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G P CHATTERJEE MEMORIAL LECTURE, 1985
Free-living Soil Amoebae as Human Pathogen

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The discovery made within the last two decades, that small free-living aerobic amoebae belonging to the genera *Acanthamoeba* and *Naegleria* cause fatal human disease affecting the central nervous system, has changed the whole concept of amoebiasis. The subject of amoebiasis can no longer be defined in terms of a single anaerobic amoeba, *Entamoeba histolytica.*

The present review deals with some of the work of others as well as with my own experience in the subject.

Field Soil as Abode for Free-living Aerobic Amoebae

It is not necessary, in dealing with soil protozoa at the present time, to discuss the views held by Ehrenberg, Dujardin, Stein, Bütschli and other earlier workers, regarding the distribution of free-living protozoa. The prevalent idea was that these protozoa, in active (trophic) state, could only exist in fresh water or in the sea.

A fertile field soil contains organic matter, mainly of plant origin, which contains starches, fats, organic acids, proteins and amino compounds. They, therefore, supply nitrogenous and carbohydrate needs of bacteria. Besides nitrogen and carbohydrates, various inorganic salts are present in soil solution which are also needed for bacteria. Small free-living amoebae usually feed on bacteria and, thus, they are well provided with food in soil. Soil reaction also plays important part in the distribution of microorganisms in soil, but Nasir (unpublished data) found that the lowest *p*H value at which development took place was 3.9 for amoebae, while they were still active at *p*H 9.5, which was the highest alkalinity tested (cited by Sandon 1927, p. 55).

Interest in soil protozoa began after the publication of Russell and Hutchinson's (1909) theory of the effect of partial sterilization of soil on the production of plant food. This theory attempted to explain 'soil sickness' as being due to excessive numbers of active (trophic) protozoa which by their phagocytic action restricted the bacterial processes going on in the soil and the remedial effect of partial sterilization by killing these protozoa by sterilising agents. Although this theory has not been fully accepted (Singh & Crump 1953), it aroused great interest in soil protozoa.

D Ward Cutler and his colleagues, under the inspiring leadership of Sir John E Russell, started pioneering work on soil protozoa at Rothamsted Experimental Station, England since 1919. This work

laid the foundation of a new branch of science known as soil protozoology. Cutler (1920) developed a culture method for the count of active and cystic protozoa in soil. Cutler et al. (1922), in their quantitative investigation of the bacterial and protozoan population of a Rothamsted field soil at daily intervals for a year, conclusively showed that large numbers of active amoebae were present, and rapid fluctuations in their numbers and in the bacterial numbers took place daily and seasonally; these were not clearly related to weather conditions. The frequency of occurrence of high numbers of active *Naegleria gruberi* (above 100,000 per gram of soil) was significantly related to that of low bacterial numbers (below 30 millions per gram). Cutler et al. (1922) thought that this may be due to the feeding of amoebae on bacteria. Why the numbers of active amoebae were low when the numbers of bacteria were high could not be explained. Ciliates were present in very small numbers as cysts in soil.

A detailed account of the historical development of soil protozoology and the possible role that soil protozoa may play in soil are given by Singh (1960, 1963, 1975).

Isolation and Culture of Small Free-living Amoebae

At the time when the surveys of bacterial and protozoan numbers in Rothamsted field soil were made, the quality of the bacterial food supply was not considered. It was important to discover whether amoebae may be affected by the quality of bacterial food or, conversely, their numbers may be affected by the proportion of edible and inedible bacterial species, as are available in soil. Singh (1941) investigated the feeding of amoebae of different species of bacteria on non-nutrient agar by devising suitable methods. Bacteria used in these experiments differed widely in their characters. About half of them were eaten by amoebae and showed a range of edibility from some that were readily and completely consumed to others that were partially attacked (Singh 1941, 1942, 1945, 1946). The work on selectivity of bacterial food by amoebae revealed that a species of bacteria, such as *Klebsiella pneumoniae* (*Aerobacter aerogenes*) or *Escherichia coli*, which was readily and completely eaten by a species of amoeba was also very good food for other species of amoebae and amoeboid organisms. This discovery led Singh (1946, 1955) to the use of non-nutrient agar and a species of readily edible bacteria for the isolation and clonal cultivation of amoebae and amoeboid organisms from soil and other substrates and for the enumeration of their numbers from these substrates. The method of isolation and culture of small free-living amoebae developed by Singh has been universally accepted as the procedure of choice for the isolation and culture of pathogenic and non-pathogenic small free-living amoebae from soil and other substrates and from human cerebrospinal fluid (CSF) and human brain tissues postmortem from cases of amoebic meningo-encephalitis (see Singh, 1975 for the literature). Since small free-living amoebae need no nutrient except a suitable edible species of bacteria supplied on non-nutrient agar, *p*H 6.6–6.8, it is quite unnecessary to add NaCl, $MgCl_2.6H_2O$, $FeSO_4.7H_2O$, Na_2HPO_4 and KH_2PO_4 to non-nutrient agar, as done by Neff (1957), and NaCl, $MgSO_4.7H_2O$, $CaCl_2 2H_2O$, Na_2 HPO_4 and KH_2PO_4, as advocated by Page (1967a).

Among free-living amoebae, strains of human pathogenic *Naegleria* have been found to be inhibited by 0.5% NaCl incorporated in non-nutrient agar (Singh & Das 1970, Carter 1970, Culbertson 1971), though this has been disputed by Cerva (1978) (see Schuster 1979 for the reference) and Griffin (1983). Thus it is safer to use

non-nutrient agar without NaCl or antibiotics for the isolation and culture of amoebae.

From the work described above, it is clear that various nutrient media, as used by different workers for the isolation and culture of small free-living amoebae, are not reliable for use from substrates having mixed microbial population (see Singh 1955, 1975, Singh & Dutta 1984). Non-nutrient agar discourages the growth of inedible bacteria and toxogenic micro-organisms coming from these substrates.

Distinguishing Characters of *Acanthamoeba* and *Naegleria*

There is still great confusion whether the pathogenic amoeba, not producing flagellate stage, should be placed in the genus *Acanthamoeba* or *Hartmannella*. Thus Martinez (1983) says that Singh (1952) and Singh and Das (1970) do not recognize *Acanthamoeba* as a distinct genus. Page (1967a) recognizes *Acanthamoeba* as distinct from *Hartmannella*. It is, therefore, of much interest to trace briefly the developmental history of these two genera.

Alexeieff (1912) created the genus *Hartmannella* on the basis of the absence of 'polar masses' during nuclear mitosis, and defined it as follows: "Pas de corps polaires dans la division nucléaire. Tout (ou presque tout) le matérial chromatique est employé à la constiution de la plaque equatoriale." Volkonsky (1931) created a subfamily Hartmannellinae in the family Amoebidae to include only those uninucleate amoebae whose resting nucleus contains a single nucleolus. He placed in it three genera; *Hartmannella* (type *H. glebae* Dobell 1914) in which the spindle is barrel-shaped or cylindrical and the cyst wall is smooth or lightly folded; *Glaeseria* (type *G. testudinis* Ivanić, 1926), with the same spindle shape, but with nuclear division occurring in the cyst; and *Acanthamoeba* (type *A. castellanii*, Douglas 1930) for amoebae with conical, pointed-ended spindle and rough cyst wall. Singh (1952), who emended the genus *Hartmannella* Alexeieff (1912) also recognised it on mitotic division pattern, and included in it those uninucleate amoebae whose resting nucleus contains a single Feulgen-negative nucleolus, and pass through a mitotic process in which the nucleolus disappears and a spindle with chromosomes arranged as an equatorial plate is formed. He (1952) agreed with Volkonsky (1931) on the creation of the genus *Glaeseria* on the basis of the nuclear division occurring in the cyst, but pointed out that spindle shape and cyst character were inadequate for separating the genus *Hartmannella* from *Acanthamoeba*, and rejected the latter genus. Singh (1952) also raised the subfamily Hartmannellinae to the status of a family Hartmannellidae, considering that the nuclear division justified this. The family Hartmannellidae was defined as follows: The resting nucleus has either a single Feulgen-negative nucleolus or several Feulgen-negative nucleoli. During mitosis the nucleolus, or nucleoli disappear, and a spindle with chromosomes arranged as an equatorial plate resembling that found in higher animals and plants develops. The nuclear membrane disappears during mitotis. Amoebae may be uni-or multi-nucleate; no temporary flagella have been discovered. *Hartmannella* Alexeieff (1912) emend. Singh (1952) was made the type genus of Hartmannellidae Volkonsky (1931) emend. Singh (1952). Ray and Hayes (1954) and Adam (1964) agreed with Singh. Pussard (1966), while agreeding with Singh (1952) in considering spindle shape as unsatisfactory for intergeneric differentiation, recognised the genus *Acanthamoeba* on the basis of its wrinkled cyst structure, which he judged to be a decisive character.

It may be stressed that Singh (1952) and Singh and Das (1970) in their classification

of the order Amoebida Kent, 1880, based on nuclear mitosis in amoebae without test, did not include locomotive form and behaviour of amoebae.

Page (1967a, b, 1968, 1974) has recognised the genus *Acanthamoeba* and has distinguished it from *Hartmannella* on locomotive form and behaviour and on cystic character of amoebae, although amoebae in both the genera have mesomitotic pattern of nuclear division. According to Page, amoebae belonging to *Acanthamoeba*, during active locomotion on glass surface, have broad, anterior hyaline lobopodium from which are produced singly or in twos or threes several hyaline projections (acanthopodia). Cysts are polyhedral or thickly bioconvex, wall consisting of more or less polygonal or stellate endocyst and more or less rippled ectocyst. Excystment takes place by removal of operculum at the point of contact between endocyst and ectocyst. Page (1968, p. 25) says—"Within the genus *Acanthamoeba*, the cysts, though very distinctive as generic character, are somewhat more confusing as a means of distinguishing species". Amoebae having *limax* locomotive form and moving usually by steady flow with cyst wall smooth and rounded, where known, were included by Page in the genus *Hartmannella*. The use of the term steady flow is not justified because Page has himself produced photographic illustration that *H. hibernica* moves in a highly eruptive manner (see also Singh & Hanumaiah 1979).

Singh and Hanumaiah (1979), who have combined the possible phylogenic classification of amoebae of Singh (1952) and Singh and Das (1970) and the contributions made by pseudopodial school of taxonomy have recognised both the genera *Acanthamoeba* and *Hartmannella* in the family Hartmannellidae only on locomotive form and behaviour of amoebae, and have rejected the cystic character because *A. glebae* (Dobell 1914) and *A. invadens* (Singh & Hanumaiah 1979) have rounded or spherical cyst wall without pores or opercula (see also Singh & Das 1970, Singh 1981, Singh & Dutta 1984, Misra & Sharma 1980). In the system of classification of Singh and Hanumaiah (1979) locomotive form and behaviour of amoebae have been used at the generic level.

Singh and Hanumaiah (1979) have defined the genera *Hartmannella* and *Acanthamoeba* as follows:

Genus *Hartmannella* Alexeieff, 1912 emend. Singh, 1952 emend. Singh & Hanumaiah, 1979.

The resting nucleus contains a single Feulgen-negative nucleolus. During mitosis the nucleolus disappears and a spindle with chromosomes arranged as an equatorial plate is formed. In active locomotion amoebae assume *limax* form. No temporary flagella are produced. Type species *Hartmannella hyalina* (Dangeard 1900).

Genus *Acanthamoeba* Volkonsky, 1931 emend. Singh & Hanumaiah, 1979.

During mitosis the Feulgen-negative nucleolus disappears and a spindle with chromosomes arranged as an equatorial plate is formed. Amoebae in active locomotion with broad anterior hyaline lobopodium from which are produced singly or in twos or threes several or many, hyaline projections (acanthopodia). Type species *Acanthamoeba glebae* (Dobell 1914)

A. glebae has been recognised as the type species according to the International Rules of Zoological Nomenclature.

Sawyer and Griffin (1975) have considered *A. glebae* as having simpler wall.

It may be pointed out that *Hartmannella* and *Acanthamoeba* are also distinct immunologically whether one uses surface or soluble protein antigens (Visvesvara & Balamuth 1975, Singh & Sharma 1983).

I hope in future the pathogenic amoeba should be called *Acanthamoeba* and not *Hartmannella.*

Page (1981) has created a new genus, *Protacanthamoeba,* for an amoeba isolated from Scottish estuary. It produces acanthopodia during active locomotion, but the cyst wall is smooth and circular to oval in outline without any preformed opercula or pores. The nuclear division, though not studied in detail, is mesomitotic. *Protacanthamoeba* is definitely synonym of *Acanthamoeba* unless it was found that it is distinct immunologically from *Acanthamoeba.*

The family Schizopyrenidae Singh, 1952 emend. Singh & Das, 1970 was created for amoebae whose resting nucleus contains a more or less central Feulgen-negative nucleolus or several Feulgen-negative nucleoli, which during mitosis from 'polar masses'. Nuclear membrane persists throughout division. 'Interzonal bodies' may be present. Amoebae may have more than one nucleus, and some genera may produce flagellate stage.

Singh (1952) recognized three genera in the family Schizopyrenidae for the amoebae showing *limax* locomotive form. Type genus *Schizopyrenus* Singh, 1952.

Feulgen-negative nucleolus dividing during mitosis to form 'polar masses'. Temporary flagellate stage is not produced. Type species *Schizopyrenus russalli* Singh, 1952.

Genus *Naegleria* Alexeieff, 1912 emend. Singh, 1952.

'Polar masses' are formed. Feulgen negative 'interzonal bodies' are present during late stage of mitosis. Temporary flagellate stage with two flagella is produced. Type species *Naegleria gruberi* (Schardinger 1899).

Genus *Didascalus* Singh, 1952.

'Polar masses' without 'interzonal bodies' are present during mitosis. Temporary flagellate stage with two flagella is produced. Type species *Didascalus thorntoni* Singh 1952.

Singh (1952) did not recognize the genus *Vahlakampfia* Chatton and Lalung—Bonnaire, 1912 as a valid genus because Vahlkampf's (1905) *limax* amoeba has distinct 'polar masses' and 'interzonal bodies' during nuclear division. Unless an amoeba is discovered that has 'polar masses' and 'interzonal bodies' and does not produce flagellate stage, *Vahlkampfia* should be considered as synonym of *Naegleria* (see also Singh & Das 1970, Singh & Hanumaiah 1979). Chang (1971) has recognized the genera *Schizopyrenus, Naelgeria* and *Didascalus* in the family Schizopyrenidae and has rejected the genus *Vahlkampfia.*

Singh and Singh (1966) showed that antisera produced against *S. russelli, N. gruberi* and *D. thorntoni* gave immobilization reaction in homologus system but not in heterologous system. Thus these genera are also distinct immunologically. Antisera against human pathogenic *Naegleria* (HB-1 strain) did not react with *D. thorntoni,* as judged by immobilization reaction (Singh & Das 1970). Thus *Naegleria* and *Didascalus* are not only distinct on the presence and absence of 'interzonal bodies' but also immunologically. The statement made by Page (1967a, 1974) that the presence or absence of 'interzonal bodies' is not a distinguishing character for *Naegleria* and *Didascalus* cannot be accepted. There is no justification for Page (1976b), Schuster (1979) and John (1982) to call *D. thorntoni* as *N. thorntoni.* Careful investigations on the nuclear division of *Naegleria* spp. have revealed the constant presence of 'interzonal bodies' (Rafalko 1947, Butt Baro & Knorr 1968, Culbertson, Ensminger & Overton 1968, Fulton & Guerrini 1969, Singh & Das 1970, Chang 1971, 1974, Gordeeva 1973, Das,

Willaert & Jadin 1974, Curson & Brown 1976, and others). The failure to find the consistent presence of 'interzonal bodies' by a few workers must have been due to faulty techniques used by them.

Immunologically *Acanthamoeba* and *Naegleria* can readily be distinguished both in terms of surface and soluble antigens (see Singh & Sharma 1983).

It is of interest to point out that Page (1974 p. 174) says: "For the benefit of non-specialists it should be emphasized that, whichever nomenclature they wish to employ, Schizopyrenidae and Vahlkampfiidae (as used here) are exact equivalents, as are *Schizopyrenus* and *Vahlkampfia*. The difference is over the validity of *Vahlkampfia* and the familial name derived from it". It should be noted that Page (1976a) has included only *limax* amoebae dividing by promitosis in the family Vahlkampfiidae. Singh and Hanumaiah (1979), Singh (1981) and Singh and Dutta (1984) have not only included *limax* amoebae but also those amoebae which during active locomotion are oval, oblong, or elipse and somewhat elongated or nearly fan-shaped in the family Schizopyrenidae, based on promitotic division.

Singh and Hanumaiah (1979) and Singh (1981) have clearly shown, based on their own detailed work and those of other workers, that amoebae, whether they are small or large, or possess different nuclear, structers or as uni- or multi-nucleate, or have different locomotive forms and behaviour, or with or without flagellate stage, or whether they are parasitic or free-living fall into three groups in the order Amoebida on the basis of their nuclear mitosis, as suggested by Singh (1952) and Singh and Das (1970). The genera of amoebae that can at present be included in the families Schizophyrenidae, Hartmannellidae and Endamoebidae Calkins, 1933 emend. Singh and Das, 1970, are given by Singh and Hanumaiah (1979), Singh (1981) and Singh and Dutta (1984). This system of classification of amoebae should enable workers to identify known and suspected pathogens, and to differentiate them from other amoebae.

Pathogenicity of Free-living Amoebae

Several reviews dealing with free-living amoebae causing fatal meningo-encephalitis in humans and in lower mammals have been published (Carter 1972, Chang 1971, 1974, Culbertson 1971, 1981, Duma 1972, Griffin 1978, Jadin 1973, John 1982, Martinez 1976, 1980, 1982, 1983, Martinez & De Jonckheere 1981, Schuster 1979, Singh 1973, 1975, Singh & Dutta 1984, and others).

It was unthought of that soil amoebae might cause disease in humans. Medical interest in these amoebae was aroused when Culbertson and his colleagues since 1958 conclusively showed that *Acanthamoeba* (strain A–1), now known as *A. culbertsoni* (Singh & Das 1970), contaminant of monkey kidney tissue culture cells, caused acute meningo-encephalitis in mice when trophozoites were administered intranasally. They postulated that a similar disease might occur in humans as the result of swimming or bathing in water heavily contaminated with pathogenic *Acanthamoeba*. Culbertson et al. also found that new isolates of *Acanthamoeba* from a variety of sources produced acute meningo-encephalitis and also chronic granulomatous encephalitis in mice after intranasal inoculation (see Culbertson 1971 for the references). A strain (HN-3), which caused chronic granulomatous disease, was identified as *A. rhysodes* (Singh 1952).

Fowler and Carter (1965) were the first to report four fatal human cases of acute pyogenic meningitis caused by free-living amoebae from Australia. Culture of the brain and meningeal exudate from all cases yielded no bacteria, nor were tubercle

bacilli, torula or viruses isolated. Fowler and Carter (1965) thought that these cases were probably due to *Acanthamoeba*. They suggested that the invasion of the brain and the meninges by amoebae was via the nasal mucosa. Butt (1966) and Patras and Andujar (1966) in the USA reported fatal human cases of meningo-encephalitis and thought that they were due to *Acanthamoeba*. Butt (1966) gave the name for the disease as primary amoebic meningo-encephalitis (PAM). All these cases, supposed to have been due to *Acanthamoeba*, are now known to be due to *Naegleria*.

Carter (1968) first isolated in Australia an amoebo-flagellate from two fatal human cases of amoebic meningo-encephalitis. According to Kudo's (1954) system of classification, Carter (1968) recommended its inclusion in the genus *Naegleria* in the family Naegleridae. In the same year Butt et al. (1968) and Culbertson et al. (1968) also reported the isolation of an amoebo-flagellate from a fatal case of Caucasion male. Both the groups of workers placed this amoeba in the genus *Naegleria*, as defined by Singh (1952), and in the family Schizopyrenidae Singh (1952). In a later study of two strains of amoebo-flagellates from fatal human cases, Carter (1970) did not find the presence of 'interzonal bodies' during mitosis. He (1970) has, in accordance with Page (1967a), now put the amoeba in the family Vahlkampfiidae, genus *Naegleria* Alexeieff, 1912 ememd. Calkins, 1913, *N. fowleri* sp. nov. Singh and Das (1970) named the human pathogenic strain (HB-1) of *Naegleria*, obtained from C.G. Culbertson, as *Naegleria aerobia* on the basis of aerobic nature of the organism. Carter (1972, pages 202-3) says—"A direct comparative study of *Naegleria fowleri* and *Naegleria aerobia* is yet to be made, but Singh and I both agree (personal communication, January 1971) that as far as can be judged from their published descriptions they are identical, with one possible exception, viz. the interzonal body described in the mitotic figure of *Naegleria aerobia* and not in *Naegleria fowleri*. However, if one believes with Page (1967a) that the interzonal body is too capricious a structure to be used as a diagnostic feature, this difference is unimportant and the two species must be considered on present evidence as a single species. In this case *Naegleria aerobia* should become the junior non-valid synonym as the journal in which it was first named (Singh & Das 1970) was published and issued after which I named *Naegleria fowleri* (Carter 1970)".

Singh (1972) pointed out that Carter (1970) described *Didascalus fowleri* because of the absence of 'interzonal bodies' during mitosis. Singh (1973) also pointed out that if the presence of 'interzonal bodies' in Carter's amoeba is found, *D. fowleri* should become the junior non-valid synonym of *N. aerobia*. It has been shown by Chang (1974) beyond doubt that the two strains of *Naegleria* isolated by Carter from human cases have 'interzonal bodies'. There is no such rule in International Rules of Zoological Nomenclature that a wrongly described amoeba should take priority. Chang (1971) suggested the name *N. invadens* for pathogenic strains of *Naegleria* on the presence of 'interzonal bodies'. *N. invadens* is definitely synonym of *N. aerobia* according to International Rules of Zoological Nomenclature.

Since the work of Fowler and Carter (1965), fatal human cases of amoebic meningo-encephalitis have been reported from Australia, Belgium, Czechoslovakia, Great Britain, India, Ireland, Japan, South Korea, New Guinea, New Zealand, Nigeria, North Western Mexico, Panama, Puerto Rico, the USA, Venezuela, Zambia and other places (see Martinez 1983 for the references).

Twenty-three cases of fatal human

meningo-encephalitis caused by *Acanthamoeba* have been reported on immunologic studies of human sera or human brain sections after tissue fixation and also on the presence of cysts (Martinez 1980, 1982). As pointed out before, cystic character is not reliable for identifying *Acanthamoeba*. Several species of *Acanthamoeba* have been implicated in the human disease. Culbertson (1981) doubts the ability of immunological methods in determining the species of *Acanthamoeba* in fixed human brain section. He (1981) has rightly pointed out that retrospective immunologic tests on fixed amoebae in sections from autopsy should not be regarded specific except for the genus and has emphasized the importance of making cultures from patients who may be suffering from meningo-encephalitis caused by *Acanthamoeba*. By the use of gel diffusion precipitin and immunoelectrophoresis tests, it has been found that the valid species of *Acanthamoeba* are related (see Singh & Sharma 1983). Willaert, Stevens and Healy (1978) claim that a species of *Acanthamoeba* can be identified in fixed human brain sections by the use of antisera poduced against highly purified plasma membranes of *Acanthamoeba* spp. (Singh & Dutta 1984).

Callicott et al. (1968) isolated *A. astronyxis* from CSF of a patient with meningitis that remitted spontaneously. Martinez (1980) has pointed out that, since CSF was not examined for motile amoebae, the organism may have been contaminents because free-living amoebae were being investigated in that laboratory. Pan and Ghosh (1971) saw motile amoebae in CSF of two patients that recovered. Cleland et al. (1982) have stated that on three occasions *A. rhysodes* was seen in CSF and cultured from a man in Nigeria who had a five year history of excessive sleeping that resulted in a confusional illness with convulsons. He made a partial recovery from the disease. There was rising serum titre of immobilizing antibody against the strain of amoeba isolated. They suggest that it was a case of chronic amoebic meningo-encephalitis. Recently, two cases of fatal human acute amoebic meningo-encephalitis have been reported from Bombay (Gogate et al. 1984). Amoebae were seen in CSF of both the patients. From one case *A. rhysodes* was isolated and from the other *A. culbertsoni* on non-nutrient agar seeded with *Escherichia coli*. Large numbers of amoebae were seen in brain sections of both the patients, but no cysts could be seen. Both the species of amoebae were highly pathogenic to mice when administered intranasally.

Since the isolation of *N. aeroba* from fatal human ameobic meningo-encephalitis cases from Australia and the USA in 1968, amoebae cultured frcm human CSF or brain tissue postmortem have turned out to be *N. aerobia*. Strains of *N. aerobia* isolated from fatal human cases have been reported to be similar immunologically, as judged by gel diffusion and immunoelectrophoresis techniques (see Visvesvara & Healy 1975 and others). According to John (1982), 108 human cases of *N. aerobia* infection have been reported. Martinez (1983) mentions about 130 cases due to *N. aerobia*. A few more human cases have been reported in 1984.

Most of the reported cases of *N. aerobia* are from developed countries rather than from developing countries. This may be due to greater awareness of the disease in the developed countries and may not be due to greater incidence (see John 1982, Martinez 1983).

According to Martinez (1980, 1982, 1983) PAM due to *N. aerobia* is an acute, haemorrhagic necrotising meningo-encephalitis which usually occurs in previously healthy young adults with a recent history of water sport activities. The most affected areas of CNS are base of frontal lobes and cerebellum. The portal of entry of amoebae is through olfactory neuroepithelium. Martinez has

suggested the name granulomatous amoebic encephalitis (GAE) for the disease caused by *Acanthamoeba*. It has been reported in chronically ill and debilitated persons and immunologically impared patients, some receiving immunosuppressive therapy. In some patients immunodeficiency has not been demonstrated. The portal of entry of amoebae to the brain is not properly understood. It appears to be haematogenous probably from a primary focus in the skin (skin ulcers) or the lower respiratory tract (lung) or eyes (corneal ulcers). The clinical picture of *Acanthamoeba* infection in humans is generally chronic. The most affected areas are the midbrain and posteriror fossa structures. Out of 23 reported cases, three did not have a typical granulomatous reaction.

The exact mechanisms of pathogenesis of *N. aerobia* is not properly understood. According to some workers amoebae attack cells directly and according to others they produce cytopathic agents (see Chang 1979, John 1982 for the references).

Two cases of fatal human meningoencephalitis caused by free-living amoebae have been described in which the amoebae in brain sections could not be identified immunologically as *Naegleria* or *Acanthamoeba* (Duma et al. 1978, Martinez et al. 1980). Duma et al. (1978) found in stained brain sections various stages of mitosis in the amoebae. They say (p.469) — "No interzonal bodies characteristic of *Naegleria* were present. Nuclear division was premitotic and the nuclear membrane persisted throughout division. The cysts in sections were rounded and characterized by a prominent, thick, slightly wrinkled poreless wall often composed of concentrical layers partially split." Thus for the characterization of pathogenic amoebae, it is of great importance to isolate them in cultute.

Eleven cases of human amoebic keratitis due to *Acanthamoeba* have been reported from the USA, Great Britain, Germany and Holland (see Bos et al. 1981, Ma et al. 1981 for the references). The causitive amoeba has been named either *A. polyphaga* or *A. castellanii.* According to Culbertson (1981, pages 40-41)— "*Acanthamoeba* and cysts thereof have been demonstrated in human corneas, in and around corneal ulcers, which may proceed to destruction of the globe. Whether the amebas initiate the ulcer, or invade it secondarily, is a question, I believe. In experimental attempts to cause amebic corneal ulcers in rabbits, I was unable (unpublished experiments) to produce them using *A. culbertsoni* following corneal epithelial scarification".

Epidemiolgy of Pathogenic Free-living Amoebae

Destruction of *N. aerobia* by normal human serum *in vitro* has been reported by Carter (1970) and Culbertson (1971). Rowan-Kelly et al. (1980) and Holbrook et al. (1980) have shown that absorption of specific antibody from the serum did not remove the amoebicidal activity. Lysis of *N. aerobia* by human serum is due to activation of complement and that the alternative complement pathway can be directly activated by the trophozoites. In humans complement activation via the alternative pathway may inhibit the spread of *N. aerobia* from the CNS to other parts of the body. Ferrante and Rowan-Kelly (1983) have also shown that normal human serum (NHS) contained an amoebicidal property for *A. culbertsoni* (strain A-1). Repeated absorption of NHS with amoebae did not remove the amoebicidal activity, indicating that specific antibody is not required. *A. culbertsoni* (A-1 strain) was able to activate the alternative complement pathway. Similar results were obtained in case of a nonvirulent strain of *A. culbertsoni* and *A. rhysodes* (strain HN-3). Thus both *N. aerobia* and pathogenic species of *Acanthamoeba* are unable to spread haematogeneously in humans.

In experimental infections with pathogenic *N. aerobia* and *A. culbertsoni* intranasally in mice, the amoebae multiply rapidly and spread into the cerebrum and cerebellum, causing fatal meningo-encephalitis. Since pathogenic *Acanthamoeba* is much more commonly found in nature as compared to pathogenic *Naegleria*, one would expect that the trophozoites of the former should enter human nose much more often than the trophozoites of the latter when humans are exposed to polluted waters. It appears that people working on human meningo-encephalitis caused by free-living amoebae do not realize that the trophozoites cannot swim in water. They are bound to round up and sink to the bottom. Unless one inhales mud from the bottom, there is rarely any chance of amoebae entering the nose and causing fatal disease effecting the central nervous system. This seems to be the case in *Acanthamoeba* meningo-encephalitis. It appears that due to a compromised immune system in chronically ill and debilitated persons and immunologically impared patients, some receiving immunosuppressive therapy, amoebae enter the brain haematogeneously probably from human skin and corneal ulcers. Thus Martinez (1980) considers *Acanthamoeba* as an opportunist organism.

Singh and Das (1972b) showed that intranasal inoculation of mice with flagellate stage of two strains of *N. aerobia*, one from fatal human case in the USA (strain HB-1) and the other isolated by Singh and Das (1972a) from a sewage sludge sample in Lucknow, caused fatal meningo-encephalitis of cent per cent of the mice in 3-6 days when 2000-2500 flagellates were given intranasally per mouse. Even 50 flagellates/mouse caused the death of two out of eight mice. It was clearly pointed out by Singh and Das (1972b) that the flagellates get converted into amoebae in the nose, the latter causing the death of mice. It appears that Culbertson (1981) does not seem to appreciate the importance of this finding in the epidemiology of human meningo-encephalitis caused by *N. aerobia*, although he found no difference in mouse pathogenicity between the two forms. The flagellates have a rigid oval shape and swim very actively in water. They are the infective stage because the trophozoites are hardly able to enter human nose from polluted waters. Thus *N. aerobia* cases are much more common compared with *Acanthamoeba*.

A proper investigation on the aetiology of human meningo-encephalitis cases in developing countries is most likely to reveal that the disease caused by *N. aerobia* is not rare. In every case of purulent (suppurative) meningitis, in which bacteria are not found, free-living amoebae should be suspected as aetiological agent. Examination of fresh CSF may show motile amoebae, making this observation mandatory for positive diagnosis and immediate treatment.

Chemotheraphy of Human Meningo-encephalitis caused by free-living Amoebae

Drugs used to cure *Entamoeba histolytica* infection in man are ineffective in the case of free-living amoebae. There is still complete absence of chemotherapeutic agents for the human disease caused by *Acanthamoeba*. Amphotericin B and miconazole intravenously and intrathecally appear to be the drug of choice for pathogenic *Naegleria* (see Culbertson 1981, Martinez 1983 for the literature).

I should like to suggest that a centre for the study of aetiology of human meningitis cases, especially in children, is most desirable in India. Such studies are likely to reveal that some of the suspected cases due to virus, and not responding to antibiotic treatment, are infact due to small free-living aerobic soil amoebae.

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