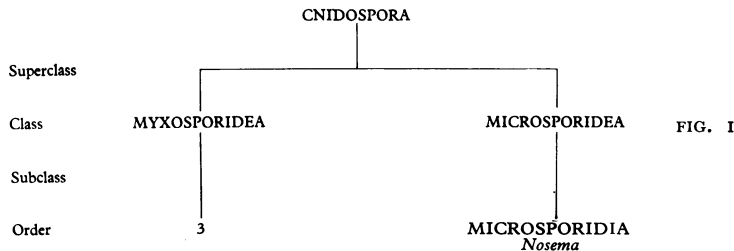


# Encephalitozoonosis (Nosematosis) of the cornea

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Infection of mammals by protozoa of the genus *Nosema*, of the class Microsporidea of the subphylum *Cnidospora* (Fig. 1) which are characterized by the possession of polar filaments, was first described in the brain and kidneys of a rabbit by Wright and Craighead (1922), and named *Encephalitozoon cuniculi* by Levaditi, Nicolau, and Schöen (1923). Since that time the parasite has been found in every major group of animals, including other protozoa, helminths, insects, fish, amphibians, reptiles, and possibly birds (*cf.* Shadduck and Pakes, 1971), but only a single acceptable example of human infection has been reported (Matsubayashi, Koike, Mikata, Takei, and Hagiwara, 1959). The patient was a 9-year-old Japanese boy who had developed meningo-encephalitis (the eyes were said to be normal); oval bodies resembling *Nosema cuniculi* were found in the cerebrospinal fluid and urine and these organisms were propagated in mice. While this diagnosis was thought to be beyond reasonable doubt by Petri (1969), it had been questioned by others on the grounds that mice (which also harbour microsporidia) were used for inoculation (Innes, Zeman, Frankel, and Borners, 1962).



The present case, wherein the cornea was invaded by microsporidia which on morphological grounds were classified as *Nosema*, is therefore of exceptional rarity and of interest in being the first report of ocular involvement in man.

The literature up to recent times is fully covered by Petri (1969) and Shadduck and Pakes (1971), who also discuss the classification of the parasite. Until the early 1960s the original name of *Encephalitozoon cuniculi* persisted, but *Nosema cuniculi* was then proposed as more appropriate (Lainson, Garnham, Killick-Kendrick, and Bird, 1964). Shadduck and Pakes (1971), however, found marked ultrastructural differences between the microsporidian parasite of the rabbit and that of *Nosema bombycis* (the type species for the genus *Nosema*, and the cause of silk-worm disease) and, therefore, suggested a return to the original name of *Encephalitozoon cuniculi* (see also Sprague and Vernick, 1971). For convenience we shall continue to use the shorter name in this report.

The spores are oval, uninucleate, thick-walled, refractile, measure  $2.7$  to  $3.0 \mu\text{m}$ .  $\times 1.2$  to  $1.8 \mu\text{m}$ , and contain a spirally coiled tubular filament and a prominent polar vacuole. They stain positively with Gram's stain, Carbol Fuchsin, and Weil-Weigert stains, while Giemsa stain shows only a dark blue nucleus lying centrally or forming a bar across the spore. They have features in common with *Toxoplasma*, subphylum *Sporozoa*, with which they have been confused, but they may readily be distinguished by their morphology, staining reactions, immunology, and pathogenicity; these differences have been described among others by Attwood and Sutton (1965), Matsubayashi and others (1959), and Innes and others (1962).

### Life cycle

The normal microsporidian life cycle, fully described by Petri (1969), is briefly as follows (Fig. 2). The ingested, or perhaps inhaled, spore invades the intestinal epithelium by discharging its contents (sporoplasm) into the cell through its extruded tubular filament, which is probably fired by the polar vacuole. Within the cell the injected sporoplasm divides by binary fission to form a schizont with 2 to 6 nuclei, which splits into unicellular organisms (meronts); these then secrete a rigid capsule and the fully-formed spore measures about  $2.5 \times 1.5 \mu\text{m}$ . The cell eventually ruptures and the cycle recommences. Petri (1969) described a sexual phase in which the nuclei of binuclear stages fuse ("autogamy").

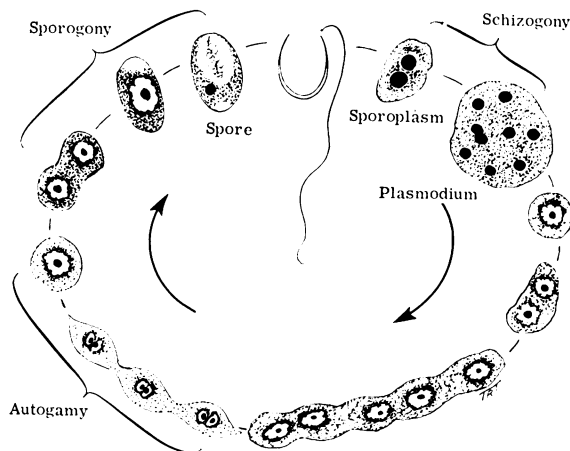


FIG. 2. Life cycle of *Nosema bombycis* (adapted from Fig. 3: Petri, 1969)

### Case report

**An 11-year-old Tamil boy** was admitted to the Eye Hospital, Colombo, complaining of defective vision in the right eye. He gave a history of having been gored by a goat 6 years previously after which the right upper lid was sutured.

#### Clinical examination

##### RIGHT EYE

Visual acuity was perception of light. The cornea was scarred and vascularized; the anterior chamber was present but no details could be seen. Granulation tissue was present over the conjunctival surface of the upper lid.

##### LEFT EYE

Visual acuity was 6/6. Follicles were present in both upper and lower conjunctival fornices.

## BLOOD PRESSURE

Normal.

*Laboratory investigations*

## BLOOD

Examination for Brucellosis and Microfilaria—negative.

## BIOPSY

Granulation tissue from the right upper lid showed follicular hyperplasia and reactive proliferation of connective tissue. The appearances suggested trachoma.

*Operation*

3 weeks later a right penetrating keratoplasty was performed and the corneal disc was sent to the Institute of Ophthalmology, London, for report.

*Histopathology of corneal disc*

Sections showed a full-thickness corneal disc with an irregular oedematous epithelium which was detached on one side. Bowman's membrane was intact. The central stroma was necrotic and surrounded by acute inflammatory cells which were seen also in the peripheral stroma where there were in addition numerous invading vessels. Lying in the deep stroma immediately above Descemet's membrane there were abundant refractile oval bodies measuring on average  $3.5 \times 1.5 \mu\text{m.}$ , some within macrophages but most lying free between the corneal lamellae. Descemet's membrane was intact, and coating its inner surface there was an organizing subacute inflammatory exudate containing numerous giant cells and pigment granules. No parasites could be identified in this exudate, indicating that they had not penetrated Descemet's membrane, and the underlying inflammatory reaction is probably attributable to diffusion of toxic products from the proliferating parasites (Fig. 3). The organisms stained weakly positive with haematoxylin, periodic acid-Schiff, and Gram's stain, and intensely with Giemsa and methylene blue, and were particularly well seen by phase contrast microscopy (Fig. 4). Smears from the cornea stained with methylene blue showed an internal structure consisting of a prominent polar vacuole and a central banded nucleus (Figs 5 and 6).

*Follow-up*

Immediately after the keratoplasty the visual acuity in the right eye was restored to 6/12; the graft gradually became opaque, however, and 2 months afterwards the acuity had deteriorated to 2/60. Otherwise the patient is entirely well and general clinical examination has shown no abnormality. A bone marrow smear was negative. A blood examination gave the following results: haemoglobin 9.4 g. (64 per cent.), white blood count 12,200 per  $\text{cm}^3$ ., polymorphs 51 per cent., lymphocytes 20 per cent., eosinophils 22 per cent., mononuclears 7 per cent.

**Comment**

The parasites found in the cornea were originally thought to be Leishman-Donovan bodies which are of a comparable shape and roughly similar size ( $4 \times 2 \mu\text{m.}$ ) and, although the evidence in the literature is in the main against a direct invasion of the cornea by *Leishmania* (Busacca, 1936; Tita, 1938; Scuderi, 1947; Marback, 1953), this seemed the most likely diagnosis in a geographical area where Leishmaniasis is known to occur. Indeed, Wenyon (1926) has specifically pointed out that *Encephalitozoon* may easily be mistaken for *Leishmania*, and in the study of microsporidia a misdiagnosis of leishmaniasis has in fact been made by protozoologists. The marked refractility of the organisms in our case, however, and their staining properties and rather smaller size, cast doubt on this diagnosis.

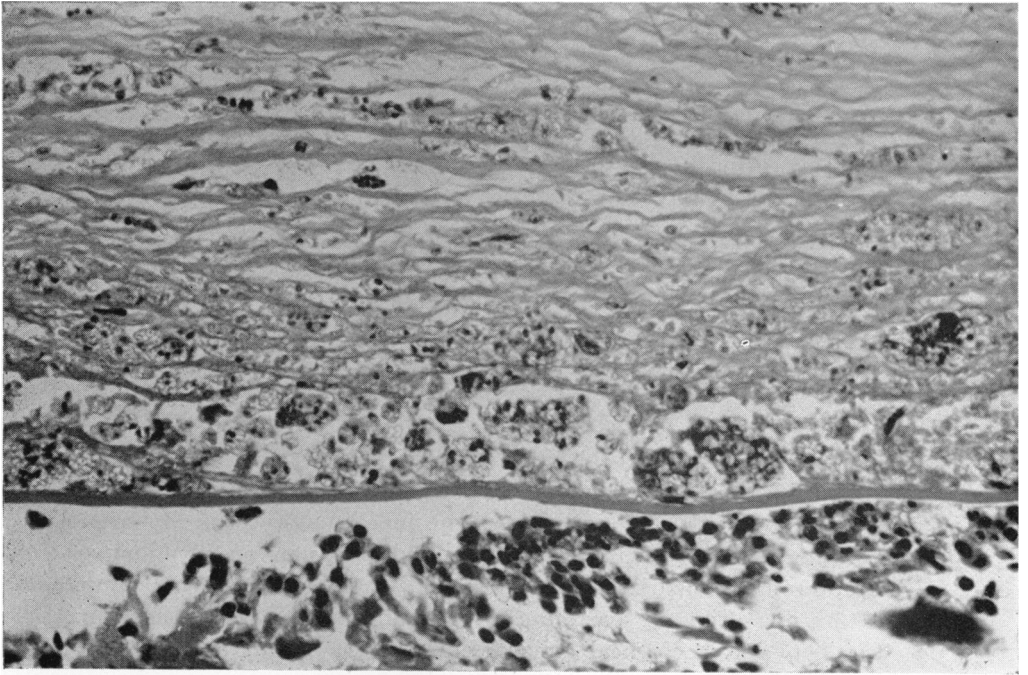


FIG. 3 The deep corneal tissue is heavily invaded with minute oval refractile bodies, some within macrophages but many lying free between the corneal lamellae and immediately upon Descemet's membrane, but there is an underlying inflammatory exudate containing giant cells. Haematoxylin and eosin.  $\times 400$

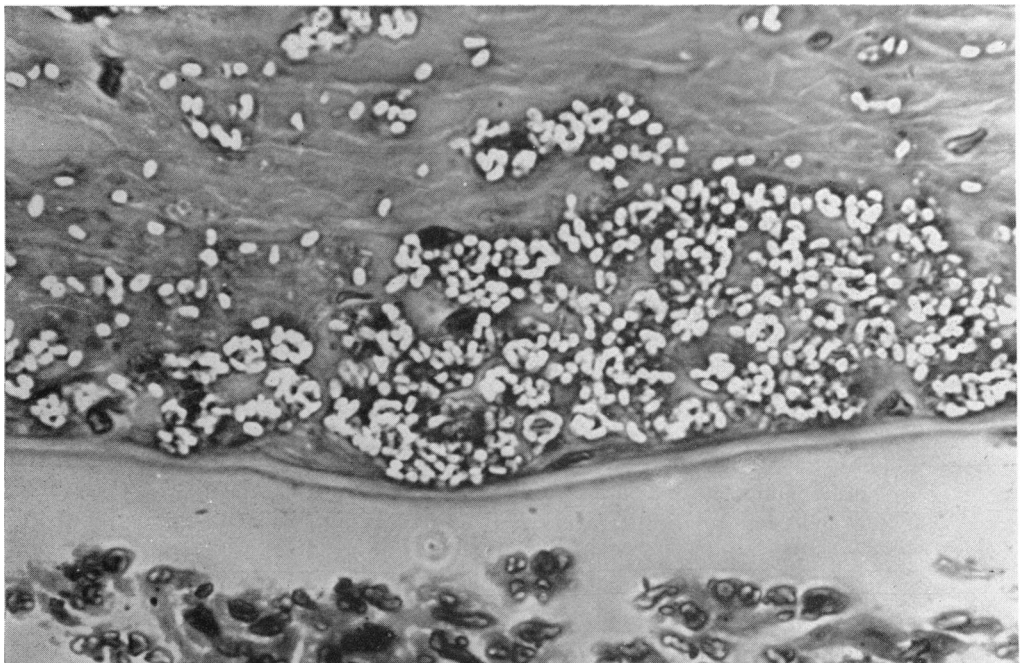


FIG. 4 Another area of the deep cornea as seen by phase contrast microscopy. The microspores are clearly seen clustered on the corneal side of Descemet's membrane. Haematoxylin and eosin.  $\times 630$

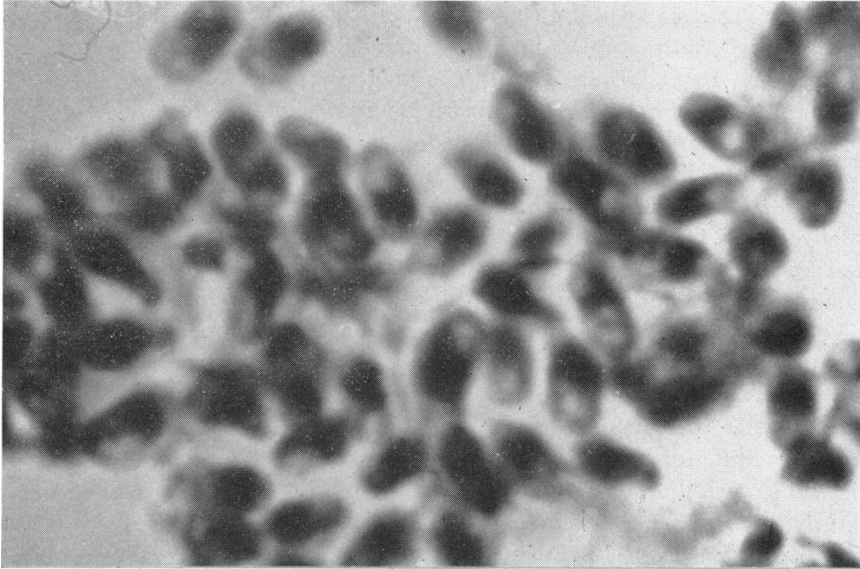


FIG. 5 *High-power view of a cluster of spores in the deep cornea. Their oval shape, polar vacuole, and banded nucleus are evident. Methylene blue.  $\times 3,000$*

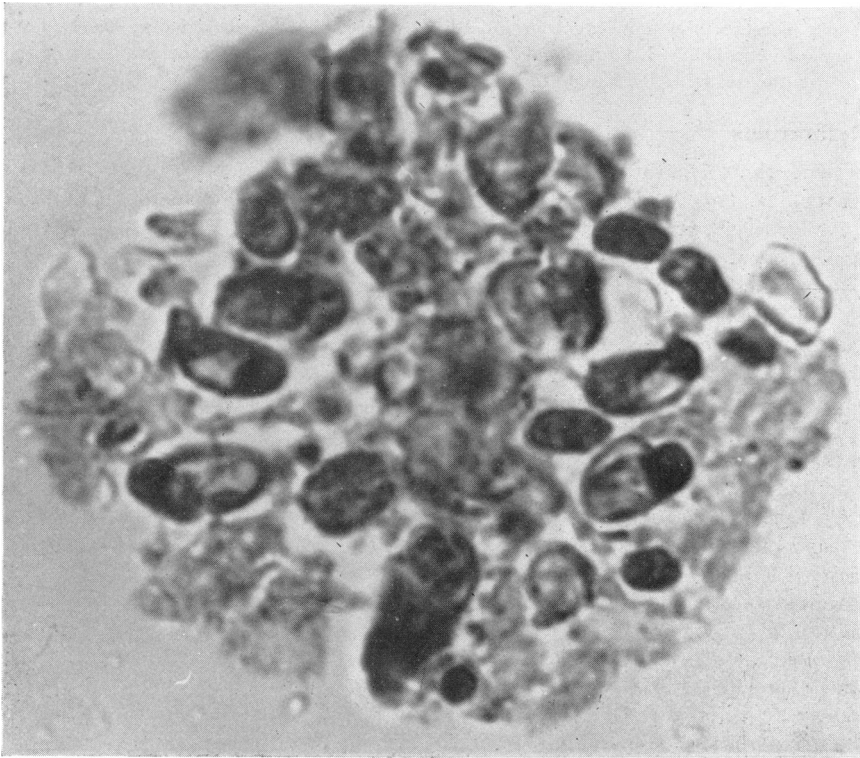


FIG. 6 *Smear preparation from corneal tissue. Several microspores, although slightly distorted, show prominent polar vacuoles and thick capsules. Methylene blue.  $\times 4,280$*

Prof. A. S. Dissanaïke of the Department of Parasitology, University of Ceylon, who in the past has especially studied microsporidian parasites of tapeworms of sheep and goats (Dissanaïke, 1957; Dissanaïke and Canning, 1957), expressed the view that the parasites were not L-D bodies but more probably microsporidian spores. Sections were then sent to Dr. L. E. Zimmerman of the Armed Forces Institute of Pathology, Washington, who consulted his colleagues (Drs. Ronald Neafie and A. J. Strano) in the Geographic Pathology and Infectious Disease Branch. They were firmly of the opinion that the organisms were microsporidia belonging to the family Nosematidae. Interestingly Drs. Neafie and Strano had recently studied a fatal case in an immunologically abnormal infant (Margileth, Strano, Chandra, Neafie, Blum, and McCully, 1973). Prof. Dissanaïke was informed of this development and he put forward the suggestion that, in view of the history of the eye being originally injured by a goat, it was just possible that the spores were those of *Nosema helminthorum*, the microsporidian parasite of tapeworms of sheep and goats. He recommended that the sections be sent for the opinion of Prof. P. C. C. Garnham, F.R.S., Emeritus Professor of Medical Protozoology, University of London. In Professor Garnham's view the parasite was undoubtedly a microsporidan and he thought it reasonable to classify it as *Nosema cuniculi* (or rather *Encephalitozoon cuniculi*); he felt the diagnosis of *Nosema helminthorum* to be rather unlikely.

We are grateful to Dr. D. S. R. Gunawardana, Medical Superintendent, Eye Hospital, Colombo, for permission to report this case, and to Dr. D. Chanmugam of The Faculty of Medicine, University of Ceylon, for providing the results of his investigations of the patient.

It is a pleasure to acknowledge our indebtedness to Prof. A. S. Dissanaïke, Dr. L. E. Zimmerman, Dr. Ronald Neafie, Dr. A. J. Strano, and Prof. P.C.C. Garnham, F.R.S., for their valuable opinions. Our thanks are also due to Mr. G. E. Knight for technical assistance and to Miss E. FitzGerald for secretarial help.

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Since this paper was submitted a further human case has been reported in which the parasites were found in a pancreatic adenocarcinoma (Marcus, Wait and Burger, 1973).