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INTRA-NASAL INFECTION OF MICE WITH FLAGELLATE STAGE OF *NAEGLERIA AEROBIA* AND ITS BEARING ON THE EPIDEMIOLOGY OF HUMAN MENINGO-ENCEPHALITIS *

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ABSTRACT

Intra-nasal infection of mice with temporary flagellate stage of *Naegleria aerobia* Singh and Das, 1970 produced meningo-encephalitis and death of the animals. No mortality in mice occurred when temporary flagellate stage of *N. gruberi* was administered intra-nasally. It is suggested that human meningo-encephalitis is caused mainly due to the entry of flagellates of *N. aerobia*, which have a rigid oval shape and move actively in water, through the intra-nasal route during swimming in fresh water.

THE pioneering work of Culbertson, Smith, Cohen and Minner[1] in 1959 on the causation of fatal meningo-encephalitis by *Hartmannella* (*Acanthamoeba*), a free-living aerobic amoeba, to laboratory animals, when given intra-nasally, gave a real stimulus in the search for free-living amoebae as the disease-producing organisms in man. The first authentic report of fatal primary amoebic meningo-encephalitis in man, caused by free-living amoebae, came from Australia in 1965 (Fowler and Carter[2]). Since that time primary amoebic meningo-encephalitis in man has been reported from widely separated parts of the world (Australia[3,4], U.S.A.[5-14], Czechoslovakia[15-17], U.K.[18,19], New Zealand[20,21], Belgium[22], Uganda[23] and India[24,25]. Amoebae isolated from human cases from 1968 onwards have turned out to be *Naegleria*. It is now generally believed that hartmannellid amoebic infection reported in man before *Naegleria* was isolated may have been caused by *Naegleria* (*see* Culbertson[26]; Chang[27]; Singh and Das[28]; Carter[29] for the literature).

The present communication deals with intra-nasal infection of mice with flagellate stage of *N. aerobia* and its bearing on the epidemiology of human meningo-encephalitis.

METERIALS AND METHODS

Pure line cultures, starting from a single cyst, of *Naegleria aerobia* (strain HB-1) Singh and Das[28], 1970 from a fatal human case of meningo-encephalitis, *N. aerobia* (strain N-1) isolated from a sewage sludge sample in Lucknow (*see* Singh and Das[30]) and *N. gruberi* (strain B), obtained from Dr. W. Balamuth, were employed in this work. Strains of *N. aerobia* were grown at 37° C on non-nutrient agar plates, pH 6·6–6·8, supplied with a young culture (2–3 days old) of *Aerobacter aerogenes* and *N. gruberi* at 25° C on non-nutrient agar containing 0·5% NaCl.

Young and actively growing cultures of *N. aerobia*, 24–30 hr old, were flooded with distilled water at 37° C. After 4–6 hr, when large numbers of flagellates were found to be actively swimming in water, the water containing the flagellates was transferred to a test-tube by a pipette. The flagellates were concentrated by centrifugation and suspended in small quantity of water. Their number was determined by a haemocytometer. The flagellates were inoculated intra-nasally into Albino mice weighing 10–12 g by the method of Singh and Das[28] used for the inoculation of trophozoites. Very sick mice with symptoms of meningo-encephalitis were killed, and the cultures of amoebae from brain tissue were made on non-nutrient agar supplied with *A. aerogenes*. Brain sections were also examined for amoebae.

Flagellates of *N. gruberi* were produced by flooding young cultures of amoebae with distilled water at 25° C.

RESULTS

The data presented in Table I show that intra-nasal inoculation of mice with temporary flagellate stage of *N. aerobia* (Figs. 4 and 5) caused death of the animals. The animals showing typical symptoms of meningo-encephalitis were sacrificed and brain smears in distilled water were examined microscopically. Large numbers of motile amoebae could be seen (Figs. 1 and 2). Careful search revealed no flagellates. This suggests that flagellates get converted into amoebae, the latter causing death of mice. It is interesting to note that the time of the death of mice inoculated with flagellates of strain HB-1 (2,500 flagellate·mouse) was similar to that reported by Singh and Das[28] when 20,000 trophozoites of strain HB-1 were inoculated intra-nasally per mouse. Administration of 2,000

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flagellates mouse of strain N-1 resulted in the death of the animals quicker than that reported by Singh and Das[30] when 20,000 trophozoites of strain N-1 were given per mouse. These findings show that inoculation of flagellates intra-nasally in mice is as effective or more effective in producing meningo-encephalitis than the inoculation of the tropic forms. Cysts of *N. aerobia* (Fig. 3) could not be seen in the brain of mice inoculated with flagellates.

Trophozites of *N. gruberi* (strain B) are known to be non-pathogenic to mice (*see* Singh and Das[28]). No death of mice occurred when flagellate stage of this organism was administered intra-nasally (Table I). Smear preparations of the brain of mice sacrificed after 14 days did not reveal the presence of amoebae. Culture of brain was also negative for amoebae.

TABLE I

Intra-nasal infection of mice with flagellate stage of Naegleria *spp.*

Naegleria	No. of flagellates inoculated/ mouse	No. of mice	Death time of mice in days
N. aerobia (strain HB-1)	2500	19	3, 3, 4, 4, 4, 4, 4, 4, 4, 4, 5, 5, 5, 5, 5, 5, 6, 6
	1000	8	5, 5, 5, 6, 7, 7, S, S
	500	6	6, 7, 7, 12, S, S
	100	7	6, 6, 7, S, S, S, S
	50	8	6, 6 S, S S, S, S, S
N. aerobia (strain N-1)	2000	6	4, 4, 5, 5, 6, 6
N. gruberi (strain B)	2500	6	S, S, S S, S, S
Control (*A. aerogenes*)	..	4	S, S, S, S

S = Mice surviving upto 14 days.

Two guineapigs, weighing 15 g. were given 5,000 flagellates/animal intra-nasally. One animal died on the 4th day and the other on the 5th day. Large numbers of motile amoebae could be seen in smear preparations of the brain tissue.

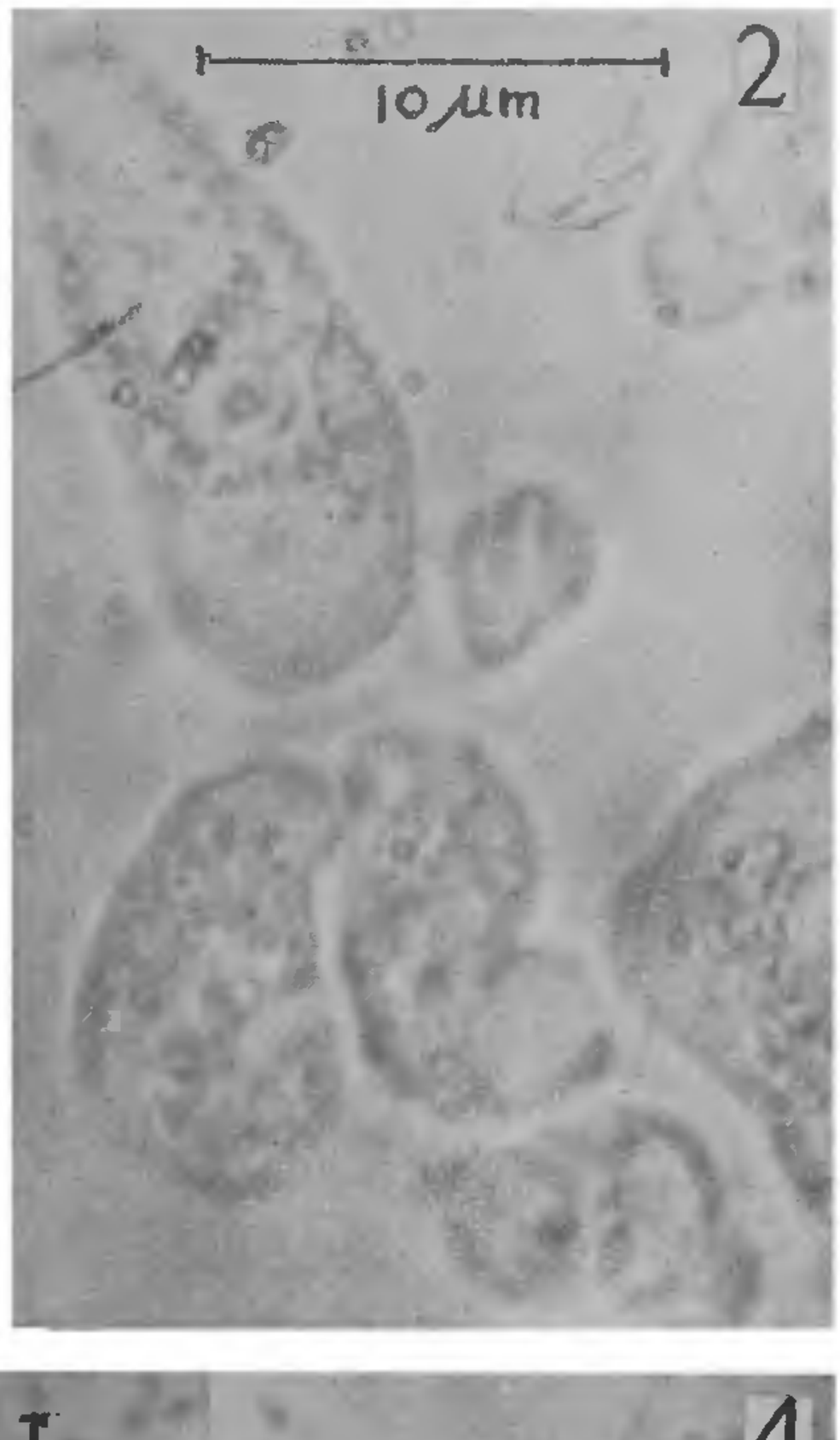
DISCUSSION

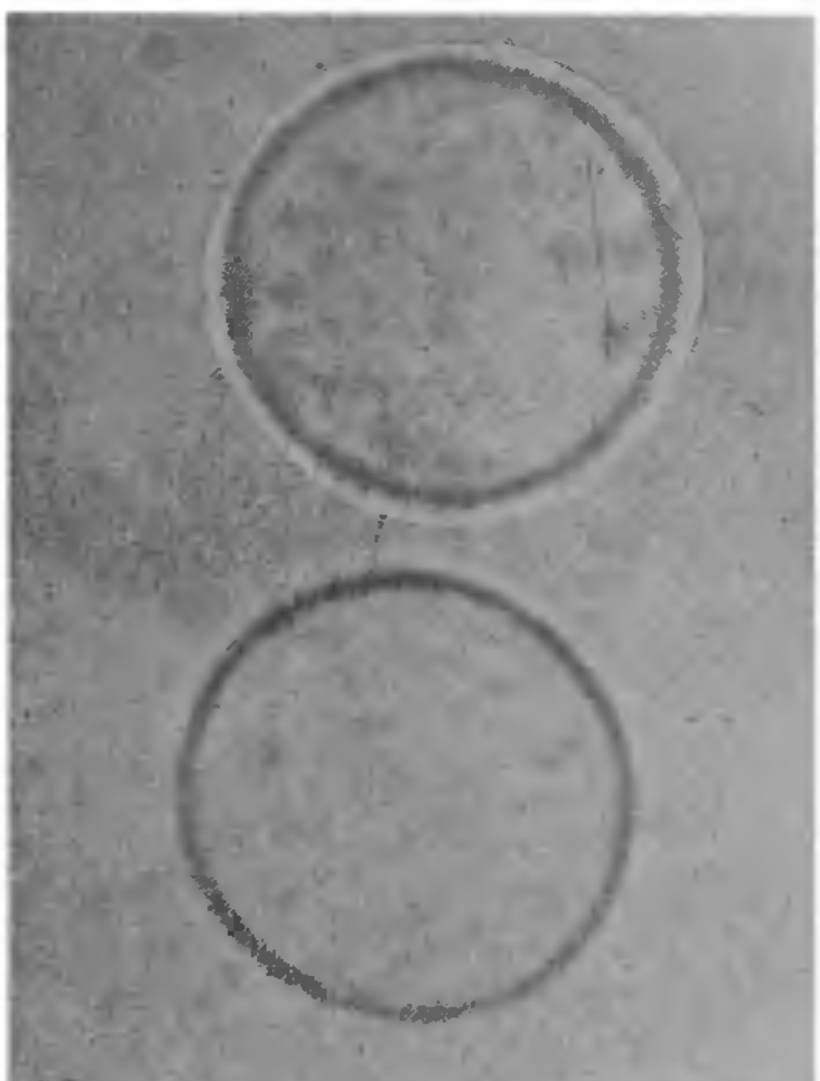
It has been shown that the intra-nasal inoculation of mice with flagellate stage of *N. aerobia* caused meningo-encephalitis and death of the animals. No mortality in mice occurred when flagellate stage of *N. gruberi*, a very common soil amoeba recorded from different parts of the world (*see* Sandon[31]), was administered intra-nasally. The trophic form of *N. gruberi* has also been found to be non-pathogenic to mice (*see* Singh and Das[28]; Chang[27]). *Hartmannella culbertsoni* Singh and Das, 1970 and *H. rhysodes* Singh, 1952 are common in soil, fresh water mud and sewage sludge because they readily produce cysts which are resistant to drying for long periods and also cause meningo-encephalitis in laboratory animals (*see* Singh and Das[28,30]; Singh[32]; Culbertson[26]). They have not been isolated, so far, from fatal primary amoebic meningo-encephalitis cases in man. The epidemiology of human meningo-encephalitis caused by *N. aerobia* is not properly understood because this amoeba has not been isolated from soil or fresh water (*see* Culbertson[26]; Carter[29]). The cysts of *N. aerobia* have little or no resistance to drying (*see also* Carter[29]) and this may explain its absence in soil. Singh and Das[30] have recently shown the common occurrence of *N. aerobia* in sewage sludge samples of Lucknow. This finding is of great epidemiological importance.

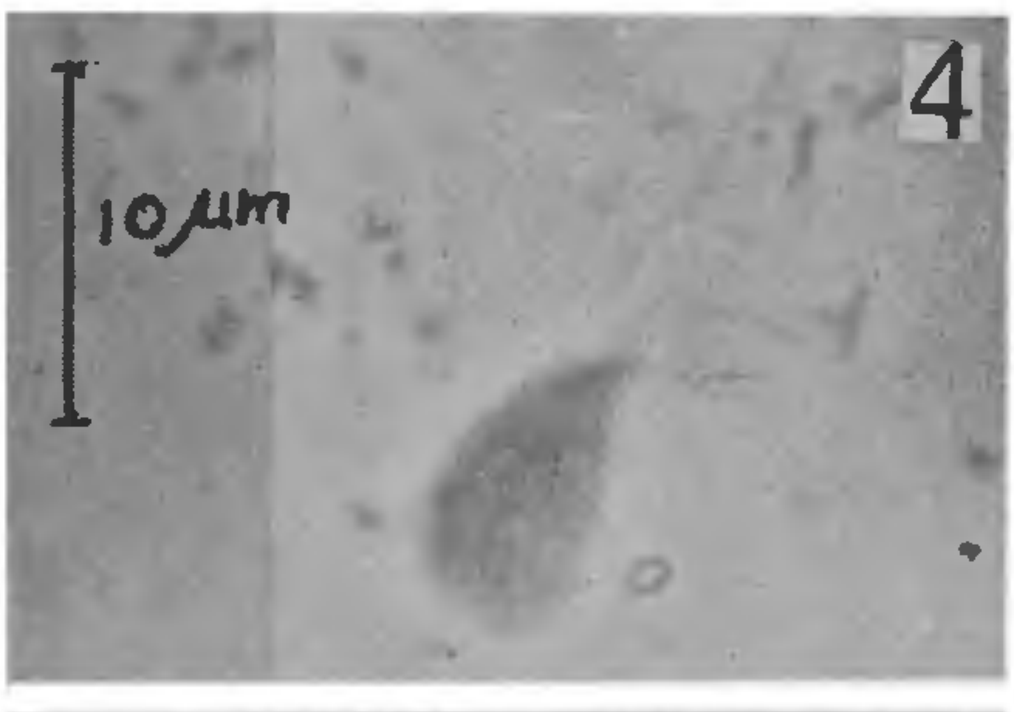
If human cases of meningo-encephalitis due to *N. aerobia* is caused by the direct entry of trophic amoebae during swimming in fresh water through the nose, as has been assumed because of animal experiments, one would expect more human cases due to *H. culbertsoni* or *H. rhysodes*. Since human cases do not happen due to *H. culbertsoni* or *H. rhysodes* or may rarely happen, it is logical to suggest that the spread of the disease by swimming in fresh water is mainly due to the entry of temporary flagellate stage of *N. aerobia* through the nose. The flagellates have rigid oval shape and move actively in water. They can easily enter the nose with water and get transformed into amoebae, the latter causing meningo-encephalitis. It is difficult to imagine that amoebae will remain suspended in water for a long time and will readily enter the nose while swimming. They are bound to round up and sink to the bottom. On finding a solid or semi-solid substrate, the amoebae will become motile, feed on suitable bacteria and other micro-organisms, if present in the substrate, and multiply. The transformation of amoebae into flagellates and then back into amoebae must be constantly occurring in nature where *N. aerobia* is able to exist. The ease with which *N. aerobia* trophozoites get transformed into flagellates at 37° C under experimental conditions may explain why human meningo-encephalitis is due to this amoeba. The work reported in this paper shows that intra-nasal inoculation of mice with flagellate stage of *N. aerobia* is more effective in causing meningo-encephalitis and death of the animals than the intra-nasal inoculation with trophic amoebae. *H. culbertsoni* and *H. rhysodes* do not produce temporary flagellate stage and, therefore, have little chance of entering the nose during swimming. These

amoebae can easily enter the nose of buffaloes and other animals, which lie in water for long periods and inhale mud, and may cause meningo-encephalitis.

Sewage sludge, which contains plenty of bacteria and other micro-organisms, seems to be one of the most suited habitat for trophic forms of *N. aerobia*







FIGS. 1–5. Figs. 1–2. Living trophozoites of *N. aerobia* (strain N-1) from infected brain of mouse. Fig. 3. Living cysts of strain N-1 with a single wall, whose outside consists of a transparent gelatinous layer. No pores could be seen. Figs. 4–5. Flagellate stage of strain N-1 showing two flagella.

to grow and multiply. When it is mixed with water, it is reasonable to suppose that plenty of flagellates will be produced. By inhaling such water or by bathing in sewage contaminated water, it is very likely that the flagellates of *N. aerobia* will get into the nose. This may explain amoebic meningo-encephalitis cases in Australia (Carter[29]) where patients had never swum at all in fresh-water or had swum in sea-water in which *N. aerobia* cannot live.

It is interesting to point out that all human cases caused by *N. aerobia* in different parts of the world occurred during the hottest season, a fact related to high range of temperature. This is also the time when people generally bathe to keep cool. There is more chance of the prevalence of primary amoebic meningo-encephalitis in countries having hot climate than in countries having very cold climate for most of the year because *N. aerobia* grows best at 37° C than at lower temperatures. During rainy season, when the temperature is high in tropical countries, children often play in muddy water and water contaminated with sewage sludge. There is likelihood of *N. aerobia* flagellates entering the nose of some of these children. A proper investigation of the aetiology of human meningo-encephalitis cases in countries having hot climate is likely to reveal that primary amoebic meningo-encephalitis is not so rare as has been assumed so far.

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