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Acanthamoeba keratitis

SIR,—Professor D L Easty and Dr D Seddon have given illuminating accounts of acanthamoeba keratitis from the points of view of the physician and the patient (23 January, p 228 and 287). It is surprising that the disease is not more well known here as the first cases¹ and the first successful drug treatments were reported from Britain.

In 1985-6 five British cases of acanthamoeba keratitis were reported to the amoebiasis unit, and it is certainly possible that other cases were undiagnosed. The amoebiasis unit (parasitology laboratory, Hospital for Tropical Diseases) has been collecting information and isolates since 1973 and we are happy to receive and confirm the identity of isolates sent to us together with case histories.

Acanthamoeba organisms are not restricted to brackish or salt water as stated by Professor Easty; they are found easily in fresh water and damp soil. Their viable cysts can be detected in air samples. Dr Seddon's comments on the hazards associated with overseas travel may possibly be misread as suggesting that travellers are a risk group for this disease, which is not the case. Certainly regular trauma, sometimes associated with contact lens use, may be responsible for the increased incidence of the disease in recent years. It is also suggestive that we and other workers have isolated acanthamoeba from contact lens washing fluid. Such salines may not be regularly renewed and the container may become contaminated with protein residues, allowing growth of bacteria.

The amoebiasis unit will, on request, post medium to microbiologists wishing to isolate acanthamoeba and carries out immunostaining techniques on histological material to aid diagnosis.

A protocol for sampling and cultivation of

acanthamoeba from the cornea is as follows.

Taking the sample—The best sample is a scrape using a fine scalpel. Punch biopsy specimens or portions of excised cornea may also be used. Swabs or washings appear to be less efficient in detecting the organism.

Medium—Plain agar plates coated with washed Escherichia coli or Klebsiella aerogenes are used. Oxoid L28 agar is autoclaved at 1.5% in 0.5% (w/v) saline and after cooling poured into small petri dishes. Leave these on the bench overnight to allow the excess water to absorb. The bacteria can be grown on plates or in broth. Take a suspension and wash by centrifugation in three changes of distilled water. Finally resuspend in distilled water to give a milky suspension. Add several drops of the bacterial suspension to the surface of the agar plates and spread. Allow to absorb at 37° C. The medium is now ready for inoculation.

Inoculation—Scrape the specimen on to the surface of the bacteria coated agar. Do not go back to the surface of the cornea using the same swab or scalpel since it will now be contaminated with live bacteria.

Incubation—Although most isolates will grow at 37°C, it is best to use 30°C routinely. The plates should be incubated in a moist box.

Examination—Examine the surface of the plate after 24 hours and then daily for four days. This is best achieved using an inverted microscope (\times 10). The plate need not be opened. Trophozoite stage amoebae can be seen to make tracks in the bacterial layer. They are about 20 μ m in diameter, move extremely slowly, but may be recognised by the filling and discharge of the contractile vacuole (period—about 2 minutes). After a few days the cysts (12-20 μ m) appear. These are easier to see and

have a polygonal shape with a thick wall. If the culture becomes positive only after more than five to six days suspect an airborne contaminant.

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- 1 Nagington J, Watson PG, Playfair TJ, et al. Amoebic infection of the eye Lancet 1974:ii:1537-40
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 Wright P, Warhurst D, Jones BR. Acanthamoeba keratitis successfully treated medically. Br J Ophthalmol 1985;69: 778-81.
- 3 Warhurst DC. Pathogenic free-living amoebae. Parasitology Today 1985;1:24-8.
- 4 Kingston D, Warhurst DC. Isolation of amoebae from the air. J Med Microbiol 1969;2:27-32.

SIR,—With Professor D L Easty's leading article (23 January, p 228) and Dr David Seddon's personal view (23 January, p 287) you gave due prominence to the severe and increasingly familiar problem of acanthamoeba keratitis. Eleven new cases were treated at this hospital last year. We would like to comment on two important aspects of this condition.

Acanthamoebae are found in soil and in standing water in this and in other countries, and should be regarded as ubiquitous. They are not confined to tropical climates.

New cases of acanthamoeba keratitis occur most frequently in wearers of soft (hydrogel) contact lenses who have neglected the proper cleaning and disinfection of their lenses. Homemade saline solutions have been incriminated, as Professor Easty indicates, and their use should be outlawed. Some commercial contact lens fluids may also fail