

Migration Patterns of Pathogenic and Nonpathogenic *Naegleria* spp.

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Four species of *Naegleria* were tested for their ability to migrate under agarose. Pathogenic *N. fowleri* strains exhibited rapid locomotion at 37°C. Environmental isolates of *N. fowleri* moved faster than clinical isolates which had been kept in axenic culture for longer periods, and this result was confirmed by using the 84-2205-7 strain kept in axenic culture for 1 or 5 months. Nonpathogenic *N. gruberi* strains migrated actively at 28°C but not at 37°C; moreover, even at 28°C, active amoebae constituted only a small proportion of the whole. The temperature-tolerant, nonpathogenic species *N. lovaniensis* moved more slowly than *N. fowleri* at 37°C. In contrast, *N. australiensis*, which is temperature tolerant as well as pathogenic for mice, migrated at a rate comparable to that of *N. fowleri*. There appears to be a direct correlation between the locomotive ability of free-living amoebae and their pathogenic potential.

Naegleria fowleri is a free-living amoeba which causes a fulminant and rapidly fatal meningoencephalitis in humans (2, 3, 11, 12, 17). Most cases are associated with swimming, and infection occurs when nasal mucosa are exposed to water contaminated by trophozoites of this species. The portal of entry is the nasopharynx, and the amoeba gains access to the brain by penetrating the nasopharyngeal mucosa and migrating along olfactory nerves through the cribriform plate (1, 13, 18, 21). One important determinant of invasiveness for *Naegleria* species may be locomotive ability. In this study, we examined the migration properties of pathogenic and nonpathogenic *Naegleria* species to determine the association between migration profile and pathogenicity.

MATERIALS AND METHODS

The clinical isolates of *N. fowleri* used in these experiments included the Northcott strain which has been used extensively in previous studies (7, 8, 18, 20) and the Damiano and Peterson strains, which are more recent isolates. We also used three environmental isolates of *N. fowleri* (K8-1, 84-2205-7, and 84-671-17) provided by Brett Robinson of the Water Resources Laboratory, Engineering and Water Supply Department of South Australia.

The nonpathogenic species of *Naegleria* used in these experiments were *N. gruberi* 1518C, originally from the collection of the Institute of Medical and Veterinary Science, Adelaide (19), and *N. lovaniensis* Ag/9/1/45D (16). We also used *N. australiensis* PP397 and *N. australiensis* subsp. *italica*, both of which are known to be pathogenic for mice (5). These were originally isolated by J. F. de Jonckheere and kindly provided to us by Brett Robinson.

Axenic culture of amoebae. The *Naegleria* strains were grown in Fulton medium A (9). All were cultured at 37°C, except *N. gruberi*, which was maintained at 28°C. All amoebae were cultured in flat-bottom tissue culture flasks and prepared by centrifugation and three washes with Page amoebic saline as previously described (15).

Migration under agarose. A 1% agarose (Calbiochem-

Behring, Australia) solution in amoebic saline was poured into tissue culture dishes (60 by 15 mm Lux) (6 ml per plate) and allowed to set at room temperature. Wells (2.5 mm diameter) were cut in the agarose. Into each well was delivered 5 µl of a suspension of amoebae (2×10^7 /ml) in Page amoebic saline. The agarose plates were then placed in a highly humidified environment at 37°C unless otherwise specified. Migration of the amoebae was observed under an inverted microscope at 1-h intervals, and the migration distance was measured with the aid of an eyepiece grid as described for neutrophils (6, 14).

RESULTS

The three environmental isolates of *N. fowleri* (84-2205-7, 84-671-17, and K8-1) migrated faster out of the wells than the two clinical isolates, the Northcott and Peterson strains (Fig. 1). *N. fowleri* 84-2205-7 moved 2.7 mm in 5 h, while *N. fowleri* Peterson moved approximately 0.5 mm in the same time period. Irrespective of the distance moved by the different *N. fowleri* strains, the amoebae appeared to have moved en masse, so that very few were left in the wells at the end of the assay period.

N. gruberi was found to migrate as well as the environmental isolates of *N. fowleri*, with migration rates of about 3.0 mm in 5 h at 28°C (Fig. 2). The migration rate was markedly depressed when the incubation temperature was 37°C. Although the migration rate of *N. gruberi* at 28°C was similar to that of the environmental isolates of *N. fowleri*, it was observed that as many as 60 to 70% of the *N. gruberi* organisms remained in the wells, and the amoebae exhibiting active locomotion constituted only a small portion of the whole.

N. lovaniensis is a recently described temperature-tolerant species which is nonpathogenic for mice (16). We compared the locomotive pattern of this species with that of a clinical isolate of *N. fowleri* (Damiano). *N. lovaniensis* moved at a much slower rate than *N. fowleri* (Fig. 3). Also, a large percentage of the *N. lovaniensis* amoebae remained in the wells, whereas most of the *N. fowleri* organisms had moved outside the wells.

We also studied the migration characteristics of another recently described species, *N. australiensis* PP397 and *N.*

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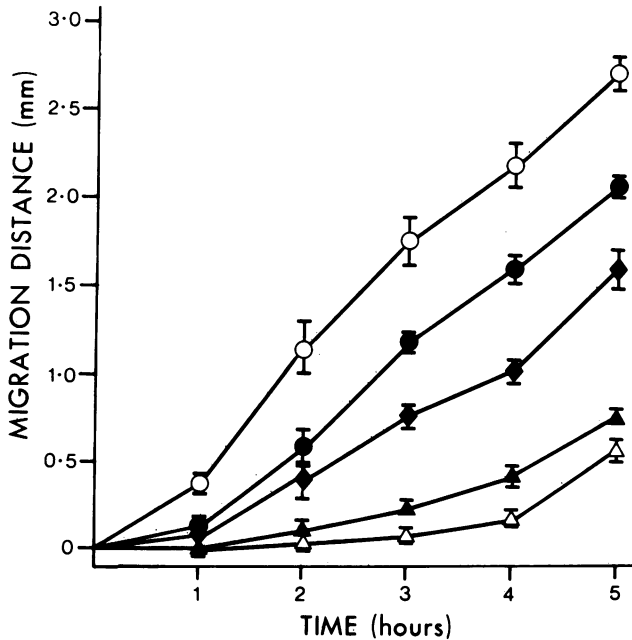


FIG. 1. Migration of three environmental isolates, 84-2205-7 (○), 84-671-17 (●), and K8-1 (◆), and two clinical isolates, Northcott (▲) and Peterson (△), of *N. fowleri* under agarose. Results represent the mean \pm standard error for four separate experiments, each performed in triplicate.

australiensis subsp. *italica*. This species is temperature tolerant, but unlike *N. lovaniensis*, it is pathogenic for mice (5). These two amoeba strains moved at a rate comparable to that of the faster-moving strains of *N. fowleri* (Fig. 3).

Since the clinical isolates Northcott and Peterson had been maintained in axenic culture longer than the environmental isolates and clinical isolate Damiano, it is possible that this factor may have been responsible for the differences. It was found that keeping environmental isolate 84-2205-7 in axenic culture for 5 months resulted in marked loss of locomotive activity, from about 2.5 to 0.7 mm per 5 h (Fig. 1 and 4). After this long-term culture, the isolate also lost some of its virulence. For example, a group of five mice inoculated intranasally with 5×10^4 fast-moving amoebae all developed *Naegleria* meningoencephalitis in 4 to 5 days and were all dead by day 8. In contrast, of another group of five mice inoculated with the slower-moving amoebae, only one developed the disease, and this mouse died on day 25. Amoebae isolated from the brain of this mouse were cultured axenically for 3 to 4 weeks and then examined for migration activity. This mouse-passaged amoeba strain had a migration rate similar to that of the original environmental isolate (Fig. 1 and 4). When this mouse-reisolated strain was inoculated into five other mice, all the animals died of *Naegleria* meningoencephalitis within 5 days.

DISCUSSION

Both clinical observation and experimental evidence indicate that pathogenic free-living amoebae gain entry into the brain by way of the nasopharynx (2, 3, 10, 12, 17). This implies that an important prerequisite for pathogenesis would be locomotive ability of a special kind; the amoebae have to make their way from the nostril, the portal of entry, into the nasopharynx and then penetrate the protective layer

of the mucosa, after which they have to migrate along the path of the olfactory nerves through the cribriform plate into the brain (1, 13, 18, 21).

The agarose technique measures the ability of motile cells to wedge themselves through the gap between the agarose gel and the plastic surface (6, 14). It is unlikely that passive forces such as surface tension, diffusion, or capillary action are responsible for the migration of free-living amoebae under agarose. First, the migration rate was reproducible for all strains in many separate experiments. Second, the finding that the same strains of *N. fowleri* kept in axenic culture for prolonged periods had marked reductions in locomotive ability would argue against this interpretation. Third, when the experiments were conducted in normal (0.9%) saline instead of hypertonic Page amoebic saline, the amoebae became rounded and did not migrate out of the wells. It is pertinent to note here that strains cultured in medium 199 supplemented with 5% heat-inactivated fetal calf serum showed migration patterns similar to those in Page amoebic saline (unpublished data), and these experiments suggest that the findings are relevant to amoebic locomotion in mammalian biological fluids. Finally, it was shown that cytochalasin B, which inhibits cellular microfilaments, also inhibited amoebic locomotion (unpublished data).

The application of the agarose technique to the study of amoebic locomotion in the present studies has provided some useful insights into possible links between motility and virulence. We found that different strains of pathogenic *N. fowleri* had different motility rates as measured by distance moved from the edge of the agarose wells. Since loss of pathogenicity in *N. fowleri* is associated with long-term

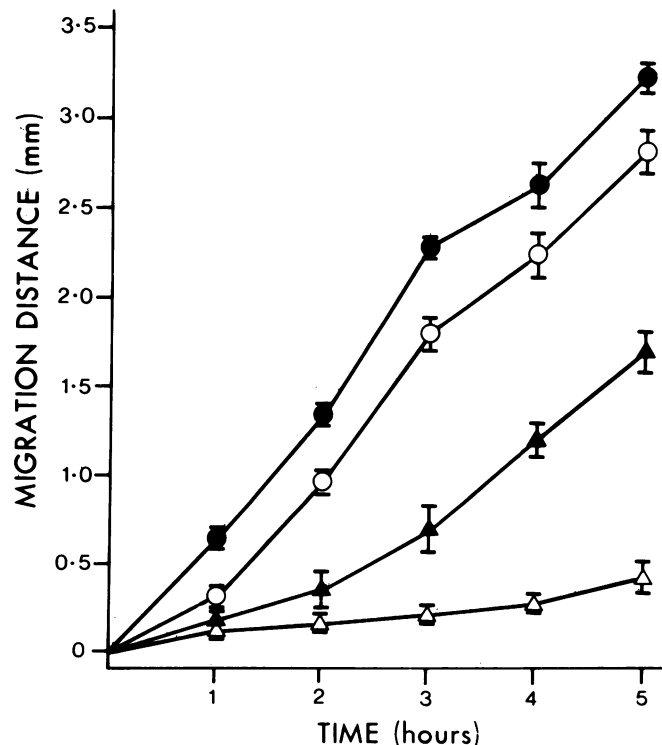


FIG. 2. Migration of *N. gruberi* under agarose at 28 to 29°C (●), 23°C (○), 34°C (▲), and 37°C (△). Results represent the mean \pm standard error for three separate experiments, each performed in triplicate.

axenic culture (22), it would be reasonable to conclude that the differences in migration rate among the various isolates are directly correlated with virulence. This line of reasoning is supported by additional data which showed that environmental isolate 84-2205-7 kept in axenic culture for 5 months also showed a marked reduction in rate as well as loss of virulence. Furthermore, repassage of the attenuated isolate in mice restored both the original migration rate and virulence. One plausible explanation for this change in locomotive behavior is the presence of all essential nutrients throughout the axenic medium so that the amoebae need not forage as actively as they would need to do in the wild.

Even more intriguing is the finding that the pathogenic species and strains of free-living amoebae were able to move en masse out of the wells into the agarose. This was consistently seen in experiments with all six strains of *N. fowleri*. In contrast, a large percentage of the nonpathogenic amoebae remained in the wells, even though a small proportion had migration rates equal to that of *N. fowleri* trophozoites. These observations suggest that pathogenic free-living amoebae may possess a greater ability to penetrate spaces and tissue planes and that this ability may be a requisite for pathogenesis of infection. The difference in migration rate found for a proportion of the nonpathogenic amoebae may be due to genetic variations.

The ability to migrate at body temperature may be another factor in pathogenesis. Thus, the inability of *N. gruberi* to be very mobile at 37°C would certainly hamper its successful invasion of body tissue, even though it has been shown to possess cytopathogenic toxins (4, 11). However, temperature tolerance does not appear to have a strong correlation with pathogenicity because of recent discoveries that heat-tolerant *Naegleria* species, such as *N. lovaniensis*, are nonpathogenic (16). This amoeba showed a poor migration rate, and this property may explain its lack of virulence.

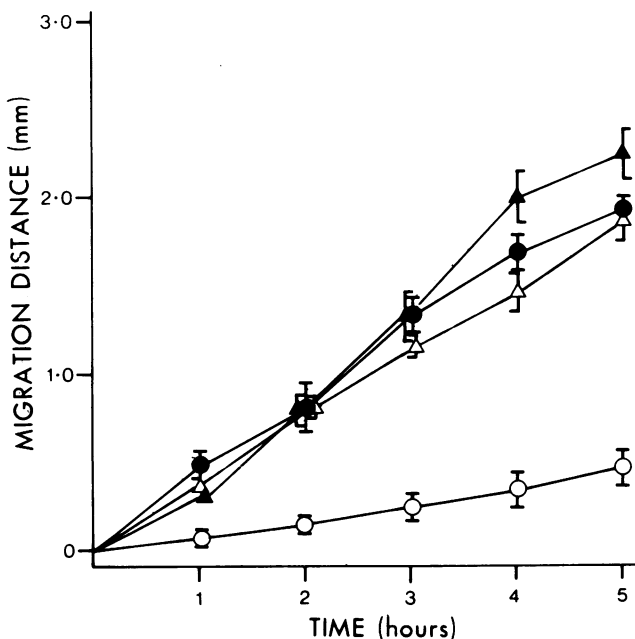


FIG. 3. Comparison of migration rates of *N. australiensis* PP397 (▲), *N. fowleri* Damiano (●), *N. australiensis* subsp. *italica* (△), and *N. lovaniensis* (○) under agarose at 37°C. Results represent the mean \pm standard error for three separate experiments, each performed in triplicate.

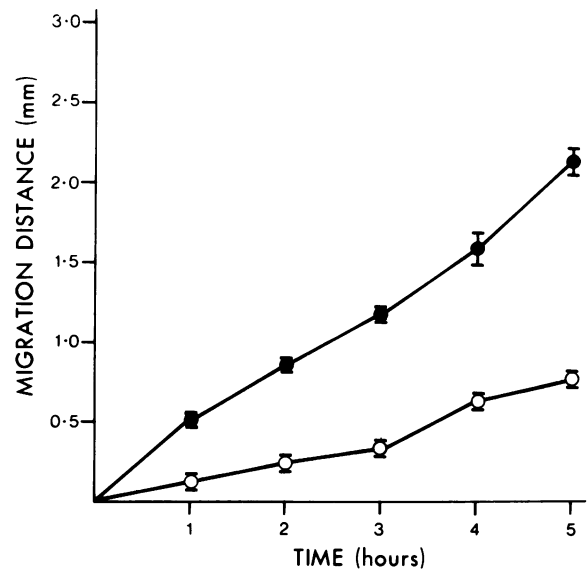


FIG. 4. Comparison of the migration rates of *N. fowleri* 84-2205-7 under agarose after 1 (●) and 5 (○) months in axenic culture. Results represent the mean \pm standard error for three separate experiments, each performed in triplicate.

The other species of *Naegleria*, *N. australiensis* PP397 and *N. australiensis* subsp. *italica*, migrated at rates comparable to that of *N. fowleri*. These recently identified *Naegleria* species have not yet been implicated in human infection, but experimental studies have shown them to be pathogenic for mice (5), and therefore the findings are consistent with our hypothesis that locomotive ability may be a marker of pathogenicity and virulence.

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LITERATURE CITED

- Carter, R. F. 1970. Description of a *Naegleria* sp. isolated from two cases of primary amebic meningoencephalitis and the experimental pathological changes induced by it. *J. Pathol.* 22:470-474.
- Carter, R. F. 1972. Primary amebic meningoencephalitis: an appraisal of present knowledge. *Trans. R. Soc. Trop. Med. Hyg.* 66:193-213.
- Culbertson, C. G. 1981. Amebic meningoencephalitis. *Antibiot. Chemother. (Basel)* 30:28-53.
- Cursons, R. T. M., T. J. Brown, and E. A. Keys. 1978. Virulence of pathogenic free-living amoebae. *J. Parasitol.* 64:744-745.
- De Jonckheere, J. F., P. Pernin, M. Scaglia, and R. Michel. 1984. A comparative study of 14 strains of *Naegleria australiensis* demonstrates the existence of a highly virulent subspecies: *N. australiensis italica* n. spp. *J. Protozool.* 3:324-331.
- Ferrante, A., L. J. Beard, and Y. H. Thong. 1980. Early decay of human neutrophil chemotactic responsiveness following isolation from peripheral blood. *Clin. Exp. Immunol.* 39:532-537.
- Ferrante, A., B. Rowan-Kelly, and Y. H. Thong. 1984. *In vitro* sensitivity of virulent *Acanthamoeba culbertsoni* to a variety of drugs and antibiotics. *Int. J. Parasitol.* 14:53-56.
- Ferrante, A., and C. Smyth. 1984. Mitogenicity of *Naegleria fowleri* extract for murine T lymphocytes. *Immunology* 51:461-468.

9. **Fulton, C.** 1970. Amoeba-flagellates as research partners: the laboratory biology of *Naegleria* and *Tetramitus*, p. 341. In D. M. Prescott (ed.), *Methods in cell physiology*, Academic Press, New York.
10. **John, D. T.** 1982. Primary amebic meningoencephalitis and the biology of *Naegleria fowleri*. *Annu. Rev. Microbiol.* **36**:101-123.
11. **Marciano-Cabral, F. M., M. Patterson, D. T. John, and S. G. Bradley.** 1982. Cytopathogenicity of *Naegleria fowleri* and *Naegleria gruberi* for established mammalian cell lines. *J. Parasitol.* **68**:1110-1116.
12. **Martinez, A. J.** 1981. Free living amoebas in human infections, p. 1-57. In W. Ball and V. Iralu (ed.), *Symposium on Clinical Parasitology: Current Concepts in a Changing Science. Monographs of the Eastern Pennsylvania Branch of the American Society for Microbiology*, Philadelphia.
13. **Martinez, A. J., R. J. Duma, E. C. Nelson, and F. L. Moretta.** 1973. Experimental *Naegleria* meningoencephalitis in mice. Penetration of the olfactory mucosal epithelium by *Naegleria* and pathologic changes produced: a light and electron microscope study. *Lab. Invest.* **29**:121-133.
14. **Nelson, R. D., P. G. Quie, and R. L. Simmons.** 1975. Chemotaxis under agarose: a new and simple method for measuring chemotaxis and spontaneous migration of human polymorphonuclear leukocytes and monocytes. *J. Immunol.* **115**:1650-1656.
15. **Page, F. C.** 1967. Taxonomic criteria for limax amoebae with descriptions of three new species of *Hartmanella* and three of *Vahlkampfia*. *J. Protozool.* **14**:499-521.
16. **Stevens, A. R., J. De Jonckheere, and E. Willaert.** 1980. *Naegleria lovaniensis* new species: isolation and identification of six thermophilic strains of a new species found in association with *Naegleria fowleri*. *Int. J. Parasitol.* **10**:51-64.
17. **Thong, Y. H.** 1980. Primary amoebic meningoencephalitis: fifteen years later. *Med. J. Aust.* **1**:352-354.
18. **Thong, Y. H., R. F. Carter, A. Ferrante, and B. Rowan-Kelly.** 1983. Site of expression of immunity to *Naegleria fowleri* in immunized mice. *Parasite Immunol.* **5**:67-76.
19. **Thong, Y. H., and A. Ferrante.** 1978. Phagocytic behaviour towards baker's yeast distinguishes pathogenic from non-pathogenic *Naegleria*. *Trans. R. Soc. Trop. Med. Hyg.* **72**:207-209.
20. **Thong, Y. H., A. Ferrante, B. Rowan-Kelly, and D. O'Keefe.** 1980. Immunization with live amoebae, amoebic lysate and culture supernatant in experimental *Naegleria* meningoencephalitis. *Trans. R. Soc. Trop. Med. Hyg.* **74**:570-576.
21. **Visvesvara, G. S., and C. S. Callaway.** 1974. Light and electron microscopic observations on the pathogenesis of *Naegleria fowleri* in mouse and brain tissue culture. *J. Protozool.* **21**:239-250.
22. **Wong, M. M., S. L. Karr, Jr., and C. K. Chow.** 1977. Change in the virulence of *Naegleria fowleri* maintained in vitro. *J. Parasitol.* **63**:872-878.