Correspondence to cultures

Although developed therapy, with no eye He had also had day. stromal opacity was visual. Case simplex 31-year-old A is amoebae and impression four patients immunofluorescent diagnosis. One is described in detail. We believe that acanthamoebic eye infection is not rare. An response to propamidine and dibromopropamidine therapy was complicated by problems of toxicity.

Although the first report of the devastating eye infection that can occur with Acanthamoeba species was described as long ago as 1974, only scattered publications have appeared since then, and reports in British journals of cases originating in this country have been few. Several papers have appeared recently in American journals which serve to highlight certain clinical features of the infection and a frequently unsatisfactory outcome, whether medical or surgical treatment or both are given. It is our impression that acanthamoeba keratitis is by no means uncommon. We report here that we have seen four patients in the past two years in whom this diagnosis was eventually confirmed. One of them is considered in detail, with a description of an immunofluorescent technique for localisation of amoebae and cysts.

Case report

A 31-year-old man was first referred in November 1984 for further management of a refractory left herpes simplex stromal keratitis. At that time he had a visual acuity of 6/24 in this eye and an extensive stromal opacity with a 1·5 mm central indolent ulcer. He was on prednisolone 1% eyedrops four times a day. He had also had a dendritic ulcer in the right eye some years earlier, which had healed uneventfully, with no visual deficit or further problems to date.

He had first presented with a dendritic ulcer in the left eye in August 1984; and this had healed with antiviral therapy, but a disciform keratitis ensued. In October 1984 he was noted to have a ring infiltrate. At this stage, while on a visit to the United States, he developed a fresh corneal abscess and hypopyon. Although cultures were negative, a presumptive diagnosis of bacterial keratitis was made, and his eye settled on topical systemic antibiotics.

His eye gradually deteriorated during December 1984, and his visual acuity fell to 6/60. He was noted to have a variable indolent epithelial defect. Treatment at this stage consisted of prednisolone eyedrops 0·3–1%, acyclovir eye ointment five times a day, gentamicin (Genticin) eyedrops four times a day and atropine eyedrops twice a day. By January 1985 he had developed stromal thinning beneath the central indolent ulcer and a bandage contact lens was fitted. Two months later the central infiltrate had extended but a clear periphery was maintained. The cornea had epithelialised by May 1985 while use of the bandage lens continued, though the eye remained inflamed, and visual acuity was hand movements. In summary, therefore, this man had had a nine-month history of a chronic stromal keratitis following a dendritic ulcer, with an indolent ulcer which was perhaps compatible with herpes simplex keratitis but was remarkably recalcitrant.

It had been impossible to quieten the eye with steroids, as is usually the case in patients with herpetic stromal keratitis. As he was experiencing very protracted pain and disability an 8 mm therapeutic penetrating keratoplasty was carried out in June 1985. At surgery it was found that the aqueous was markedly viscous, which accords with a case described by Hirst et al. The corneal disc was sent for histological and microbiological examination. Within 36 hours of culture on Escherichia coli seeded nutrient free agar, acanthamoeba trophozoites were identified on the plate. Subsequent periodic acid Schiff (PAS) staining of the corneal sections revealed multiple acanthamoeba cysts in the stroma (Fig. 1). These cysts were also identified by an immunofluorescent technique as follows.
Acanthamoeba keratitis

IMMUNOFLUORESCENT LOCALISATION OF AMOEBAE AND CYSTS
Wax-embedded sections of the corneal disc were dewaxed and brought to 50% ethanol. After they had been dried and rinsed with water marker, a 1/50 dilution of rabbit anti-Acanthamoeba serum in phosphate buffered saline (PBS) was layered on the sections. They were held at room temperature for 30 minutes in a moist box. After two 10-minute washes in PBS, fluorescein-conjugated sheep antirabbit Ig serum was added at a dilution of 1/50 and further held for 30 minutes. Two further washes in PBS were followed by a five-minute exposure to 1/10000 Evans blue and a final five-minute PBS wash. After being mounted under a cover slip in pH 7.1 glyceral mountant (Difco), the sections were examined under a Neofluor x25 objective with incident blue light in a Zeiss fluorescent microscope. Cysts and amoebic trophozoites fluoresced yellow-green.

TREATMENT
Postoperatively he was treated with chloramphenicol eyedrops qds, dexamethasone eyedrops qds, acyclovir eye ointment qds, cyclopentolate eyedrops bd, Brolene eyedrops qds, and Brolene eye ointment nocte. Brolene is the trade name for a preparation containing propamidine and dibromopropamidine which is widely available in chemists without prescription. He did well initially, maintaining a clear corneal graft for several months. In September 1985 some loose suture loops occurred which were removed. His treatment at this time was prednisolone eyedrops qds, acyclovir eye ointment tds, Brolene eyedrops qds, and Brolene eye ointment nocte. In October 1985 he was noted to have epithelial thickening and irregularity over the graft and it was felt that these changes were due to Brolene toxicity. Brolene therapy was therefore reduced and stopped over a 10-day period. However, when seen the following week he was noted to have an epithelial defect, an active anterior chamber, keratic precipitates over graft and host, and a small hypopyon. This was thought to represent a recurrence of acanthamoeba in the graft. Brolene therapy was restarted at two-hourly intervals. Within a few days his eye had quietened down considerably, and the epithelial defect had regressed. Since then he has had further epithelial defects. His visual acuity has varied between 6/18 and 6/60 postoperatively, and this limitation is mainly due to a posterior subcapsular cataract. Current therapy is prednisolone eyedrops qds and Brolene eyedrops qds.

Discussion
Acanthamoebae are free-living amoebae found in the soil, water, and air. The trophozoites are uninucleate, have small spiky acanthopodia, and move slowly on a broad front. There is a resistant cystic stage characterised by angular double-walled cysts. In outdoor air it has been found that an average of one amoebic cyst of any species was found in 9·1 m³ of air and one cyst of Acanthamoeba castellanii in 18·2 m³. Since 1974 this ubiquitous organism has been implicated as the causative agent in some cases of chronic stromal keratitis. Two groups of patients

Fig. 1 Acanthamoeba cysts in corneal disc. Periodic acid Schiff stain.
Another way in which the organism can be introduced is through trauma. Trauma, however, is not a rare cause of acanthamoeba keratitis. Acanthamoeba is more likely to be isolated in a case of keratitis that is due to trauma than to a non-traumatic keratitis, which suggests that trauma may play a role in the development of the condition. In a retrospective survey, 1975 revealed no cases of acanthamoeba keratitis. Although trauma is a known cause of acanthamoeba keratitis, it is not the only means by which the organism can be introduced.

In conclusion, the management of acanthamoeba keratitis is challenging. The treatment options are limited, and there is a need for more research in this area. The use of topical corticosteroids may be effective in reducing inflammation, but the long-term effects of these drugs are not well understood. Further studies are needed to improve the management of acanthamoeba keratitis.


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