No significant neurological deficits were noted at discharge.

In a few of the other cases of PAM, where proof that N. fowleri was the etiological agent involved and amphotericin B given at effective doses the course of the disease was often too advanced to see any effect (Van Den Driessche et al., 1973; Cursons et al., 1976, pers. comm. 1979).

Callicott et al. (1968), isolated an ameba identified as A. astronyxis from a spinal fluid sample from a patient with a purulent

meningitis that remitted spontaneously. The authors were unable to provide evidence that the organism caused the illness and was not just a cultural contaminant.

Kenney (1971), reported the case of a patient hospitalized for acute gastritis of unknown origin. Complement fixation tests revealed no antibodies to Entamoeba histolytica but did reveal antibodies to A. culbertsoni. Over the next two months a rising titer to A. culbertsoni antigen was reported. Clinical investigation by a physician did not reveal any symptomatology suggestive of cerebromeningeal involvement. The patient refused a spinal tap.

The gastro-intestinal symptoms continued and a stool examination revealed amebae which were called Iodamoeba butschlii. Because of the titre to Acanthamoeba antigen, the patient was put on antiamebic therapy consisting of Dehydro-Emetine (IM) and chloroquine. Complement fixation tests two months later demonstrated that the serum titer had decreased. This case appears to demonstrate a form of disease between the symptomless carrier state and fulminating meningo-encephalitis which may be found to be more common than at present believed.

The only human Acanthamoeba infections positively diagnosed during life under circumstances where chemotherapy could have been tried were those in the eye. Nagington et al. (1974) repeatedly isolated Acanthamoeba from two English patients with corneal ulcers. Warhust and Thomas (1975) identified the amebae as A. castellanii and A. polyphaga. One of the infections was in a 32 year old woman who had a mild unilateral keratoconjunctivitis and uveitis which did not respond to treatment which included chloramphenicol, idoxuridine, 3-fluorothymidine, gentamicin, methicillin and later on sulphadiazine (500 mg, six hourly). Six months after treatment, because of corneal ulceration, pain and loss of vision, a corneal graft was performed but the graft was rejected. The other infection described was in a 59 year old farmer with an identical clinical condition which required enucleation of the eye after one year. Treatment in this case included chloramphenicol, acetylcysteine, 3-fluorothymidine and clotrimazole eye drops.

Jones et al. (1975) cultivated A. polyphaga from corneal ulcers of two patients in Houston, Texas. They reported suppression of the amebae with paromomycin. Griffin (1978) reported seeing similar material at the Armed Forces Institute of Pathology, Washington D.C. and it seems likely that Acanthamoeba in the eye will not prove to be strikingly rare or unusual.