Mycosis of the Eye and Its Adnexa
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with a contribution by R. Rüchel, Göttingen

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Dedicated to
my wife Cordula and our daughters Verena and Corinna
Chapter 1

An Overview of Fungal Pathogens of Ophthalmological Importance

R. Rüchel

1.1 Introduction

Fungi were initially thought to be plants that lacked chlorophyll, but following the proposal of Whittaker [1], they are now considered to be an independent fifth kingdom of life in parallel with the Monera (bacteria and certain prokaryotic algae), Protoctista (protozoa, nucleated algae, slime moulds, etc.), Plantae and Animalia. Fungi (mycetes) are eukaryotes, in contrast to the bacteria (prokaryotes). Accommodation of the various fungi in a natural system of classification has proved to be difficult in many cases, due to a lack of sexual states, and many of the medically relevant fungi therefore remain classified as ‘imperfect fungi’ and have been artificially placed in the formal phylum Deuteromycota. The classification of the fungi is permanently under revision, as true relationships are elucidated. The following overview of fungi focuses on pathogenic members of the kingdom, and is based on the comprehensive treatise on the mycoses by Kwon-Chung and Bennett [2]. The characteristics of the medically relevant fungi are displayed in an atlas by de Hoog and Guarro [3]. As a laboratory manual for mycological bench work, the manual of Evans and Richardson [4] can be recommended.

Fungi grow asexually as yeasts (unicellular organisms that multiply by budding or fission) or as filaments (hyphae). A multitude of hyphae is called the mycelium. The sexual forms in particular, which for unknown reasons do not occur in warm-blooded hosts, produce a variety of distinct morphologies, which lend themselves to mycological differentiation. For practical purposes, the medically relevant fungi may be divided into yeasts, moulds (fungi forming aerial mycelia) and dermatophytes (ringworm fungi, fungi that will grow on keratin).

In systematic mycology, the fungus kingdom consists of four phyla (divisions): (1) Zygomycota; (2) Ascomycota; (3) Basidiomycota, and (4) the form
phylum Deuteromycota (fungi imperfecti). This last phylum comprises fungi which, due to an apparent lack of a sexual reproductive phase, cannot at present be affiliated with certainty to any of the three genetically defined phyla. The phyla are subdivided into the classes: (1) Zygomycota thus comprise the classes Zygomycetes and Trichomycetes; (2) Ascomycota comprise the classes Ascomycetes and Hemiascomycetes; (3) Basidiomycota comprise the classes Holobasidiomycetes and Heterobasidiomycetes; and (4) the form phylum Deuteromycota comprises the form classes Blastomycetes, Coelomycetes and Hyphomycetes (fig. 1.1). The classes are further subdivided into orders, families, genera, species and varieties.

1.2

**Zygomycota**

The phylum Zygomycota contains the medically important class of Zygomycetes, which in turn contains, among others, the orders Mucorales and Entomophthorales. The Mucorales comprise the families Mucoraceae, Cunninghamellaceae, Syncephalastraceae and Saksenaeaceae, while the order Entomophthorales comprises the families Entomophthoraceae and Basidiobolaceae. The zygomycetes are mainly saprophytes, living on dead organic matter. The family Mucoraceae is of particular ophthalmological interest, as these fungi are typical agents of rhinocerebral mucormycosis (zygomycosis), an infection that often causes blindness due to occlusion by the fungus of the central artery of the optic nerve. In the environment, zygomycetes reproduce sexually by formation of zygospores and (mainly) asexually by production of sporangiospores within sporangia. In the host, zygomycetes typically grow by formation of broad, wrinkled hyphae. These hyphae are mostly nonseptate (coenocytic), which is an important criterion for microscopic identification in clinical specimens. The main agents of mucormycosis are *Rhizopus microsporus*, *R. oryzae* and *Absidia corymbifera*. The prognosis for invasive mucormycosis is poor [5].

The Entomophthorales usually produce septate hyphae, and on solid media produce ballistospores, which are forcibly discharged, for example on the cover of the Petri dish. Both of the families Entomophthoraceae and the Basidiobolaceae have medical importance. *Basidiobolus ranarum*, as well as the Entomophthoraceae *Conidiobolus coronatus* and *C. incongrus*, are human pathogens. The mycoses caused by these fungi are called entomophthora-

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1 The critical comments of Professor Charlotte Thielke, formerly of the Department of Botany, Free University of Berlin, are gratefully acknowledged.
Fig 1.1. Some medically important relations in the Fungus kingdom. Deuteromycota (in parentheses) have been placed according to their proven or suggested sexual affiliations.
mycoses, but various other terms including phycomycosis have also been used. Painless subcutaneous granulomas, in the case of Conidiobolus spp. originating from the nasal submucosa, are typically observed.

1.3

**Ascomycota**

The phylum Ascomycota comprises the classes Ascomycetes and Hemiascomycetes. Together with certain species of the Deuteromycetes, which show ascomycete traits, these fungi make up most of the pathogens. The ascomycota are characterized by development of asci, or sacs that contain sexual spores, called ascospores. Their natural habitat is soil or water. The most well-investigated organism among these fungi is bakers’ yeast (*Saccharomyces cerevisiae*), which belongs to the class of Hemiascomycetes, and more precisely to the order Endomycetales. Other yeast-like fungi in this order are the ‘perfect’ analogues (teleomorphs) of certain *Candida* spp. Teleomorphs of *Geotrichum* spp. also belong to the Endomycetales; these are often isolated from the human digestive tract, but are considered nonpathogenic. Their abundant formation of aerial mycelia on solid media is a typical source of misinterpretation as a mould.

The ascomycetes show branched, septate mycelia. The intricacies of ascospore formation is beyond the scope of this overview, but several orders of ascomycetes merit a mention. The order Onygenales includes the families Arthrodermataceae and Onygenaceae, among others. The former family contains the genus *Arthroderma*, which comprises the perfect states of dermatophytes. The corresponding anamorphic genera that are encountered as pathogens are *Trichophyton* and *Microsporum*, the most important agents of ringworm. Also among the Onygenaceae, the genus *Ajellomyces* comprises the perfect states of *Histoplasma capsulatum* and *Blastomyces dermatitidis*, which are among the most pathogenic fungi causing systemic mycoses.

In the family Microascaceae of the order Microascales are found *Pseudoallescheria boydii* as a pathogenic species, whose two anamorphic states are *Scedosporium apiospermum* and *Graphium eumorphum*. The fungus is an agent of systemic mycoses and white grain mycetoma (mycetoma being a granulomatous fungal infection originating from subcutaneous lesions). In patients who have experienced near-drowning, *S. apiospermum* may cause cerebral abscess [6].

The order Eurotiales comprises, in the family Trichocomaceae, the perfect states of certain aspergilli and penicillia. The most important pathogenic moulds among them, however (*Aspergillus fumigatus, A. flavus, A. niger* and *Penicillium marneffei*) are not known in their perfect state.

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1 An Overview of Fungal Pathogens of Ophthalmological Importance
The order Ophiostomatales comprises *Ophiostoma stenoceras*, which may represent the perfect state of *Sporothrix schenckii*, a pathogen that has been associated with wood splinters and that preferentially causes cutaneous or lymphatic mycoses, though cases of endophthalmitis have also been described.

The order Sordariales comprises several rare opportunistic pathogens of the genus Chaetomium.

The order Hypocreales comprises, among others, the genus *Nectria*. *N. haematococca* is the perfect state of *Fusarium solani*. The form genus *Fusarium* comprises plant pathogens and producers of mycotoxins. These fungi are the most important causes of human keratitis and may also cause systemic infection [7, 8]. *F. oxysporum* and *F. verticilloides* are also medically important, though their perfect states are not known.

Finally, the ascomycetous order Dothidiales needs mentioning. It comprises agents of mycetoma in the genera *Leptosphaeria* and *Neotestudina*, as well as *Piedraia hortae*, the agent of black piedra, a disease of the hair. Anamorphic species of the form genus *Alternaria* are also related to the Dothidiales, and *A. alternata* has been associated with mycoses of the eye.

1.4 Basidiomycota

The phylum Basidiomycota is distinguished by the exogenous formation of sexual spores (basidiospores) on elongated projections called basidia. Their dikaryotic mycelia show typical clamp connections. Most of the basidiomycota are saprophocytes or plant pathogens. The phylum comprises the classes of Holobasidiomycetes, including the ‘higher fungi’ (mushrooms) and the Heterobasidiomycetes. The most common illness caused by mushrooms is mycetism (mushroom poisoning). Infections by basidiomycota are rare. Among the holobasidiomycetes, only infections by *Schizophyllum commune* have been observed. Among the heterobasidiomycetes, the genus *Filobasidiella* in the order Filobasidiales contains *F. neoformans*, the perfect state of the yeast *Cryptococcus neoformans*, which is a major cause of invasive infections in patients with defective T lymphocytes [9].

Other medically relevant yeasts with basidiomycetous affiliation are the anamorphic genera *Malassezia* (saprophytes of the skin that very occasionally cause systemic infections), *Rhodotorula* (perfect state *Rhodosporidium*, which has occasionally been involved in keratitis, dacryoadenitis, and endophthalmitis), *Trichosporon* (the causative agent of white piedra, an infection of the hair, or of invasive mycoses in compromised hosts), and *Sporobolomyces* (perfect state *Sporidiobolus*).
Many fungal species could not be accommodated in the past (or even now) in the genetic classification system outlined above, because they lack a recognizable sexual reproductive cycle and thus a ‘teleomorphic’ or ‘perfect’ state. These fungi are therefore considered to be ‘imperfect’ (fungi imperfecti) or ‘anamorphic’. Certain fungi may even show different asexual morphology (synanamorphs). For various imperfect fungi, a corresponding perfect state has subsequently been identified. Some of these were previously thought to be independent organisms, and accordingly had already been allocated different names. In recent years, as a perfect state for a previously known imperfect fungus was discovered, the epithet of the anamorphic name has been maintained in the nomenclature, e.g. *C. neoformans*, *F. neoformans*.

Most anamorphic fungi are essentially considered as defective ascomycetes, a view derived from a commonality of certain enzymic activities. These organisms may have lost constituents of the sexual cycle in the course of evolution and thus became imperfect. Molecular genetic analysis of the fungi imperfecti may eventually establish the true place of the anamorphic species in the natural system. For the present, they have arbitrarily assembled in the formal phylum Deuteromycota (fig. 1.2).

The Deuteromycota comprise the form classes Blastomycetes (yeast-like anamorphic fungi), Coelomycetes (producing particular containers for asexual spores), and Hyphomycetes (fungi that produce asexual conidia at random directly from their hyphae).

The blastomycetes comprise the form order Sporobolomycetales with members of little medical relevance, and the form order Cryptococcales, with many relevant species among the form genera *Candida*, *Cryptococcus*, and *Rhodotorula*. *C. albicans* is often harbored on mucosa in the healthy individual. In the compromised patient, *C. albicans* is the most important opportunistic fungus; it is also an important agent of endophthalmitis and keratitis [10].

The form class Coelomycetes comprises, in the form order Sphaeropsidales, several medically relevant genera, e.g. *Phoma* and *Hendersonula* (agents of phaeohyphomycosis) and *Pyrenochaeta*, an agent of mycetoma.

The form class Hyphomycetes comprises, in the form order Moniliiales, the medically relevant form families Moniliaceae and Dematiaceae. Among the Moniliaceae are the form genera *Acremonium*, *Aspergillus*, *Fusarium*, *Paecilomyces*, and *Penicillium*, many species of which are relevant for the ophthalmologist as agents of keratomycosis [8, 11, 12]. *A. fumigatus*, *A. flavus*, *P. marneffei*, and *F. solani* are also important agents of invasive mycosis [13, 14].
Fig. 1.2. Selected medically important relations in the form phylum Deuteromycota (fungi imperfecti). The mycological classification of the Hyphomycetes beyond the class level is preferably based on the type of conidiogenesis (thallic, holothallic, thallic-arthric, blastic, holoblastic, enteroblastic). The histological classification of the hyphomycoses is based on the presence or absence of melanin pigmentation of fungal elements (phaeohyphomycosis, hyalohyphomycosis).

The form family Dematiaceae (with dark elements due to generation of melanin) also contains medically relevant species among the form genera Bipolaris, Cladosporium, Curvularia, Exophiala, Phialophora, and Cladosporium (Cladophialaphora) [15].

The histological classification of the corresponding hyphomycoses is based on the detection of pigmented hyphae in the host tissue; these are present in phaeohyphomycosis and absent in hyalohyphomycosis. The presence of septate hyaline hyphae is often interpreted as aspergillosis, though this is not necessarily correct. Branched hyaline hyphae may also represent other fungi, e.g. Scedosporium spp. or Fusarium spp. It is therefore prudent to describe the findings as hyalohyphomycosis, and to attempt differentiation and antifungal testing of the organism, which may resist amphotericin. For the exact classification of the Hyphomycetes, recognition of the type of conidiogenesis (thallic, blastic) has been recommended [2].
It must also be remembered that actinomycetes (*Actinomyces* spp. and *Nocardia* spp.) may be mistaken for fungi, though they are in fact filamentous gram-positive bacteria.

1.6  
**Indigenous Fungi Causing Invasive Mycoses**

The incidence of severe fungal infections (mycoses) has been steadily increasing in the last decade, not least as a result of modern intensive care measures. In the former Federal Republic of Germany alone, data from a German university hospital suggested 36,000 cases of invasive mycoses in 1986, and invasive fungal infections were contributory factors in about 7,000 deaths [Müller, pers. commun.]. Invasive fungal infections may involve the eyes, especially if the infection colonizes multiple organs (systemic or disseminated fungal infections).

The typical causative agents of invasive mycoses in temperate northern latitudes are yeasts of the genus *Candida*, *C. neoformans* and certain moulds. The medical and microbiological aspects of these fungi are briefly presented below, together with some rare fungal agents that should be taken into account in a differential diagnosis. The microbiological aspects are presented by de Hoog and Guarro [3], by Campbell et al. [16], and (in German) by Seeliger and Heymer [17]. The clinical and pathological aspects of invasive mycoses were comprehensively presented by Kwon-Chung and Bennett [2], by Richardson and Warnock [18], and (in German) by Gemeinhardt et al. [19].

1.6.1  
**Candida albicans**

In temperate regions, *C. albicans* is still by far the most important causative agent of invasive mycoses [20, 21], though other species, e.g. *C. glabrata* and *C. krusei* are isolated increasingly often from clinical specimens.  

*C. albicans* is the only fungus considered part of the microflora of human mucous membranes [22]. Other fungi are at most capable of temporary colonization of healthy subjects. The intestinal colonization rate of *C. albicans* in healthy human subjects is estimated as about 50%. In common with all other indigenous agents of invasive mycoses, *C. albicans* is an opportunistic pathogen that becomes clinically relevant only if the host’s immune defence mechanism has been weakened.

Due to its persistence on the mucous membranes of the pharynx, in the lower intestinal tract and in the vagina, *C. albicans* is the typical agent of
mucosal candidiasis (thrush). Dissemination from candidal lesions deep in the tissues then gives rise to disseminated candidiases, when the number of granulocytes in the blood may fall below 500/μl. Such severe forms of granulocytopenia are most likely to occur in patients with cancer after antineoplastic chemotherapy. Disseminated candidiasis in these patients resembles the clinical picture of bacterial septicaemia. In a small proportion, eye involvement may result from an episode of candidemia [10]. *Candida* endophthalmitis may occur in intravenous drug abusers and in patients with candidiasis after abdominal surgery [23]. In contrast, in AIDS patients, who have selective cellular immunodeficiency, candidal infections are almost always restricted to the mucosa and submucosa.

The commensalism of *C. albicans* reduces the diagnostic value of demonstrating fungi on mucous membranes; however, quantitative culturing methods may indicate candidiasis. *C. albicans* can be easily cultured under aerobic conditions in many bacteriological nutrient media, as well as on malt agar or Sabouraud glucose agar. Cream-colored colonies become visible after 24 h at 37°C and at the end of 3 days may reach diameters up to 3 mm.

Even at low magnification, *C. albicans* can be identified as a yeast-like fungus by its large gram-positive yeast cells (synonyms: blastoconidia, blastospores) (fig. 1.3). *C. albicans* is a dimorphic yeast and forms filiform cell aggregates, described as pseudomycelia, which represent chains of yeast cells
that have failed to detach from each other. The formation of a pseudomycelium is an indication of poor growing conditions. In infected tissue, a pseudomycelium is often present in the center of the Candida colony, while blastoconidia predominate at the periphery (fig. 1.4).

Under poor growing conditions in vitro, *C. albicans* forms chlamydospores (fig. 1.3), which can be used as an identifying mark. Chlamydospores do not appear to have any pathogenetic importance, as they have seldom been identified in infected tissue.

Tubules known as germ tubes are another identifying mark. Germ tubes will grow in a few hours incubated at 37 °C in plasma. This process does not represent the usual budding of yeast that is associated with complete septum formation; at the point of emergence from the yeast cell, the germ tube lacks the constriction typical of the budding process (fig. 1.5). Germ tubes also form in infected tissue and are considered indicative of the presence of a *C. albicans* virulence factor, as invasion of epithelium takes place chiefly via germ tubes (fig. 1.5).

Like most pathogenic fungi, *C. albicans* is sensitive to polyene antifungal antibiotics (amphotericin B, nystatin and natamycin). By far the most common serotype of *C. albicans*, serotype A, is usually sensitive to flucytosine, but commonly develops secondary resistance after prolonged treatment. The rare serotype B of *C. albicans* usually exhibits primary resistance to flucytosine.
Patients with AIDS are likely to have a higher proportion of serotype B isolates [24].

*C. albicans* used to be fully sensitive to triazole antifungal agents (fluconazole, itraconazole), but resistance to fluconazole is increasingly found. This may indicate cross-resistance with other triazoles and necessitates sensitivity testing of isolates in cases of doubt. Fluconazole is valuable in ophthalmology, by virtue of its low protein binding and good penetration of tissues and body fluids [25].

### 1.6.2 Candida tropicalis

*C. tropicalis* is second in medical importance of the *Candida* yeasts [21]. In patients with granulocytopenia, *C. tropicalis* may be a more common cause of invasive infections than *C. albicans* [26].

*C. tropicalis* is an especially undemanding saprophyte with a worldwide distribution in the environment. It is not part of the normal human microflora, and its presence in clinical specimens is invariably an indication of a local or generalized weakness of the immune defences. Once *C. tropicalis* becomes established on mucous membranes, it appears to be more difficult than *C. al-
bicans to eliminate. The invasiveness of *C. tropicalis* appears to exceed that of *C. albicans* [27].

*C. tropicalis* is macroscopically and microscopically indistinguishable from *C. albicans*, but only exceptionally forms chlamydospores and germ tubes. Its precise identification is based on metabolic parameters.

*C. tropicalis* is sensitive to polyene antifungal antibiotics, though primary resistance to flucytosine is commonly found. Triazole antifungal agents are effective.

1.6.3 Candida parapsilosis

*C. parapsilosis* probably ranks third in medical importance of the *Candida* yeasts. Invasive infections caused by *C. parapsilosis* are rare and are usually due to contamination of infusion solutions. *C. parapsilosis* accounts for 25% of cases of candidal endocarditis, however, which is disproportionately high. Many of those affected are drug abusers. *C. parapsilosis* also occurs as a contaminant of ophthalmological irrigation fluids.

*C. parapsilosis* has been isolated from meat and other foodstuffs as well as from the environment. This yeast is often found on the skin of healthy subjects, and consequently is a relatively common cause of fungal infections of the nails [28].

*C. parapsilosis* is macroscopically and microscopically indistinguishable from *C. albicans* or *C. tropicalis*. It forms neither chlamydospores nor germ tubes. It is identified on the basis of its metabolic capabilities.

*C. parapsilosis* is usually sensitive to polyene antibiotics, to flucytosine and usually also to triazole antifungal agents.

1.6.4 Candida glabrata (Torulopsis glabrata) and *C. krusei*

*C. glabrata* is a yeast found in the environment, which occurs relatively commonly as a transient member of the microflora in the urogenital tract [21]. *C. glabrata* is a typical opportunistic pathogen of low virulence. Invasive infections caused by *C. glabrata* are rare and should always suggest dissemination from an infected catheter.

*C. glabrata* forms exceptionally small blastoconidia and in many respects resembles *C. albicans*. It never forms mycelia, however, and for this reason was originally classified in the genus *Torulopsis*. The accurate identification of

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1 An Overview of Fungal Pathogens of Ophthalmological Importance
C. glabrata requires investigation of metabolic capabilities. The fungus has been isolated from clinical specimens increasingly often during the recent years. It tends to acquire resistance to fluconazole.

C. krusei is a waste decomposer from the environment which may cause invasive mycoses in humans. Being primarily resistant to the triazoles, it tends to be selected in patients under prolonged fluconazole therapy, such as AIDS patients. Although it forms suggestive elongated yeast cells, its proper identification relies on metabolic capabilities.

1.6.5

Rare Agents of Invasive Yeast Infections

Other yeasts may cause invasive mycoses to the point of septicemia in patients with severe and prolonged immune deficiency, with defects of gut mucosa, and with extensive burn wounds. These microorganisms include C. lusitaniae, Trichosporon spp. or even baker’s yeast (S. cerevisiae) [29]. A potential blood-mediated colonization of the eyes must be borne in mind in all such cases.

1.6.6

Mixed Infections

Mixed infections involving several yeasts are apparently very rare. Dual infections with Candida sp. and Aspergillus sp., on the other hand, are continually reported. Mixed infections with C. albicans and Staphylococcus aureus or enterococci are similarly not uncommon [30].

1.6.7

Cryptococcus neoformans

C. neoformans is an opportunistic yeast, which in the pre-AIDS era was very occasionally isolated as the causative agent of life-threatening meningoencephalitis. The course of cryptococcosis with central nervous system (CNS) involvement is insidious and appears to have often been confused with tuberculosis. Classic cryptococcosis is only indirectly associated with eye involvement (diplopia, papillary oedema). Cryptococcosis is more common in patients with AIDS, in whom it is not confined to the CNS but is likely to become disseminated. In Germany, about 5% of patients with AIDS develop crypto-
cocciosis, which in immunocompromised subjects tends to recur despite adequate treatment [Staib, per. commun.].

The causative agent, *C. neoformans*, is distributed worldwide, but in temperate northern latitudes is found in the faeces of birds, e.g. pigeons and parrots. The fungus is inhaled with dust particles and induces a transient bronchopulmonary syndrome. This is followed in immunocompromised patients by dissemination via the blood vessels.

*C. neoformans* obtained from body fluids, especially from cerebrospinal fluid, can be visualized by simple microscopy in an India ink smear preparation, due to its thick capsule (fig. 1.6). An antigen test (latex slide agglutination test) sensitively and reliably demonstrates the polysaccharides of the capsule in all body fluids, and titres of 1:1,000 are often exceeded. Strains of *C. neoformans* with poor capsule formation have been described, however, so even low antigen titres must be regarded with suspicion.

Staib’s medium (*Guizotia abyssinica* creatinine seed agar) is a selective culture medium that permits the identification of individual *Cryptococcus* colonies, which become brown due to phenol oxidase activity. *C. neoformans* never develops mycelial forms, is usually spherical, and can be distinguished from the nonpathogenic species of the genus by growth at 37 °C. *C. neoformans* differs from *Candida* spp. in possessing marked urease activity.
Cryptococcosis is treated with a combination of amphotericin and flucytosine. Triazole antifungal agents are also used, particularly as follow-up treatment and to prevent recurrences.

1.6.8
Aspergillus fumigatus

*A. fumigatus* is the most important causative agent of fungal infections. Other species of the same genus (*A. niger, A. flavus, and A. nidulans*) are found much more rarely [31]. Among the author’s patients, cases of aspergillosis have occurred more frequently in the 1990s. They represent the largest group of invasive mycoses in the author’s hospital at present.

Aspergillus spp. are opportunistic causative organisms of pulmonary mycoses. The development of aspergillosis is favoured by, for example, a history of pulmonary tuberculosis, long-term treatment with cortisone, or alcoholism, but hardly ever by AIDS. *Aspergillus* spp. may colonize cavities to form macroscopic aggregates or aspergillomas (fungus ball).

Blood-borne dissemination may occur in highly immunocompromised patients, and as many as 50% of cases of invasive mycosis in neutropenic patients may be due to *Aspergillus* spp. Metastases of pulmonary aspergillosis most often affect the CNS. Aspergillosis should thus always be borne in mind when high-risk patients present with encephalitis. *Aspergillus* spp., like other moulds, can penetrate arteries, which can lead to massive hemorrhage or embolism. Hematogenic eye involvement is very rare. Invasive aspergilloses from infected nasal sinuses may cause indirect eye involvement with signs of congestion [31].

Aspergillus spp. are easy to culture. They grow under aerobic conditions at up to 50 °C on almost any solid culture medium. They form true septate mycelia, with radially arranged hyphae that fork at acute angles (fig. 1.7). In culture they form aerial mycelia (`mould`), the ends of which bear conidiophores with chains of conidia (spores), which have given rise to the fungus’ popular name of `sprinkler mould`. Conidiophores are only rarely found in the lungs, and when they are this indicates involvement of aerated areas of that organ (fig. 1.8).

The highly resistant conidia of this ubiquitous fungus are air-borne and inhaled, and thus represent the infective agent. Increases in dust have been associated with a higher incidence of infections. This means that finding *Aspergillus* spp. in human specimens may be due to contamination. The *Aspergillus* isolate should nevertheless be investigated (repetition of the attempt at culturing, serodiagnosis). An exception is colonization of the
Fig. 1.7. Pulmonary aspergillosis in immunosuppression. Grocott methenamine-silver staining. Photograph by Dr. M. Bergholz, Göttingen. Bar = 5 μm.

Fig. 1.8. Pulmonary aspergillosis in chronic alcoholism. The typical conidiophores (arrow) show that the infection extends to aerated parts of the organ. Grocott methenamine-silver staining. Photograph by E. Bothmann, Göttingen. Bar = 15 μm.
external auditory meatus by \textit{A. niger}, which may be suitable for topical treatment.

\textit{Aspergillus} spp. are sensitive to amphotericin. Combination therapy with fluconazole serves mainly to reduce the nephrotoxicity of amphotericin. Of the new triazoles, itraconazole is active against \textit{Aspergillus} spp. [32], though in the author’s experience, fluconazole may fail to induce a response and is thus to be avoided.

1.6.9

\textit{Mucormycosis}

Mucormycoses (synonyms: zygomycosis, phycomycosis) are rare but often fatal infections. The ubiquitous family Mucoraceae includes pathogenic species of the genera \textit{Mucor}, \textit{Absidia}, \textit{Rhizopus} and \textit{Rhizomucor}. The spores of Mucoraceae, unlike those of \textit{Aspergillus} spp., are enveloped by a membrane in a sporangium. The intact sporangia give the fungi their typical microscopic appearance (fig. 1.9). On maturation, the membrane tears and the spores float in the air and are inhaled. As in the case of aspergillosis, the opportunistic Mucoraceae may colonize the paranasal sinuses and already damaged lungs.
Fig. 1.10. Section of human lung with the large, irregularly arranged hyphae of a zygomycete after staining with an optical brightener and fluorescence microscopy. Ca. × 400.

The one common form of mucormycosis, the invasive pulmonary type, primarily affects patients with granulocytopenia and immunosuppressed patients after deferoxamine therapy [33]. Such cases have a very poor prognosis, as Mucoraceae are particularly likely to invade major arteries, resulting in infarct pneumonia or massive hemorrhages, the cause of which is generally recognized only postmortem. An embolism of unknown origin should always suggest the possibility of mucormycosis. Hematogenic involvement of the eyes, however, is unlikely. In generally immunocompromised patients, mucormycosis may emanate from the gut.

The other typical condition is rhinocerebral mucormycosis of diabetic patients, in which the condition may spread from an infected paranasal sinus to the base of the skull. Phlegmon of the orbit may arise from rhinocerebral mucormycosis, which is most likely to originate from colonization of a paranasal sinus. The ensuing blindness is due to fungal obliteration of the central artery supplying the optic nerve. This condition is not confined to diabetic patients with ketoacidosis, but may also be induced by deferoxamine.

Mucoraceae may be cultured on simple media at 37 °C under aerobic conditions, but culturing from biopsies is often unsuccessful. The mycelia of Mucoraceae are often only faintly visualized with the routine stains; they are exceptionally large, usually nonseptate, and irregularly arranged (fig. 1.10). Rectangular branching of the hyphae predominates, and typical sporangia
can be detected only if aerated surfaces are involved. Fluorescent staining with optical brighteners, e.g. calcofluor white, is particularly suitable for the rapid detection of typical zygomycetous mycelia in tissue.

Polyene antibiotics (amphotericin, nystatin) are the only antifungal agents that are clinically effective against Mucoraceae.

1.6.10

Fusariomycosis

_Fusarium_ spp., especially _F. solani_, also cause invasive mycoses that occasionally involve the eyes. These moulds are plant pathogens that form typical sickle-shaped conidia on their aerial mycelia. _Fusarium_ spp. may become dangerous to man, particularly in patients with bone marrow aplasia. Some isolates have been found with resistance to azole as well as polyene antifungal agents, and to flucytosine. The mortality of fusariomycosis is high [14]. In ophthalmology, _Fusarium_ spp. are important agents of keratitis, e.g. among wearers of contact lenses.

1.6.11

_Scedosporium_ spp.

_Scedosporium_ spp. are agents of keratomycosis. Among the so-called ‘emerging fungal pathogens’, _S. apiospermum_ (the perfect form being _P. boydii_) and _S. prolificans_ (perfect form unknown) have been noted as agents of systemic infections in patients with leucopenia. _S. apiospermum_ is also a causative agent of white grain mycetoma, and it was recently identified as the cause of cerebral abscesses in patients who had experienced near-drowning [34].

Isolates of either fungus may resist all antifungal agents at present available.

1.6.12

_Histoplasmosis_

Finally, mention must be made of histoplasmosis [2], the causative agent of which, _H. capsulatum_, is endemic in the region of the Mississippi and central Africa, but has been occasionally carried to Europe. _H. capsulatum_ is one of the fungi described as dimorphic. It occurs in the environment in its mycelial form, is taken up via conidia into the lungs, and there becomes parasitic as a yeast. Disseminated forms with endophthalmitis have been described,
particularly in patients with AIDS. A relevant history of travelling calls for serodiagnosis and a cutaneous histoplasmin test.

Other systemic mycoses of the Americas are coccidioidomycosis (causative agent, *C. immitis*), blastomycosis (causative agent, *B. dermatitidis*) and paracoccidioidomycosis (causative agent, *P. brasiliensis*).

1.7 Causative Agents of Superficial Mycoses

In addition to the fungi described above that cause invasive mycoses, those that have been isolated in local (usually superficial) infections of the eyes are also of interest to ophthalmologists. Such fungi generally originate from the environment; they are only temporarily able to colonize the eye and become pathogenic only if they penetrate the tissues via an injury.

According to Sundaram et al. [11], most of the infective agents found in the outer eye are *Aspergillus* spp., followed by *Fusarium* spp. and *Penicillium* spp., and then *Curvularia* spp., *C. albicans*, *Mucor* spp., *Drechslera* spp., *Cladosporium* spp., *Pullularia* spp. and *Pseudallescheria* spp. The pathogenic species of *Aspergillus*, *Fusarium*, *Mucoraceae* and *Candida* have, as infective agents of invasive mycoses, been described above. Kwong-Chung and Bennett [2] have published detailed descriptions of the fungi listed below.

*Penicillium* is a genus of ubiquitous saprophytes; its name, derived from the Latin for paint brush, describes the typical form of its conidiophores. *Penicillium* resembles *Aspergillus*, but is hardly ever found as a causative agent of invasive infections. Only *P. marneffei*, which occurs in southeast Asia, must be considered a potential agent of invasive mycoses in AIDS patients. *P. rugulosum* has been isolated from eyes. Some *Penicillium* spp. are important sources of antibiotics.

*Scopulariopsis brevicaulis*, a mould related to *Penicillium*, attacks chiefly the toenails.

*Acremonium kiliense* (*Cephalosporium acremonium*) is a saprophytic mould, which has occasionally been identified as the cause of mycetoma. It may also cause keratitis, but has no significance as an agent of invasive mycoses.

*Alternaria* spp., *Curvularia* spp., and *Exophiala (Wangiella) dermatitidis* are examples of saprophytic moulds that produce dark, melaninized hyphae (and sometimes spores) in the infected tissue. Such fungi are therefore called dematiaceous fungi. The corresponding diseases are the phaeohyphomycoses (chromomycoses). Some of them are agents of mycetoma, others may cause CNS infection, but they have little relevance for the ophthalmologist.
Other members of the Dematiaceae are agents of chromoblastomycosis. These mycoses afflict the skin and subcutaneous tissue. They present with so-called sclerotic bodies, which are thick-walled pigmented round cells, which may divide by fission, but not by budding. Agents of chromoblastomycosis are *Cladosporium carrioni*, *Fonsecaea* spp., and *Phialophora verrucosa*.

The fungal genera *Exophiala* (synonym, *Wangiella*) and *Phialophora* may cause chromomycosis. Some of them have a particular affinity for the CNS.

Infections by nonpigmented hyphae are described collectively on histological grounds as hyalohyphomycoses, the foremost agents being *Aspergillus* spp., followed by *Fusarium* spp. *Acremonium* spp., which are important agents of keratitis, are also hyalohyphomycetes.

The dematiaceous fungi have been reviewed by Dixon and Polak-Wyss [35]. A dimorphous fungus, *Sporothrix schenckii*, is usually transmitted via splinters of wood. Its yeast form can provoke sporotrichosis, which is primarily a chronic subcutaneous infection affiliated to the lymphatic system causing ulcers. *Rhinosporidium seeberi* occasionally causes chronic infections in the region of the eye. The taxonomic classification of this fungus is uncertain. Culturing of the fungus so far succeed only on epithelial cell cultures.

The typical keratinophilic dermatophytes of the genera *Microsporum*, *Trichophyton* and *Epidermophyton* may also be transferred from infected skin to the eyes.

*Pityrosporum orbiculare* (synonym *Malassezia furfur*) and *P. ovale* (*M. pachydermatis*) are lipophilic yeast-like fungi of the skin which may cause pityriasis versicolor. They may also cause blepharoconjunctivitis.

Reports are being continually received, particularly from warm latitudes, of other fungi causing injury-mediated infections in the outer eye. These are fungi living in the environment, which in subjects in poor general health living in conditions of poor hygiene may become pathogenic. The same may be true of typical fungal laboratory contaminants of the genus *Paecilomyces* (related to *Penicillium*), which have been found in infections of the eye and in endocarditis.

1.8

**Miscellaneous**

1.8.1

*Actinomycetales*

Despite their name, Actinomycetales are not fungi but gram-positive bacteria. The misleading description is due to the microscopic appearance of these bacteria, which simulates the presence of fungal mycelia. Actinomycetales
have a typical bacterial (prokaryotic) structure and are sensitive to the typical antibacterial antibiotics and resistant to systemic antifungal agents.

Where the diagnosis has to be based on the microscopic findings alone, there is a considerable risk of confusing the bacteria with mycelium-forming fungi. Actinomycosis should be suspected, however, if individual structures of the mycelium can be detected in smears or biopsy material only at a magnification of $\times800$ or above. As a rule, true fungi form larger mycelia that can be clearly distinguished using a 40-power microscopic lens.

Two groups of Actinomycetales are distinguished: anaerobic Actinomycetales, which include the important genus of *Actinomyces*, and aerobic Actinomycetales (genus *Nocardia*), which are not an important cause of eye infections. Those of particular interest to the ophthalmologist are the actinomycetes that occasionally cause canaliculitis. This condition, in contrast with the classic, destructive actinomycoses, is generally a noninvasive, single-agent infection. The infective agent is commonly *Arachnia propionica* [36]. The infection tends to originate in the endogenous reservoir of the many actinomycetes that occur in the oral cavity.

Material for testing (concretions from the tear duct) should be transferred immediately to a suitable transport medium (e.g. Port-a-Cul transport agar, Becton-Dickinson). The specimen should be inoculated deep into the agar with the aid of the swab supplied in the pack, so as to avoid killing the microorganisms with oxygen from the air.

Anaerobic actinomycetes are generally sensitive to penicillin, whereas metronidazole, which is typically active against anaerobic organisms, is not always effective in this condition. On the other hand, canaliculitis does not usually require systemic antibacterial therapy.

1.8.2

*Collection of Specimens and Culturing of Fungi*

When collecting specimens for culturing, it should be borne in mind that the causative organism is most likely to be found at the margins of a lesion, while commonly only secondary microflora occur in the center. It is occasionally recommended to clean the surface cautiously before collecting a specimen. As the fungal origin has often not been established, however, and there is also the possibility of a mixed infection, the use of a cleanser, e.g. alcohol, may destroy pathogenic bacteria, resulting in false-negative findings.

Consideration should be given to the direct inoculation of culture media. Ready-prepared special agars for fungi are commercially available, the most generally suitable of which is Sabouraud glucose agar. In view of the possibility
of mixed infections, it is recommended that 2 blood or chocolate agars are 
inoculated at the same time; with aerobic/anaerobic incubation, this will permit 
the growth of most bacterial pathogens. The proliferation of fungi on such 
culture media is often suppressed by the quicker growing bacteria. Only yeasts 
and some Mucoraceae can match the rate of bacterial growth, so results 
can be expected after only 3 days. Many moulds need 1 week, and some 
dermatophytes may require almost 2 months for typical colonies to develop. 
Once a fungal agar has been inoculated, it should be incubated at about 36 °C. 
If a second fungal agar is available, the latter should be incubated at 26 °C; 
this also applies to bacteria.

The evaluation and further differentiation of cultures should always be un-
dertaken by a specialist, and specimens are generally sent to a specialist labora-
tory. This requires transfer of the specimen to a commercially available transport 
agar set. Part of the test material should be pressed deep into the agar in order 
to exclude air. Although pathogenic fungi are aerobic, mixed infections with 
aerobic microorganisms cannot be ruled out. All microorganisms are to a 
greater or lesser extent at risk from drying out, and the use of dry swabs for the 
transport should thus be regarded as no more than an emergency solution.

If sufficient test material is available, it can also be inoculated directly on 
to blood cultures (aerobic/anaerobic). Any remaining material can be used 
for smears on glass slides, which are then air-dried before dispatch. Suitable 
transport containers for slides are obtainable from specialist suppliers.

Lactophenol blue staining is commonly used for direct visualization of 
fungal elements. Ready-prepared lactophenol blue solution is commercially 
available. As a rule, fungal cells react with this stain within a few minutes at 
room temperature. Surplus staining solution does not need to be removed 
before microscopy. Gram staining is commonly used for the rapid detection 
of yeasts and most bacteria. Fluorescent staining with strongly basic solutions 
of optical brighteners is recommended for the simultaneous maceration of 
tissue and demonstration of any fungus therein [37]. In the case of solid material 
for testing, an aliquot should also be sent to a histopathology laboratory, where 
sections can be used for specific fungal staining (periodic acid-Schiff, Gomori 
methenamine) and immunofluorescence staining.

References

3 de Hoog, G.S. and J. Guarro, Atlas of clinical fungi. 1995: Baarn, Centraalbureau voor Schimmel-
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**Glossary**

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anamorph</td>
<td>Asexual form of fungal fructification, imperfect state (see teleomorph)</td>
</tr>
<tr>
<td>Arthroconidium (arthrospore)</td>
<td>Asexual propagule formed by fragmentation of hyphae</td>
</tr>
<tr>
<td>Blastococonidium (blastospore)</td>
<td>Asexual propagule formed by budding</td>
</tr>
<tr>
<td>Budding</td>
<td>Asexual form of propagation, daughter cell is blown out from a parental cell.</td>
</tr>
<tr>
<td>Candidosis</td>
<td>Synonym for candidiasis; infection due to Candida spp.</td>
</tr>
<tr>
<td>Chlamydospore</td>
<td>Thick-walled asexual spore that develops intercalarly or terminally. Among the Candida spp., only <em>C. albicans</em> produces chlamydospores</td>
</tr>
<tr>
<td>Columella</td>
<td>Inflated end of a hypha within the sporangium of certain zygomycetes</td>
</tr>
<tr>
<td>Conidium</td>
<td>Asexual propagule formed exogenously by the conidiophore</td>
</tr>
<tr>
<td>Conidiophore</td>
<td>Specialized hypha that produces conidia, e.g. in the genus <em>Aspergillus</em></td>
</tr>
<tr>
<td>Dematiaceous fungi</td>
<td>Fungi that produce dark pigmented (melaninized) elements</td>
</tr>
<tr>
<td>Dermatophyte</td>
<td>Fungus that grows exclusively on keratinized tissues (skin, hair, nail), e.g. <em>Trichophyton</em> spp.</td>
</tr>
<tr>
<td>Dichotomous branching</td>
<td>Branching of hyphae with forking in pairs</td>
</tr>
<tr>
<td>Dimorphic fungi</td>
<td>Fungi that appear in 2 forms: as a yeast at 37 °C and as a mould at 30 °C or below, e.g. <em>H. capsulatum</em></td>
</tr>
<tr>
<td>Endospore</td>
<td>Spore formed within a specialized cell (sporangium)</td>
</tr>
<tr>
<td>Filamentous growth</td>
<td>Production of true or pseudomycelia</td>
</tr>
<tr>
<td>Germ tube</td>
<td>Short true hypha emanating from a spore or a yeast cell, typical element of <em>C. albicans</em></td>
</tr>
<tr>
<td>Hypha</td>
<td>Vegetative filament of a fungal mycelium; <em>true hyphae</em> are produced by many filamentous fungi, <em>pseudohyphae</em> are chains of yeast cells</td>
</tr>
<tr>
<td>Hyalohyphomycosis</td>
<td>Infection by a fungus forming nonpigmented mycelia in the tissue</td>
</tr>
<tr>
<td>Imperfect state</td>
<td>Asexual form of a fungus (synonym, <em>anamorph</em>)</td>
</tr>
<tr>
<td>Macroconidium</td>
<td>Large asexual propagule, distinctive in the dermatophytes</td>
</tr>
<tr>
<td>Microconidium</td>
<td>Small asexual propagules, barely distinctive</td>
</tr>
<tr>
<td>Term</td>
<td>Description</td>
</tr>
<tr>
<td>-----------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Mould</td>
<td>Fungal saprophyte that produces abundant aerial mycelia and/or spores, e.g. aspergilli</td>
</tr>
<tr>
<td>Mycelium</td>
<td>Network of hyphae which, in most instances, constitutes the thallus of a fungus; aerial mycelia are typically formed by the moulds, submersed mycelia (nutritional mycelia) grow in the media</td>
</tr>
<tr>
<td>Mycetoma</td>
<td>Chronic granulomatous infection by fungi (eumycetoma) or by certain bacteria</td>
</tr>
<tr>
<td>Mycotoxin</td>
<td>Toxin formed by a fungus in food products, e.g. aflatoxins of <em>A. flavus</em></td>
</tr>
<tr>
<td>Phaeohyphomycosis</td>
<td>Infection by pigmented (melaninized) hyphae</td>
</tr>
<tr>
<td>Phialides</td>
<td>Specialized cells that produce conidia, e.g. in the aspergilli</td>
</tr>
<tr>
<td>Perfect state</td>
<td>Sexually reproducing form of a fungus (synonym, teleomorph)</td>
</tr>
<tr>
<td>Rhizoid</td>
<td>Short, root-like array of hyphae in the genus <em>Rhizopus</em></td>
</tr>
<tr>
<td>Ringworm</td>
<td>Mycosis of the skin (see <em>tinea</em>)</td>
</tr>
<tr>
<td>Sporangium</td>
<td>Container for asexual <em>sporangiospores</em>: closed off by a rigid membrane, e.g. in certain <em>zygyomycetes</em></td>
</tr>
<tr>
<td>Spore</td>
<td>Asexual propagule, exogenously formed by a conidiophore, or endogenously by a sporangium</td>
</tr>
<tr>
<td>Teleomorph</td>
<td>Sexual form of fungal fructification (synonym, perfect state; see anamorph)</td>
</tr>
<tr>
<td>Thallus</td>
<td>The vegetative body of a fungus (usually formed by the mycelia)</td>
</tr>
<tr>
<td>Thrush</td>
<td>Mucosal candidiasis, which gives rise to creamy pseudomembranes</td>
</tr>
<tr>
<td>Tinea</td>
<td>Mycosis of epidermis (ringworm), nails or hair</td>
</tr>
<tr>
<td>Vesicle</td>
<td>Inflated end of a hypha within a conidiophore of aspergilli</td>
</tr>
<tr>
<td>Yeast</td>
<td>Strictly meaning baker’s yeast (<em>S. cerevisiae</em>). A general term for unicellular fungi that reproduce by budding or fission. Related fungi are referred to as yeast-like</td>
</tr>
</tbody>
</table>
Chapter 2

Antifungal Agents

The antifungal agents used in ophthalmology can be divided into several groups on the basis of their chemical structure: (1) polyenes; (2) flucytosine, and (3) azoles. In addition, several antiseptic agents are used.

The ocular findings from animal studies are mainly presented in chapter 7 (see pp. 162–196).

2.1 Polyenes

Because of their toxicity, the polyene antibiotics natamycin (pimaricin) and nystatin are only used topically. They are not absorbed from the gut, however, and can thus be given orally in the treatment of fungal infections of the gastrointestinal mucosa. They are used as aerosols in the treatment of mucosal mycoses of the respiratory tract. In severe cases, amphotericin B may also be given by intravenous or intravitreous injection.

The polyene antibiotics are capable of penetrating ergosterol, a constituent of the plasma membranes of fungi, in such a way that hydrophilic pores develop [1]. This formation of ‘leaks’ explains the efficacy of these agents against both resting and growing fungi [2–6].

2.1.1 Amphotericin B

Amphotericin B was developed as early as the 1950s from Streptomyces nodosus [3, 7] and still represents the antifungal agent of first choice [8, 9] and the standard against which new agents are measured [10, 11].

Systemic use of amphotericin B is associated with the major side effect of nephrotoxicity, with reduced glomerular filtration, hypokalemia and acidosis [3, 8, 12, 13]. Renal damage can be alleviated or even prevented by saline infusions [14] or parenteral administration of flucytosine [15]. Combination
with flucytosine allows the recommended dose (1 mg/kg body weight) to be reduced by 50%.

Amphotericin B may only be administered in 5% glucose solution, as the drug precipitates in physiological saline. A liposomal formulation has also been developed, and this is better tolerated and has more favorable pharmacokinetics than the original formulation [16–21]. Tolerability may also be improved by administration of amphotericin B in a fat emulsion [22–24]. The dose is 1 mg/kg body weight administered as a mixture of 2 mg of amphotericin B per 1 ml fat emulsion (Intralipid). However, in a randomized clinical study any reduction of side effects could not be demonstrated [25], this formulation has not been registred and pulmonary embolism may develop due to enlarged lipid particles [26]. Amphotericin B is well tolerated in colloidal dispersion in sodium cholesteryl sulphate without the risk of renal toxicity [27]. At present, however, it is not yet clear whether these new formulations of amphotericin B are equivalent to or better than the earlier simple amphotericin B preparations.

After systemic administration, amphotericin B penetrates the aqueous humor and to a certain extent the vitreous humor [28], where the concentration may exceed the minimum inhibitory concentration (MIC) for the pathogen [29, 30]. Systemic administration is usually advisable in endogenous endophthalmitis involving fungemia, in order to include any extraocular foci. In exogenous endophthalmitis, on the other hand, direct injection of amphotericin into the anterior chamber or the vitreous body is initially preferable to parenteral administration, because it achieves higher concentrations and avoids systemic side effects. As a rule, a dose of 5–10 μg (maximum) is injected into the center of the vitreous cavity following pars plana vitrectomy [31–43], after which the electroretinogram may be temporarily reduced [42, 44]. The injection may be repeated, but candidal endophthalmitis has been successfully treated with a single dose of 5 μg without vitrectomy [45]. Measurements of drug clearance in rabbits showed that the half-lives in unmodified phakic eyes, Candida-infected eyes, aphakic eyes and aphakic vitrectomized eyes to be 9.1, 8.6, 4.7 and 1.4 days after a single intravitreous injection of 10 μg [44].

Instructions for the preparation of amphotericin solution for intraocular injection are given in table 2.1. Repeated injections (7.5 μg once daily) into the anterior chamber are well tolerated [46, 47]. A subconjunctival injection is possible [47, 48], but may result in the formation of nodules and yellowish discoloration [49].

Fungal infections of the cornea are treated with eye-drops (5 mg/ml) [50–65]. Twelve patients, 4 with Fusarium spp., responded successfully to amphotericin B at a concentration of only 0.15% [66]. Amphotericin has also been used in the form of an eye ointment [67, 68], but this may cause a burning sensation. Instructions for preparation of eye-drops and eye ointment are
Table 2.1. Preparation of amphotericin B for intraocular injection (75 μg/ml)

- Add 10 ml of water for injection to a bottle containing 50 mg of amphotericin B dry substance (Bristol-Myers Squibb); shake the bottle until the solution is clear (50 mg of amphotericin B)
- Take up 1 ml of the solution in a 10 ml syringe (5 mg of amphotericin B)
- Make up to 10 ml with water for injection, mix well, then discard the contents apart from 1 ml (0.5 mg of amphotericin B)
- Dilute this amount (500 μg) with water for injection to make 6.7 ml and shake well (74.62 μg of amphotericin B)
- Aspirate 0.1 ml of this mixture into an insulin syringe (7.5 μg of amphotericin B)

Table 2.2. Preparation of amphotericin B drops

0.5% (5 mg/ml) drops
- Add 10 ml water for injection to a bottle of 50 mg of amphotericin B dry substance (Bristol-Myers Squibb); shake the bottle until the solution is clear
- Remove some of the solution with a syringe and instill directly into the conjunctival sac
- The solution may be kept in a refrigerator for 1 week when using a sterile workbench
- If the mixture is to be transferred to eye-drop bottles, filter through a 0.2 μm pore-mesh as it is a colloidal suspension

0.15% drops
- Take 3 ml of the 0.5% solution and make up to 10 ml with water for injection
- Shake well

Table 2.3. Preparation of amphotericin B 0.5% ointment (5 mg/g)

- Add 3 ml water for injection to a bottle containing 50 mg of amphotericin B dry substance (Bristol-Myers Squibb); shake the bottle until the solution is clear
- Blend amphotericin B solution 1.5 ml, Eucerin anhydr. (sterile) 1.0 g, Bepanthen Augensalbe® (eye ointment) to 5.0 g using a sterile workbench
- The ointment may be in a refrigerator for 1 week

given in tables 2.2 and 2.3. With topical use, it must be remembered that amphotericin B does not penetrate the corneal epithelium, thus necessitating corneal abrasion (see chapter 7, p. 172).

The spectrum of activity of amphotericin B covers most fungi, including *C. albicans*, most species of *Aspergillus*, *H. capsulatum*, *Cryptococcus* spp. and *Blastomyces* spp. [3, 9]. Some isolates of *C. albicans*, however, have been found to be resistant in vitro (by MIC values) and in vivo [69]. Variable sensitivity has been shown by species of *Curvularia*, *Alternaria*, *Wangiella* and *Clado-
Sporium [9]. *P. boydii* is often resistant. *S. brevicaulis* proved susceptible to a combination of topical amphotericin B and chloramphenicol [70, 71]. Mixed results were reported for *P. lilacinus* [72, 73]. Despite sensitivity of the organisms, progressive inflammation developed in invasive *A. fumigatus* keratitis [74] and in *F. solani* keratitis [75], presumably due to insufficient penetration.

2.1.2

**Natamycin (Pimaricin)**

This substance was isolated in 1955 from *Streptomyces* spp. that were found in the vicinity of Pietermaritzburg, Natal, South Africa. The name pimaricin is derived from that of the town, and the names natamycin and *S. natalensis* from that of the province from which the species was obtained [76]. The antibiotic is available as a suspension in concentrations of 1–5% and is well tolerated. In assessing the reports in the literature, it has to be borne in mind that in the USA and the UK, the 5% solution is used almost exclusively. In central Europe, on the other hand, natamycin is so far available for ophthalmological use only in the form of a 1% eye ointment.

Natamycin was very effective in vitro against *C. albicans*, *A. fumigatus* and *F. solani* [77, 78]. Its activity against *Fusarium* spp., in particular, was clearly greater than that of theazole derivatives [79]. Natamycin 5% is being used clinically with good results in keratomycoses, especially if these are still superficial [40, 58, 62, 80–89]. In keratitis caused by *Fusarium* spp. or *Aspergillus* spp., natamycin is superior to amphotericin B, while amphotericin is more effective in candidal keratomycosis [81, 84, 90].

Natamycin 2.5% in combination with miconazole has been successfully used in corneal infections caused by *Paecilomyces* spp. [91], though the fungus was said to be sensitive only to miconazole [92]. Natamycin 1% has also been prescribed [93, 94] sometimes in combination with nystatin [56, 95–97]. There are no reports of unequivocal effects, as in the case of the 5% concentration.

Experimental studies of natamycin are described in chapter 7 (see p. 173 and 179).

2.1.3

**Nystatin**

Nystatin has been applied topically to the eye in a concentration of 100,000 IU [98]. A suspension is prepared from the pure substance with sterile, isotonic phosphate buffer solution (table 2.4). Nystatin is well tolerated [99].
Table 2.4. Preparation of nystatin drops (100,000 IU/ml)

- To prepare the buffer solution, mix 20 ml of sodium dihydrogen phosphate solution (8.0 g NaH₂PO₄/1,000 ml H₂O) with 80 ml disodium hydrogen phosphate solution (9.47 g Na₂HPO₄/1,000 ml H₂O) and, after addition of 0.44 g sodium chloride sterilize by autoclave.
- Shake the contents of a bottle of pure substance (Candio-Hermal®, Hermal) with 5 ml of the isotonic, isohydric phosphate buffer solution described.
- This preparation is a suspension and should be shaken before use.
- The pure substance can also be used to prepare an ointment (100,000 IU nystatin/g ointment).

Nystatin has been prescribed for candidal keratomycoses [51, 62, 100–103] as well as for those caused by *Aspergillus* spp. [52, 104–107]. In one study, nystatin (drops and 5000 IU subconjunctivally) was used in keratomycoses that had been confirmed by culturing but were not described in detail. It proved successful in 53% of 30 cases, compared with 75% of 20 patients treated with amphotericin [99]. In another group in this study, 34% of 35 patients were cured with 2% miconazole.

Nystatin is not a first-line drug, as other agents are more effective; however, it may serve as an alternative in some cases.

2.2

**Flucytosine**

Flucytosine (5-fluorocytosine, 5-FC) is derived from a fluorinated pyrimidine derivative, which is converted by fungi into 5-fluorouracil [57, 108]. Its toxic effects are due to its antimetabolite activity. Thrombocytopenia is a known adverse effect of flucytosine. A dose of 160 mg/kg body weight is recommended for systemic administration. Flucytosine is effective against *C. albicans* [77, 109] and *Cryptococcus* spp. [5]. The development of resistance cannot, however, be ruled out [110, 111].

Oral administration of flucytosine, alone or in combination with amphotericin, has often been used or recommended in fungemia with or without fungal endophthalmitis [6, 8, 34, 40, 42, 75, 112–128]. A 1.5% concentration of flucytosine may be applied topically to the eye [55, 129]. Oral doses of flucytosine, as well as topical applications as 1% drops, have been successfully used in candidal keratitis [57, 108, 130]. The treatment should be continued for a period of 12–32 weeks [57]. A keratomycosis caused by *A. alternata*
Table 2.5. Use of flucytosine 1% drops

- A dose of the infusion solution (Ancothil®, Roche) (2.5 g/250 ml) may be aspirated directly into a sterile syringe and instilled into the conjunctival sac
- The solution must be stored at 15–23 °C to avoid conversion to 5-fluorouracil (not visible)

Table 2.6. Preparation of clotrimazole 1% oily drops

- Dissolve the drug (US Ph. 21, obtainable from various suppliers) in sterile oil in a waterbath under aseptic conditions in the proportions clotrimazole, 0.1 g, and castor oil, 10.0 g
- Filter the solution through a 0.2-μm filter into sterile eye-drop bottles

cleared up completely in response to oral flucytosine plus topical application of natamycin and thimerosal [131]. Topical flucytosine has also been used in fungal blepharocconjunctivitis [130].

The use of flucytosine eye-drops is described in table 2.5.

2.3 Azoles

The antifungal agents of this drug category are chemically derived from the imidazole ring and are usually substituted in the 2 position. They have a broad spectrum of activity which includes dermatophytes, e.g. Trichophyton, Microsporon and Epidermophyton, as well as other pathogens such as Candida spp. and Cryptococcus spp. Some azoles were inactive in vitro against Fusarium spp. [79], though their use has been reported to be successful after several weeks in vivo [132, 133]. A general overview has been published by [134–137], respectively. Development of resistance has occurred and must be taken into account [137].

2.3.1 Clotrimazole

Clotrimazole (1-(α-2-chlorotrityl)imidazole) was one of the first azoles in clinical use. It can be used as a 1% concentration in castor oil or as an ointment (tables 2.6, 2.7). The use of polyethylene glycols or cremophor as vehicles
Table 2.7. Preparation of clotrimazole 1% ointment

- Dissolve clotrimazole (as in table 2.6) in sterile oil with heating and mix with white soft paraffin
- The proportions are clotrimazole, 0.1 g, arachis oil (sterile), 5.0 g, white soft paraffin for ophthalmic use, 10.0 g

causes epithelial damage [138]. Complete healing was recorded in several cases of *A. fumigatus* keratitis [58, 139] and in keratomycoses caused by *Candida* spp. [58] or by *Penicillium* spp. [140, 141]. In 1 case each of infection with *Monosporium apiospermum*, *P. rugulosum* and *A. ianus*, as well as 2 cases of *T. famata*, the lesions healed completely after treatment with clotrimazole as single agent for 3–8 weeks [140]. The same treatment was successfully used in keratomycosis caused by (1 case each) *A. fumigatus*, *P. rugulosum* and *Cladosporium* sp. [141]. Clotrimazole also proved effective against *S. brevicaulis* in vitro [266].

Clotrimazole has also been prescribed for topical application in combination with oral ketoconazole [142]. The risk of resistant fungal strains must, however, be taken into account [110], and for this reason clotrimazole is now generally considered to be a second- or third-line drug.

### 2.3.2 Miconazole

Miconazole was usually instilled into the eye in a 1% solution, but could also be administered by subconjunctival injection (5–10 mg) [40, 143] or systemically (20 mg/kg). Miconazole had also been given by intravitreous injection [143]. Resistance did occur [34, 110], but the in vitro efficacy of miconazole exceeded that of natamycin and nystatin [83].

The clinical use of topical miconazole was successful in keratomycoses caused by *Aspergillus* spp. [57, 132, 144] or by *Cryptococcus* sp. [145], as well as in combination with oral ketoconazole, 200 mg/day, against *Drechslera* spp. and *Curvularia* spp. [132]. In an open prospective study, 1% miconazole was successful in 55 of 85 patients (64.7%) with a mean healing time of 22 days [146]. Efficacy was most pronounced against *Candida* spp. (4 of 5), followed by *Aspergillus* spp. (14 of 20) and *Fusarium* spp. (3 of 6). A 2% miconazole ointment had also been prescribed [99, 147]. Miconazole appears to be the only effective agent against *P. lilacinus* [91, 92]. Topical as well as systemic miconazole was effective against *S. brevicaulis* keratitis [148]. Intraocular blas-
tomycosis cleared up only when subconjunctival miconazole, 5 mg/0.5 ml, was added to the treatment with intravenous amphotericin B [149]. On the other hand, a case of keratitis caused by P. boydii failed to respond even to 1,200 mg 3 times daily in combination with hourly applications of 1% miconazole and 2 further subconjunctival injections of miconazole, resulting in the loss of the eye. The organism was sensitive to the drug (MIC 0.5 mg/l) [150].

Systemic doses of miconazole were used with good results in Botryodiplodia theobromae keratitis [151], and in Aspergillus keratitis [152], as well as in orbital abscess caused by P. boydii [153]. The compound was also effective in C. albicans endophthalmitis [32, 122] and in postoperative Aspergillus endophthalmitis [154]. On the other hand, endophthalmitis resulting from coccidioidomycosis worsened during treatment with miconazole, but improved when this was replaced by amphotericin B [155].

It had been recommended to administer an antihistamine before parenteral administration. The adverse effects were probably attributable to the vehicle, Cremophor EL [156] and consisted of nausea, pruritus, cardiac dysrhythmia and, in some cases, phlebitis [157, 158].

Systemic miconazole has failed to fulfil the original expectations [159]. Meanwhile the manufacturer has discontinued the production of the intravenous solution with the result that topical ophthalmic use with this formulation is no longer possible.

2.3.3 Ketoconazole

This imidazole derivative has been prescribed for topical as well as systemic use (200–400 mg daily). A general overview is provided by [160]. Apart from nausea and vomiting, liver damage may result from systemic administration [161]. The adverse effects are dose related [8, 129].

Oral doses of ketoconazole have proved especially effective in keratomycosis due to Fusarium spp. [133], particularly in combination with topical miconazole [132]. A case of Botryodiplodia keratitis that had failed to respond to ketoconazole cleared up when miconazole was given intravenously [162]. In a study involving 30 cases of keratomycoses (including 11 Aspergillus, 6 Fusarium and 3 Curvularia), ketoconazole was given orally in doses of 600 mg/day, 400 mg/day or 200 mg/day for 5 days each; it proved very effective in 20 cases, and no adverse effects were reported [266]. Oral ketoconazole was effective in a case of Candida endophthalmitis involving the anterior sections of the eye which had failed to respond to natamycin [163]. It was ineffective, however, in experimental Aspergillus keratitis [164].
Table 2.8. Preparation of ketoconazole 2% oily drops (20 mg/ml)

- Dissolve 1 tablet (200 mg) of ketoconazole (Nizoral®, Janssen) in 10 ml of castor oil using a mortar and pestle
- Shake well, do not filter, and do not freeze

Following diagnostic and therapeutic vitrectomy the intravitreous concentration of ketoconazole 8 h after an oral dose of 600 mg was shown to be 0.92 mg/l, which was above the MIC for the offending organism C. parapsilosis [165]. It has accordingly been recommended that systemic ketoconazole is used in the treatment of fungal endophthalmitis [40], particularly as it has no serious side effects [165, 166]. It has been claimed that a combination with intravitreous amphotericin B [165] or with oral flucytosine [115, 267] was beneficial in candidal deep mycosis. On the other hand, microbiologists warn against the combination of ketoconazole and amphotericin B, because Candida spp. resistant to ketoconazole but sensitive to amphotericin B developed resistance in vitro to the latter antifungal agent [166].

As with miconazole, ketoconazole has failed to fulfil early expectations of efficacy in invasive mycoses and has been largely superseded by itraconazole and fluconazole [159]. It has been recommended that eye-drops are prepared by pulverising a 200-mg tablet and adding 10 ml of sterile saline solution [167] or 5 ml of 4.5% boric acid solution with 5 ml hydroxypropylmethylcellulose [168]. This results in a solution of low pH, however, so it is preferable to suspend the lipophilic ketoconazole in castor oil or ground-nut oil (table 2.8). Ketoconazole 2% eye-drops have been found effective against Aspergillus spp., F. solani and Alternaria spp. [168], as well as in combination with oral ketoconazole, 200 mg/day, against Penicillium spp. [167].

2.3.4

Itraconazole

Itraconazole has proved very effective in systemic Aspergillus infections [169–174]. The MICs are favorable even in infections with problem organisms, e.g. C. glabrata, C. krusei and C. neoformans. Itraconazole is particularly effective in infections with dermatophytes; the only gaps in the spectrum are Fusarium spp. [79, 170] and Zygomyces spp. [175]. Itraconazole is regarded as the treatment of choice in histoplasmosis [176]. In cryptococcosis it is recommended as a good alternative to the standard combination of amphoter-
icin B and flucytosine [177]. After administration of a mean itraconazole dose of 5.1 mg/kg body weight/day, serum levels varied widely in the same individual and between different individuals (range 117–1127 mg/ml) [171]. Absorption of orally administered itraconazole capsules can be increased in patients with achlorhydria or otherwise reduced gastric acidity by concurrent ingestion of a cola beverage (pH 2.5) [178]. Drinking cola is not necessary if itraconazole is taken in liquid form due to the low pH of the solution.

Itraconazole, 200 mg/day orally, was used in 40 cases of keratomycosis (19 caused by F. solani and 15 by Aspergillus spp). The Aspergillus infections responded particularly well to the treatment [179, 180]. Similar results were obtained in a patient with Aspergillus keratitis who was treated with itraconazole, 400 mg/day, plus topical amphotericin B [181]. In a case of keratomycosis caused by S. brevicaulis, the fungus was resistant to amphotericin B but sensitive to itraconazole [182]; however, despite a dose of 200 mg twice daily, the case required penetrating keratoplasty. In a case of keratomycosis caused by A. flavus, itraconazole finally induced resolution after the condition had failed to respond to oral ketoconazole and topical amphotericin B [183]. P. boydii, on the other hand, proved resistant (MIC > 50 mg/l) [150]. Corneal penetration of topical itraconazole has been studied in the rabbit [184].

2.3.5

Fluconazole

This triazole antifungal drug, which has been available since the mid-1980s, is suitable for systemic (200–400 mg/day) as well as topical use. It is marked by low plasma protein binding and good pharmacokinetics [135, 185–187]. Experimental studies have shown that after systemic administration fluconazole penetrates the eye [188, 189] and the cerebrospinal fluid [161, 190, 191]. The concentration in the aqueous humor of patients 2 h after an oral dose of 200 mg were 2–7 and 5.4 μg/ml (mean, 3.7 ± 2.17 μg/ml), measured by high-performance liquid chromatography [192]. In 1 case of P. boydii endophthalmitis, the drug concentration in the vitreous humor was 55% of the plasma level [193]. Following oral doses of 400 mg/day, concentrations of fluconazole were 15 μg/ml in the vitreous humour and 19 μg/ml in the plasma [194]

Although the in vitro activity of fluconazole is little if any better than that of other imidazole derivatives [195, 196], the in vivo results are far more favorable [197–200], so that fluconazole, with its low incidence of side effects, is now regarded as a drug of choice for susceptible organisms [11, 159, 177, 201–203]. Sensitive pathogens include in particular C. albicans and Crypto-
Table 2.9. Use of fluconazole 0.2% drops (2 mg/ml)

- Aspirate a dose of the infusion solution Difucan® (100 mg of fluconazole/50 ml) into a syringe under sterile conditions and instill directly into the conjunctival sac
- Difucan may also be transferred, under sterile conditions, to eye-drop bottles; store in a refrigerator

C. albicans spp. Another major use is in the prevention of invasive candidiasis [201]. In a study comparing the efficacy of fluconazole and amphotericin B plus flucytosine in 40 surgical patients with systemic candidiasis, the median elimination time of the pathogens was 8.5 days in the fluconazole group and 5.5 days in the combination group. Side effects necessitating a change of therapy occurred twice in the combination group. The cure rates did not differ between the 2 regimens [10].

Following widespread use of fluconazole, drug resistance is developing, particularly in non-\textit{C. albicans} species [204, 205]. In addition, fluconazole is not sufficiently active against \textit{Aspergillus} spp. Interactions with other drugs and hormones are considerably less marked than those of ketoconazole, however, and in addition the compound causes few adverse effects and is well tolerated [187].

Systemic fluconazole has been successfully used in \textit{Candida} endophthalmitis [126, 194, 206–218]. Oral fluconazole has also been used successfully in \textit{Cryptococcus laurentii} endophthalmitis [219].

Fluconazole 0.2% eye-drops (table 2.9) are well tolerated [Behrens-Baumann 1990, unpubl.data]. The use of an ophthalmic gel is recommended at night (table 2.10). Due to its good water solubility, fluconazole penetrates well into the deep corneal stroma and into the aqueous humor. Experimental data (pharmacokinetics, keratomycoses and endophthalmitis) are described in chapter 7.

2.3.6

\textit{Econazole, Voriconazole and Other Azole Antifungal Agents}

2.3.6.1

\textit{Econazole}

Econazole has been recommended as 1% eye-drops or ointment for the treatment of keratomycoses caused by \textit{Fusarium} spp., \textit{Penicillium} spp. or \textit{Aspergillus} spp. [57, 220]. It shows marked activity in vitro against \textit{C. albicans} and
Table 2.10: Preparation of fluconazole 0.2% gel

- The proportions are hypromellose (Methocel® E 4 M premium), 0.3 g, Difucan i.v. solution to 10.0 g
- Transfer the Difucan under sterile conditions to a 50-ml bottle and heat to about 50 °C
- Mix with Methocel and shake well
- Allow the gel to cool in a refrigerator before transferring it to sterile eye-ointment tubes
- Store in the refrigerator no longer than 3 days

is the most effective of the azoles against Fusarium spp., though it is less potent than natamycin [79]. In vivo, however, it was ineffective in 2 cases of Fusarium keratitis [221].

2.3.6.2 Voriconazole

Voriconazole (UL-109,496), a novel broad-spectrum azole, has been shown to be effective in vitro against filamentous and dimorphic fungi including Aspergillus spp., Fusarium spp., P. boydii, Rhizopus spp., S. schenckii, B. dermatitis, Histoplasma spp., Paecilomyces spp., C. parapsilosis, C. krusei and C. albicans. The MIC values were similar to or lower than those of itraconazole or amphotericin B [222]. These results were confirmed by other studies including C. glabrata and C. krusei, which are inherently resistant to fluconazole [223, 224]. The drug was highly effective after oral administration in systemic cryptococcosis as well as in aspergillosis and candidiasis in guinea pigs [225–229]. In man, voriconazole had good activity in chronic invasive aspergillosis, against acute invasive aspergillosis and in oropharyngeal candidiasis [229–231]. Visual disturbances (enhanced brightness of light or blurred vision) have been reported but were fully reversible, sometimes with continuing administration of the study drug [229–231].

2.3.6.3 Saperconazole and Thiabendazole

Saperconazole is highly effective against Aspergillus spp. in vitro as well as against C. albicans [79, 232]. The pharmacokinetics after topical, subconjunctival and oral administration have been investigated in rabbits. Peak levels following subconjunctival injection in normal corneas (12.91 ± 2.02 µg/g) were
approximately twofold greater than those following sustained topical administration (6.19 ± 0.16 µg/g) and in debrided corneas were one-third higher than those following topical therapy in nondebrided corneas. Clearance was almost complete by 8 h. Levels following oral administration were low and probably subtherapeutic in all ocular tissues that were evaluated [233]. One case of *Fusarium* keratitis failed to respond to saperconazole [221]. Thiabendazole [57, 77, 234, 235] is no longer used.

2.4 Miscellaneous Antifungal Agents

A history of antifungal agents used in the past is provided by Gale [236]. These agents include silver sulphadiazine 1% [237], potassium iodide 1 g/ml [129, 238–240], and cycloheximide 0.02% [241]. Phenylmercury nitrate, 2 mg/100 ml, was used in vitro as a fungicide against *Aspergillus* spp., *Scedosporium* spp., and *C. albicans* [242]. *Antiseptic* agents have also been investigated (chlorhexidine, povidone-iodine, propamidine and polyhexamethylene biguanide) [243]. Chlorhexidine and povidone-iodine were the most effective in vitro, but in a small in vivo pilot study, chlorhexidine gave better results than povidone-iodine. In another study chlorhexidine gluconate 0.2% was significantly superior over natamycin in a masked randomized clinical trial with 60 patients [244]. This conclusion has been confirmed recently [245].

*Terbinafine* is one of a new generation of antifungal drugs that is effective in the treatment of dermatophyte infections. It is an allyl amine that inhibits the enzyme squalene epoxidase in fungal cell membranes. Terbinafine proved successful in 5 nonimmunocompromised cases of bronchopulmonary aspergillosis, in contrast with amphotericin B, itraconazole, and miconazole [246]. A case of pulmonary *P. boydii* infection refractory to itraconazole also responded to oral terbinafine [246]. White spots on the retina of monkeys and dyschromatopsia in one patient have been reported as side effects [247].

*Benomyl* (methyl-1-butylcarbamoyl)-1-benzimidazole carbamate, is a fungicide that is widely used on many commercial food crops and ornamental plants. The compound has produced ocular and craniocerebral malformations, including retinal dysplasia, cataracts, microphthalmia and anophthalmia [248].

*Pradimicines*, a novel class of broad-spectrum antifungal compounds, are currently undergoing preclinical and phase I clinical trials. The pradimicin BMS-181184 has in vitro antifungal activity against *Candida* spp., *C. neoformans*, *Aspergillus* spp. and zygomycetes, whereas *Fusarium* spp. are comparatively resistant [249].
Table 2.11. Recommended drugs for fungal infections of the eye

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Recommended drug for ophthalmic use</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
<td>Alternaria spp.</td>
<td>fluconazole</td>
<td>Koc, 1997 [250]</td>
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<tr>
<td>Aspergillus spp.</td>
<td>amphotericin B</td>
<td>Valluri, 1993 [251], Levin, 1996 [252], Heier, 1995 [253]</td>
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<tr>
<td>Blastomyces spp.</td>
<td>amphotericin B</td>
<td>Gottlieb, 1995 [254]</td>
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<td></td>
<td>amphotericin B plus miconazole</td>
<td>Mason, 1993 [149]</td>
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<tr>
<td>C. albicans</td>
<td>fluconazole</td>
<td>Meunier, 1994 [201], Philipps, 1997 [202], Urbak, 1994 [203]</td>
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<td></td>
<td>voriconazole (in vitro)</td>
<td>Cauwenbergh, 1994 [170]</td>
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<td></td>
<td></td>
<td>Hitchcock, 1996 [225], Peye, 1996 [255], Richardson, 1996 [224]</td>
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<td>C. immitis</td>
<td>amphotericin B</td>
<td>Blumenkranz, 1980 [155]</td>
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<td></td>
<td>fluconazole</td>
<td>Luttrull, 1995 [209]</td>
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<tr>
<td>Cryptococcus spp.</td>
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<td>Custis, 1995 [219]</td>
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<td>intraconazole</td>
<td>Just-Nübling, 1994 [177]</td>
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<tr>
<td>Curvularia</td>
<td>natamycin 5%</td>
<td>Dorey, 1997 [257]</td>
</tr>
<tr>
<td>Fonsecaea spp.</td>
<td>intraconazole</td>
<td>Barton, 1997 [258]</td>
</tr>
<tr>
<td>Fusarium spp.</td>
<td>natamycin 5%</td>
<td>Rosa, 1994 [89]</td>
</tr>
<tr>
<td></td>
<td>amphotericin B</td>
<td>Wood, 1976 [66]</td>
</tr>
<tr>
<td>Histoplasma spp.</td>
<td>intraconazole</td>
<td>Negroni, 1989 [176]</td>
</tr>
<tr>
<td></td>
<td>amphotericin B</td>
<td>Djistra, 1989 [259]</td>
</tr>
<tr>
<td>Mucor (Rhizopus) spp.</td>
<td>amphotericin B</td>
<td>Lehrer, 1980 [260], Ferry, 1983 [261]</td>
</tr>
<tr>
<td>Ovadendron spp.</td>
<td>amphotericin B</td>
<td>Lee, 1995 [262]</td>
</tr>
<tr>
<td>Phaeohyphomycosis</td>
<td>natamycin 5%</td>
<td>Kanungo, 1996 [263]</td>
</tr>
<tr>
<td>(Exserohilum) spp.</td>
<td>miconazole</td>
<td></td>
</tr>
<tr>
<td>Paecilomyces spp.</td>
<td>miconazole</td>
<td>Kozarsky, 1984 [92], Pflugfelder, 1988 [40]</td>
</tr>
<tr>
<td>Penicillium spp.</td>
<td>ketoconazole</td>
<td>Fromtling, 1988 [135]</td>
</tr>
<tr>
<td></td>
<td>miconazole</td>
<td>Ishibashi, 1984 [152], Ruben, 1991 [264], Nunery, 1985 [265]</td>
</tr>
<tr>
<td>S. brevicaulis</td>
<td>miconazole</td>
<td>Del Prete, 1994 [148]</td>
</tr>
</tbody>
</table>

2.5 **Recommended Drugs for Fungal Infections**

The drugs currently recommended for use against various fungal infections are shown in table 2.11. These recommendations are based on the current literature as cited.
References


Raah, W., Mykosebehandlung mit Imidazolderivaten. 1978: Springer Verlag.


References


References


Chapter 3

Periocular Fungal Infections

3.1 Palpebral Involvement

Fungal infections of the eyelids have been described several times. The eyelids alone may be affected, or their involvement may be part of a generalized dermatomycosis [1–4]. Table 3.1 lists fungi that have been implicated in palpebral disease. Both aspergilloma [5] and sporotrichosis [2] can simulate a chalazion, and coccidioidomycosis may be mistaken for an infected basal cell carcinoma. Aspergillomas respond well to direct injection of amphotericin B [5]. In North America, generalized blastomycosis affects the eyelids in a quarter of the patients [6] and commonly causes a cicatricial ectropium [7–9].

As from the conjunctivae (see chapter 4, p. 68), it may be possible to isolate fungi from the lids in the absence of a manifest infection [10, 11]. A fungal infection of the lid margins may resemble a staphylococcal infection [12]. Nelson et al. [13] successfully treated Pityrosporum blepharitis with topical ketoconazole, but equally good results were obtained in the control group of this double-blind study, whose sole treatment consisted of cleansing with baby shampoo. It thus seems that mechanical cleansing of the lid margins may be the key aspect of the management of chronic blepharitis [14].

3.2 Infections of the Lacrimal Ducts

Since the time of von Graefe, who in 1854 was the first to describe actinomycosis of the canaliculi [15], there have been repeated reports in the literature of infections and stenoses of the tear ducts caused by this microorganism (table 3.2). The incidence in Central Europe is probably considerably less than 2% of the dacryostenoses, a figure quoted by Wissmann [16] for the population of Breslau, Silesia in 1913. The fact that actinomycetes (Streptothrix spp., Leptothrix spp., Nocardia spp.), despite their names, are not fungi but
Table 3.1. Reported fungal infections of the eyelids

<table>
<thead>
<tr>
<th>Fungal Infection</th>
<th>Year and Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. boydii</td>
<td>Persaud, 1968 [104]</td>
</tr>
<tr>
<td>Aspergillosis</td>
<td>Harrell, 1955 [5]</td>
</tr>
<tr>
<td></td>
<td>Timm, 1963 [105]</td>
</tr>
<tr>
<td>Blastomycosis</td>
<td>Blodi, 1958 [7]</td>
</tr>
<tr>
<td></td>
<td>Harrell, 1959 [106]</td>
</tr>
<tr>
<td></td>
<td>Noojin, 1951 [107]</td>
</tr>
<tr>
<td></td>
<td>Witorsch, 1968 [8]</td>
</tr>
<tr>
<td></td>
<td>Kreibig, 1940 [108]</td>
</tr>
<tr>
<td></td>
<td>Barr, 1986 [109]</td>
</tr>
<tr>
<td></td>
<td>Bongiorno, 1974 [110]</td>
</tr>
<tr>
<td>C. albicans</td>
<td>Starr, 1986 [9]</td>
</tr>
<tr>
<td></td>
<td>Ostler, 1993[111]</td>
</tr>
<tr>
<td></td>
<td>Roth, 1998 [112]</td>
</tr>
<tr>
<td>Coccidioidomycosis</td>
<td>Faulkner, 1962 [1]</td>
</tr>
<tr>
<td></td>
<td>Irvine, 1968 [3]</td>
</tr>
<tr>
<td></td>
<td>Starr, 1986 [9]</td>
</tr>
<tr>
<td>Paracoccidioidomycosis</td>
<td>Belfort, 1975 [113]</td>
</tr>
<tr>
<td>(South American blastomycosis)</td>
<td>de Moraes Silva, 1988 [114]</td>
</tr>
<tr>
<td>Pityrosporum spp.</td>
<td>Romano, 1978 [115]</td>
</tr>
<tr>
<td></td>
<td>Starr, 1986 [9]</td>
</tr>
<tr>
<td>Rhinosporidiosis</td>
<td>Duggan, 1928 [116]</td>
</tr>
<tr>
<td></td>
<td>Kuriakose, 1963 [117]</td>
</tr>
<tr>
<td></td>
<td>Sharma, 1958 [118]</td>
</tr>
<tr>
<td></td>
<td>Gupta, 1966 [119]</td>
</tr>
<tr>
<td></td>
<td>Starr, 1986 [9]</td>
</tr>
<tr>
<td>Sporothrix spp.</td>
<td>Hill, 1930 [120]</td>
</tr>
<tr>
<td></td>
<td>Starr, 1986 [9]</td>
</tr>
<tr>
<td>Sporotrichosis</td>
<td>Gifford, 1922 [121]</td>
</tr>
<tr>
<td></td>
<td>Gordon, 1947 [2]</td>
</tr>
<tr>
<td>Trichophytosis</td>
<td>Wirtz, 1922 [122]</td>
</tr>
<tr>
<td></td>
<td>Ostler, 1971 [123]</td>
</tr>
<tr>
<td></td>
<td>Starr, 1986 [9]</td>
</tr>
</tbody>
</table>

Infections of the Lacrimal Ducts
Table 3.2. Publications on an actinomycosis (not a fungus, see text)

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Year</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brinckerhoff</td>
<td>1942</td>
<td>[124]</td>
</tr>
<tr>
<td>Förster</td>
<td>1869</td>
<td>[125]</td>
</tr>
<tr>
<td>von Graefe</td>
<td>1856</td>
<td>[126]</td>
</tr>
<tr>
<td></td>
<td>1869</td>
<td>[127]</td>
</tr>
<tr>
<td>Grüter</td>
<td>1933</td>
<td>[128]</td>
</tr>
<tr>
<td>Hoffmann</td>
<td>1962</td>
<td>[17]</td>
</tr>
<tr>
<td>Kipp</td>
<td>1883</td>
<td>[129]</td>
</tr>
<tr>
<td>Müller</td>
<td>1960</td>
<td>[130]</td>
</tr>
<tr>
<td>Pine et al.</td>
<td>1961</td>
<td>[131]</td>
</tr>
<tr>
<td>Pine and Hardin</td>
<td>1959</td>
<td>[132]</td>
</tr>
<tr>
<td>Purgason</td>
<td>1992</td>
<td>[20]</td>
</tr>
<tr>
<td>Richards</td>
<td>1973</td>
<td>[133]</td>
</tr>
<tr>
<td>Savir et al.</td>
<td>1978</td>
<td>[134]</td>
</tr>
<tr>
<td>Thies</td>
<td>1931</td>
<td>[135]</td>
</tr>
<tr>
<td>Wirtz</td>
<td>1922</td>
<td>[122]</td>
</tr>
<tr>
<td>Wissmann</td>
<td>1913</td>
<td>[16]</td>
</tr>
</tbody>
</table>

Bacteria was first emphasized in the ophthalmological literature by Hoffmann [17] (see also p. 21–22).

As with the eyelids, several fungi can cause infections of the nasolacrimal duct, and these are listed in Table 3.3. Dacryostenosis caused by true fungi is probably rare. C. albicans was isolated from only 2 of 236 patients (1.2%) with dacryocystitis [18]. Busse [19] found that only 2 cases of approximately 4,000 occlusions of the lacrimal canaliculi were caused by infections with C. albicans, with 1 case of canaliculitis and 1 case of diverticulitis of the lacrimal sac [19]. Candida spp. have also been reported in 2 cases of dacryocystitis, without dacrolith formation [20].

Dacryoliths, or brittle, hard concretions in the lacrimal canaliculi however, are characteristic of the condition. They may occasionally fill the entire lumen. It is often possible to express these fungal concretions with the aid of two glass rods. Where this is not feasible, an incision has to be made in the canaliculus to remove the concretion (1-snip or 2-snip procedure). This should be followed by microsurgical apposition of the wound margins. It is always advisable to irrigate with an antifungal agent, e.g. amphotericin B 0.5%.

3.3 Fungal Infections of the Orbit

Various types of fungus have been described in infections of the orbit [21, 22]. The most commonly diagnosed conditions are mucormycosis and...
Table 3.3. Fungal infections of the nasolacrimal duct

<table>
<thead>
<tr>
<th>Fungal Infections</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blastomyces spp.</td>
<td>Ostler, 1993 [141]</td>
</tr>
<tr>
<td>Cephalosporium spp.</td>
<td>Ostler, 1993 [141]</td>
</tr>
<tr>
<td>Curvularia</td>
<td>Brook, 1998 [147]</td>
</tr>
<tr>
<td>Penicillium spp.</td>
<td>Pine, 1961 [131]</td>
</tr>
<tr>
<td>Streptomyces spp.</td>
<td>Savir, 1978 [134]</td>
</tr>
<tr>
<td>Trichophyton spp.</td>
<td>Bergaust, 1965 [157]</td>
</tr>
</tbody>
</table>

aspergillosis. Other pathogens are listed in table 3.4. After injury to an eyelid, a 4-year-old child developed an orbital and brain abscess caused by P. boydii. The child was successfully treated with surgical debridement and intravenous miconazole [23]. In a similar case, the organism was resistant to amphotericin B, but after surgical debridement responded to a 6-week course of intravenous
Table 3.4. Fungal infections of the orbits

<table>
<thead>
<tr>
<th>Fungal Infection</th>
<th>Year and Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternaria spp.</td>
<td>Bartynski, 1990 [98]</td>
</tr>
<tr>
<td>Aspergillus spp.</td>
<td>See chapter 3.3.2</td>
</tr>
<tr>
<td>Bipolaris (Drechslera) spp.</td>
<td>Jacobson, 1992 [95]</td>
</tr>
<tr>
<td></td>
<td>Manning, 1991 [96]</td>
</tr>
<tr>
<td></td>
<td>Klapper, 1997 [97]</td>
</tr>
<tr>
<td>Blastomyces spp.</td>
<td>Vida, 1974 [101]</td>
</tr>
<tr>
<td>C. immitis</td>
<td>Rodenbiker, 1980 [100]</td>
</tr>
<tr>
<td></td>
<td>Jou, 1995 [158]</td>
</tr>
<tr>
<td>Curvularia spp.</td>
<td>Brummund, 1986 [99]</td>
</tr>
<tr>
<td></td>
<td>Berry, 1984 [159]</td>
</tr>
<tr>
<td></td>
<td>Heier, 1995 [70]</td>
</tr>
<tr>
<td>Histoplasma spp.</td>
<td>Olurin, 1969 [102]</td>
</tr>
<tr>
<td>Mucor spp.</td>
<td>See chapter 3.3.1</td>
</tr>
<tr>
<td>Penicillium spp.</td>
<td>Moriss, 1970 [103]</td>
</tr>
<tr>
<td>Pseudallescheria spp.</td>
<td>Anderson, 1984 [23]</td>
</tr>
<tr>
<td></td>
<td>Nunery, 1985 [24]</td>
</tr>
<tr>
<td>Sporothrix spp.</td>
<td>Morax, 1910 [160]</td>
</tr>
</tbody>
</table>

3.3.1 Mucormycosis

Mucormycosis (phycomycosis, zygomycosis) is usually caused by fungi of the genera Absidia, Rhizomacor or Rhizopus. Infection may be centered on one area, such as an orbit or paranasal sinus (orbital, rhinocerebral), stomach (gastrointestinal), lung (pulmonary) or skin (cutaneous), or it may be more widespread (disseminated) [26]. Most cases of mucormycosis are associated with an underlying disease, though it has been reported in healthy persons [27]. Diabetes mellitus is the most common underlying disorder, especially if complicated by metabolic acidosis [26, 28–30]. Other predisposing factors

miconazole [24]. A case of periorbital necrotizing fasciitis caused by Cryptococcus sp., also after an eyelid injury, healed after surgical debridement and treatment with fluconazole [25].
include cancer chemotherapy, organ transplantation with immunosuppressive therapy, treatment with corticosteroids, and leukemia and lymphoma [30, 31]. Treatment with deferoxamine represents an additional risk factor, as the fungi need iron for growth [32–40]. More than 100 cases of rhino-orbital-cerebral mucormycosis have been reported [28, 41, 42], and invasion of the optic nerve has also been described [43, 44].

The first clinical symptoms are often orbital pain associated with sinusitis or pharyngitis [30, 45], followed by rapidly increasing proptosis. One characteristic of mucormycosis is the invasion of blood vessels by fungal hyphae and subsequent infarction [28, 46–48], which not uncommonly leads to a sudden loss of vision due to occlusion of the central artery [39, 49, 50]. Later, black discoloration of the mucous membranes or skin appears as a characteristic sign [30]. The disease often takes a fulminating course, so that it is misdiagnosed as a bacterial infection [51]. The initial symptoms and signs of the disease in a series of 16 patients are listed in table 3.5 [30].

The prognosis is serious, particularly as the condition is often complicated by cerebral involvement. Preceding or concurrent bacterial infections are common [30].

Surgical excision of infected tissues in addition to antifungal chemotherapy has been life saving [29, 39]. Debridement must be radical to be beneficial; persistence of unsuspected areas of infection may lead to serious postoperative complications, e.g. hemorrhage [52]. In appropriate cases a frozen section-guided surgical debridement may provide an alternative to traditional radical surgical excision [53]. Drug treatment consists of systemic administration of amphotericin B, possibly combined with flucytosine [26, 30, 39, 54]. Hyperbaric oxygen may also be useful [55]. A case resistant to amphotericin B (MIC, 64 mg/l in vitro) was successfully treated with oral ketoconazole, 600 mg/day.

Table 3.5. Initial symptoms and signs in 16 patients with mucormycosis [30]

<table>
<thead>
<tr>
<th>Symptom or sign</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinusitis, pharyngitis or nasal discharge</td>
<td>11</td>
</tr>
<tr>
<td>Orbital or periorbital pain</td>
<td>6</td>
</tr>
<tr>
<td>Abrupt visual loss</td>
<td>4</td>
</tr>
<tr>
<td>Black eschar of skin, nasal mucosa or palate</td>
<td>3</td>
</tr>
<tr>
<td>Cellulitis of face or lids</td>
<td>5</td>
</tr>
<tr>
<td>Proptosis</td>
<td>2</td>
</tr>
<tr>
<td>Numbness of homolateral side of face</td>
<td>1</td>
</tr>
</tbody>
</table>

1 Several signs or symptoms developed concurrently in some patients.
for 5 months [56]. Where appropriate, treatment may also be attempted with itraconazole or fluconazole [42]. This treatment may be supported by repeated intraconazole instillation of amphotericin B, 1 mg/ml, every 12 h via an Abocath No. 18 catheter [50].

Mucormycosis has also been diagnosed in the eye [30, 57] and was once misdiagnosed as Coats’ disease [58]. It has also, though doubtfully, been reported in the cornea [59].

3.3.1.1 Case Report [39]

A woman aged 24 years was diagnosed in 1988 as having acute myeloid leukemia, FAB classification type M2. The initial treatment consisted of 2 cycles of daunorubicin, cytarabine and etoposide chemotherapy. After complete remission was achieved in August 1988, and in the absence of an allogeneic bone marrow donor, autologous marrow was removed and then transplanted after myeloablative conditioning therapy with busulphan and cyclophosphamide. Despite her continuing complete remission, the patient developed chronic pancytopenia as her bone marrow function did not recover. The essential regular transfusions of erythrocytes and thrombocytes (red cells every 14 days, platelets every 5 days) resulted in an accumulation of iron which from October 1990 onwards, with ferritin levels of 10,000 ng/ml, required intermittent treatment with deferoxamine, 5 g, which was given by intravenous injection at the end of every infusion.

Although she had been treated throughout as an out-patient, the woman was admitted in mid-May 1991 because of violent mid-frontal headache that had increased within 1 week, with swelling of the left periorbital region that had developed during the previous 24 h and pyrexia (38.5 °C axillary). Corrected visual acuity of both eyes was unimpaired. Pupillary reaction to both direct and indirect light was normal. The motility of both eyes was undiminished, but there was a slight pain on movement on the left side.

The suspected diagnosis based on the clinical findings was frontal and maxillary sinusitis on the left side, and on the day of admission the patient was commenced on antibiotic therapy with 2.2 g amoxycillin with clavulanic acid 3 times daily. Orbital phlegmons developed within 24 h of this treatment, which was therefore changed to rifampicin, 300 mg twice daily, and ciprofloxacin, 200 mg twice daily, but the infection advanced.

Within the next 2 days, the vision in the affected eye deteriorated rapidly to blindness, due to occlusion of retinal arteries (fig. 3.1.).

Computed tomography (CT) revealed extensive tissue proliferation, isodense to soft tissue, which most nearly resembled an abscess or an orbital phlegmon. There was also a protrusion of the bulb on the left side, and infiltration of the medial and inferior rectus muscles and marginally also of the optic nerve.

As the condition failed to respond to antibiotics, and the CT findings indicated considerable deterioration, orbital decompression was carried out by a paranasal incision on the left. The orbital plate of the ethmoid bone was removed, and the orbit incised. Extensive mucosal biopsies were taken from the region of the paranasal sinuses, and R. rhizopodiformis was cultured from these.

On the basis of these findings, parenteral combination therapy with amphotericin B and flucytosine was initiated 7 days after the start of the clinical symptoms. Amphotericin
B was given as a single test dose of 1 mg and then immediately in a dose of 1 mg/kg/day. Flucytosine was infused in the standard dose of 150 mg/kg/day. This treatment was continued unchanged for 10 days and then for another 12 days at half the dose of amphotericin B. The total dose of amphotericin B was only 0.8 g. Apart from shivering, which was controlled by intravenous injection of pethidine, 50 mg, the treatment caused no serious adverse effects. Figure 3.2 shows the clinical condition of the patient at the start of the antifungal treatment after orbital decompression.

The earlier progression of clinical symptoms stabilized within 24 h of the start of antifungal therapy, and then slowly subsided. However, blindness of the left eye due to occlusion of the central artery, which had occurred only 5 days after the clinical manifestation of the mucormycosis, remained irreversible.

3.3.2
Aspergillus and Other Fungal Organisms

Orbital aspergillosis usually arises from nasal and paranasal fungal sinusitis. The spectrum of Aspergillus infections is complex and may present in 4 fundamental patterns [60–63]: allergic, noninvasive, invasive and fulminant. Host immune defences are of crucial importance in determining susceptibility to aspergillosis. Table 3.6 lists the known risk factors for aspergillosis [63].

The first 2 modes (allergic, noninvasive), which are usually less aggressive, are typically found in nonimmunocompromised individuals. Allergic Asper-
Fig. 3.2. Proptosis immediately after orbital decompression and before the start of antifungal treatment.

Table 3.6. Risk factors for aspergillosis [63]

<table>
<thead>
<tr>
<th>Risk Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total neutrophils less than 1000/mm³</td>
</tr>
<tr>
<td>T cell defects, e.g. AIDS [161]</td>
</tr>
<tr>
<td>Defects of phagocytosis</td>
</tr>
<tr>
<td>Haematological malignancy</td>
</tr>
<tr>
<td>Steroids or other immunosuppressive agents</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Prosthetic devices or trauma</td>
</tr>
<tr>
<td>Excessive environmental exposure, e.g. nearby demolition or restoration of buildings, outdoor work, compost heaps</td>
</tr>
<tr>
<td>Residence in an endemic area, e.g. Sudan</td>
</tr>
<tr>
<td>Advanced age?</td>
</tr>
</tbody>
</table>

_Gillus_ sinusitis is a cause of chronic sinusitis in atopic but otherwise normal individuals [64, 65] and is thought to be a combination of type I and type III immunological reactions to _Aspergillus_ antigens via granulomatous inflammation. This saprophytic condition may take a benign course, or may result in a progressive sinus wall erosion with rapid visual loss [66]. Noninvasive disease results in the formation of an aspergilloma (a fungus ball) and behaves like chronic sinusitis [63]. In some cases, the course of the disease may extend over several years [67]. Occasionally, chronic inflammation induced
by Aspergillus may cause tissue destruction without actual invasion by the organisms [68].

The other 2 forms (invasive, fulminant) are typically associated with immunocompromised patients. The invasive form behaves like a malignant neoplasm, presenting with granulomatous inflammation and fibrosis. Exceptionally, invasive aspergillosis may occur even in otherwise healthy individuals [69]. Fulminant aspergillosis is rapidly destructive with tissue necrosis and vascular invasion and is often fatal. Thus, there is a wide spectrum of disease [63, 68, 70–72]. Prolonged Aspergillus infections may also lead to the production of mycotoxins, such as aflatoxins and ochratoxins. These mycotoxins may produce optic neuritis and ophthalmoplegia in cases of noninvasive sinus mycoses [73].

The clinical symptoms include orbital inflammation and a red proptotic eye with or without associated pain [45, 59, 63, 67, 68, 70–77]. In addition, ophthalmoplegia may develop [71, 78]. Embolization of vessels of the optic nerve [79], or direct involvement of the nerve may occur [66, 80, 81]. Bilateral involvement has also been described [82].

The differential diagnosis includes infectious, neoplastic and noninfectious granulomatous processes. In particular, orbital pseudotumor and the early stages of Tolosa-Hunt syndrome may mimic orbital aspergillosis, leading to treatment with corticosteroids with a fatal outcome [78, 80, 83].

Both CT and magnetic resonance imaging (MRI) are useful in establishing the diagnosis. The presence of dense intraluminal calcifications on a CT scan is highly indicative of aspergillosis [84, 85], particularly if the density exceeds 2,000 Hounsfield units [86]; however, calcifications are present in only 50% of infected patients [73]. Fine-needle aspiration [87] or a biopsy may be performed.

Treatment consists of radical surgical debridement [63, 67, 75, 88, 89], if necessary with orbital exenteration [90]. Medical treatment may also be attempted with amphotericin B plus flucytosine or with itraconazole [63, 70, 91–93]. Itraconazole has fewer side-effects than amphotericin B and has been used successfully [89, 94]. Direct injection of amphotericin B into the cavity of the abscess without surgical debridement has been reported to be an effective palliative treatment of an orbital mass induced by A. fumigatus in a patient with AIDS [77].

Other fungi mimicking aspergillosis are Bipolaris spp., which include most Drechslera species. The infections particularly resemble allergic Aspergillus sinusitis [95–97]. Other pathogens described in this context, inducing slowly progressive exophthalmos, include Alternaria spp. [98], Curvularia spp. [99], C. immitis [100], B. dermatitidis [101], Histoplasma spp. [102] and Penicillium spp. [103]. Fulminant invasive aspergillosis may be mimicked by rhino-orbital-cerebral mucormycosis.
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References
Chapter 4

Mycoses of the Anterior Segment of the Eye

4.1 Prevalence of Fungi in the Conjunctival Flora

In a similar way to bacteria [1, 2], fungi may also occur in the conjunctival flora without necessarily causing any manifest inflammation. A detailed overview is given in table 4.1. The variations in the percentage of positive smears recorded can be explained mainly by differences in the ages of the subjects [3, 4]. Fungal colonization of the conjunctiva increased with age, and it is also likely that regional and seasonal factors play a part [5]. In some reports, positive smears were related to individuals rather than to the total number of smears. Some data [5] are difficult to understand and have not been included in the table. Repeated investigations at intervals of 4 weeks or 1 week [4, 5] seldom found the same species in the 2 smears, which may indicate that fungi in the conjunctiva are mostly airborne microorganisms, i.e. a transient flora. It is also conceivable that the conjunctiva has a fungistatic action. A factor isolated from bovine conjunctiva, which was not described in detail, reduced the number of viable cells of *Candida albicans* by 80%, while lysozyme had no discernible effects on the fungi [6].

Topical applications of corticosteroids for 3 weeks were followed by an increase in the prevalence of fungi from 18.8 to 67% [7], though these findings were not confirmed in another study, in which a significant difference from the control group was only found after use of betamethasone/neomycin drops [4]. After topical application of hydrocortisone or tetracycline for 4 weeks, fungal colonization rates of the conjunctivae were 41.2 and 28.7%, respectively, in originally fungus-free eyes [8]. Yeasts were isolated from the conjunctival sacs of 13.3% of 37 women with Sjögren’s syndrome, which was a higher prevalence than in a comparable population with normal eyes (same sex and similar age distribution [4]). Fungal colonization rates in patients with non-fungal underlying illnesses were also higher than in those with healthy eyes [9] (see 4.4.1.5).
Table 4.1. The prevalence of fungi in the conjunctival flora without inflammation

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Number of conjunctival smears</th>
<th>Positive conjunctival smears, %</th>
<th>Age of patients</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fazakas [250]</td>
<td>1935</td>
<td>160</td>
<td>23.3 ±</td>
<td>–</td>
<td>Hungary</td>
</tr>
<tr>
<td>Fazakas [251]</td>
<td>1953</td>
<td>993</td>
<td>25.4 ±</td>
<td>–</td>
<td>Hungary</td>
</tr>
<tr>
<td>Mitsui et al. [7]</td>
<td>1955</td>
<td>65</td>
<td>18.5 ±</td>
<td>–</td>
<td>Japan</td>
</tr>
<tr>
<td>Hammeke et al. [3]</td>
<td>1960</td>
<td>312</td>
<td>10.3 ± adults</td>
<td>–</td>
<td>Arkansas/USA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.8 ± children</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.9 ± neonates</td>
<td></td>
</tr>
<tr>
<td>Janke et al. [252]</td>
<td>1961</td>
<td>342</td>
<td>11.1 ±</td>
<td>–</td>
<td>Austria</td>
</tr>
<tr>
<td>Ainley et al. [253]</td>
<td>1965</td>
<td>43</td>
<td>27.9 ± 40–85 years</td>
<td>–</td>
<td>UK</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>30 aged over 60</td>
<td></td>
</tr>
<tr>
<td>Nema et al. [13]</td>
<td>1966</td>
<td>180</td>
<td>22.2 ±</td>
<td>–</td>
<td>India</td>
</tr>
<tr>
<td>Marchlewitz et al. [12]</td>
<td>1966</td>
<td>2,346</td>
<td>6.6 ± 20–30 years</td>
<td>–</td>
<td>GDR</td>
</tr>
<tr>
<td>Williamson et al. [4]</td>
<td>1968</td>
<td>1,106</td>
<td>2.9 ± total</td>
<td>–</td>
<td>Scotland</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.8 ± 0–9 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7.0 ± 60–69 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30 to &gt; 60 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saxena et al. [254]</td>
<td>1971</td>
<td>54</td>
<td>8.7 ±</td>
<td>–</td>
<td>India</td>
</tr>
<tr>
<td>Dasgupta et al. [97]</td>
<td>1973</td>
<td>200</td>
<td>8.5 ±</td>
<td>–</td>
<td>India</td>
</tr>
<tr>
<td>Romano et al. [64]</td>
<td>1975</td>
<td>280</td>
<td>2.5 ± &gt;16 years</td>
<td>–</td>
<td>Israel</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>24</td>
<td>&lt;2 months</td>
<td></td>
</tr>
<tr>
<td>Sandhu et al. [255]</td>
<td>1980</td>
<td>103</td>
<td>31.0 ±</td>
<td>–</td>
<td>India</td>
</tr>
<tr>
<td>Sandhu et al. [105]</td>
<td>1981</td>
<td>128</td>
<td>26.6 ±</td>
<td>–</td>
<td>India</td>
</tr>
<tr>
<td>Ando et al. [9]</td>
<td>1982</td>
<td>664</td>
<td>6.6 ±</td>
<td>–</td>
<td>Japan</td>
</tr>
</tbody>
</table>

The lid margins are more often colonized than the conjunctivae [5], as is also the case with bacteria [1, 10]. Of 302 positive fungal smears, 80% were isolated from the lid margin, 15% from the conjunctiva and 5% from both sites [11]. In this study, smears were examined from a group of young native Americans (aged 14–20 years); 36% of 115 smears were positive, and in a similar but younger population (aged 8–15 years) from a different region, fungi were demonstrated even in 86% of 28 smears.
The two largest studies, with 2346 smears [12] and 1106 smears [4] from central Europe showed the incidence of *Penicillium* spp. to be the highest, followed by *Candida* spp. and *Aspergillus* spp. Species from other genera, however, were also isolated, e.g. *Scopulariopsis* spp. and *Cladosporium* spp; the latter species were subject to regional variation [12]. A study from India [13] also showed a variety of fungi (including species of *Aspergillus*, *Penicillium*, *Candida*, *Fusarium*, *Alternaria*, *Hormodendrum* and *Mucor*).

4.2

**Fungal Conjunctivitis**

Fungal conjunctivitis is usually found in association with keratomycosis, but it may also occur separately. In a personal experiment, acute conjunctivitis was induced by inoculation with *C. albicans* [14]. There have been reports of conjunctivitides caused by *Candida* spp. [15–18] that sometimes caused pseudomembranous exudates, as well as some caused by *Blastomyces* spp. [19], by *Sporotrichum* spp. [20, 21] and by *Coccidioides* spp. [22, 23]. *Rhinococcidioides* may, over the course of a few months, lead to vascular proliferations [24–32] and to scleritis [33]. Conjunctivitis due to *A. niger* has been reported after foreign body injuries [34] and in concomitant trachoma [35]. [36] reported conjunctivitis in 3 children who had received subconjunctival doses of 3% saline because of retinal disease. The saline solutions had been contaminated with yeasts (*Endomyopsis fibular*) and moulds (*Monoverticillium ramigenum*). The conjunctivitis cleared up spontaneously. The injection solutions caused the same symptoms in the eyes of rabbits.

*C. neoformans* has been isolated from subconjunctival granulomas in patients with AIDS [37, 38].

4.3

**Fungal Scleritis**

A report by von Graefe [39] in 1857 provided the first description of a case of fungal scleritis after surgery for strabismus carried out by Dieffenbach. The ‘fungal granulations’ caused by the condition were described by von Virchow as ‘continuously’ associated with the sclera. A case of fungal scleritis due to *A. fumigatus* after injury caused by a wood splinter has also been described [40]. The author experimentally induced the same infection in rabbit eyes. Another patient, 2 months after injury caused by the branch of a tree, developed scleritis that healed only after cryotherapy and dura mater grafting;

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A. fumigatus was isolated from the biopsy of the scleral nodule [41]. After injury caused by a cow’s tail, a patient developed a scleral abscess with nodules and necrosis and exudative retinal detachment. After 6 months of treatment with flucytosine 1% eye-drops and fluconazole, 200 mg/day orally, the scleral inflammation was completely resolved and the retina was attached [42]. A scleral infection with A. niger, possibly caused by drug abuse or self-mutilation, progressed to endophthalmitis [43]. The reverse – first endophthalmitis, then scleritis – has also been reported [44]. Scleritis due to A. flavus developed after uncomplicated cataract surgery [45]. Resolution of the inflammation was achieved with oral itraconazole after progression to multifocal scleral nodulation and necrosis had occurred during treatment with topical amphotericin B and oral ketoconazole. In a case of uncomplicated phacoemulsification and posterior chamber intraocular lens implantation in the left eye via a 5.5 mm, superior, scleral tunnel incision Rhizopus scleritis developed in a diabetic man. Severe destruction of the globe ensued despite topical, subconjunctival, and intravenous amphotericin B, in combination with hyperbaric oxygen therapy. Histopathological examination of the enucleated globe was consistent with Rhizopus infection [46]. After recurrent pterygium operations with bare scleral technique, one patient developed posterior scleritis, which was found to be caused by P. boydii [47]. The infection mimicked autoimmune posterior scleritis, which prompted the administration of systemic immunosuppressants. A biopsy was required before the condition was correctly diagnosed. Despite oral itraconazole and subconjunctival injections of miconazole, the eye showed no clinical response, necessitating enucleation. In another case Scedosporium prolificans scleritis followed pterygium surgery with β-irradiation (3 × 8 Gy) 7 years previously [48]. In a series of intrascleral dissemination of infectious scleritis following pterygium excision one of 18 patients had Aspergillus [49]. The infection could not be cured and the globe was enucleated.

Table 4.2 summarizes published reports on fungal scleritis.

4.4 Keratomycosis

After the first description of a case of Aspergillus keratitis by Leber [50] and others around the turn of the century [51–53], relatively few reports of keratomycoses appeared in the first half of the 20th century. The early 1950s, however, saw a substantial increase. Thus, while [54] found 122 cases in the world literature for the period 1879–1950, they recorded the same number of cases for the period of only 1950–1962. Chick and Conant [55] found 148 cases in the literature, of which 42 had occurred between 1879 and 1916, 84
<table>
<thead>
<tr>
<th>Author(s) and reference</th>
<th>Predisposing condition</th>
<th>Fungal isolate</th>
<th>Medical antifungal therapy</th>
<th>Surgical therapy</th>
<th>Outcome (visual acuity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Köllner, 1906 [40]</td>
<td>trauma, piece of wood as foreign body</td>
<td><em>Aspergillus or Trichophyton</em></td>
<td>Oral potassium iodide</td>
<td>Excision</td>
<td>‘much improved’</td>
</tr>
<tr>
<td>Chaillous, 1912 [256]</td>
<td>systemic sporotrichosis</td>
<td><em>Sporotrichum</em></td>
<td>i.v. and topical amphotericin B</td>
<td>none</td>
<td>‘completely healed’</td>
</tr>
<tr>
<td>Podedworny and Snie, 1964 [257]</td>
<td>cataract extraction</td>
<td><em>Paecilomyces</em> sp.</td>
<td>Implant removal</td>
<td>None</td>
<td>Light perception</td>
</tr>
<tr>
<td>Lincoff et al., 1965 [258]</td>
<td>diabetes mellitus; scleral buckling operation</td>
<td>probably <em>M. mycosis</em></td>
<td>Implant removal</td>
<td>None</td>
<td>Light perception</td>
</tr>
<tr>
<td>Milauskas et al., 1967 [259]</td>
<td>diabetes mellitus; scleral buckling operation</td>
<td>‘fungus organisms with budding and branching forms’</td>
<td>Excision/irradiation</td>
<td>Light perception</td>
<td>Enucleation</td>
</tr>
<tr>
<td>Stenson et al., 1982 [44]</td>
<td>i.v. drug use; systemic <em>Aspergillus</em> disease 5 years previously</td>
<td><em>Aspergillus oryzae</em></td>
<td>Topical natamycin and amphotericin B, oral fluocytosine, i.v. amphotericin B</td>
<td>Biopsies</td>
<td>Complete resolution</td>
</tr>
<tr>
<td>Margo et al., 1988 [260]</td>
<td><em>Pterygium</em> excision/irradiation</td>
<td><em>Aspergillus</em> sp.</td>
<td>Topical natamycin, topical miconazole, oral fluocytosine</td>
<td>None</td>
<td>Enucleation</td>
</tr>
<tr>
<td>Reynolds and Alfonso, 1991 [261]</td>
<td>not reported</td>
<td><em>Acremonium</em></td>
<td>Topical natamycin, 5%</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>Moriarty et al., 1993 [262]</td>
<td><em>Pterygium</em> excision/irradiation (22 Gy)</td>
<td><em>Scedosporium inflatum</em></td>
<td>Topical natamycin, oral fluconazole, i.v. amphotericin B</td>
<td>Biopsies</td>
<td>6/18</td>
</tr>
<tr>
<td></td>
<td><em>Pterygium</em> excision/irradiation (24 Gy)</td>
<td><em>Fusarium</em></td>
<td>Topical natamycin, oral ketoconazole</td>
<td>Debridement</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td><em>Pterygium</em> excision/irradiation</td>
<td><em>Petriellidium boydii</em></td>
<td>Topical natamycin, oral ketoconazole, i.v. amphotericin B</td>
<td>Two penetrating grafts</td>
<td>6/36</td>
</tr>
<tr>
<td></td>
<td><em>Pterygium</em> excision/irradiation</td>
<td><em>Petriellidium boydii</em></td>
<td>Topical natamycin, i.v. amphotericin B</td>
<td>Two penetrating grafts</td>
<td>Enucleation</td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus niger</em></td>
<td>Amphotericin B, oral and topical fluconazole</td>
<td>Vitrectomy, lensctomy</td>
<td>Enucleation</td>
<td></td>
</tr>
<tr>
<td>Sullivan et al., 1994 [263]</td>
<td><em>Pterygium</em> excision/irradiation</td>
<td><em>Scedosporium prolificans</em></td>
<td>Topical natamycin and amphotericin B, systemic itraconazole and ketoconazole</td>
<td>Enucleation</td>
<td></td>
</tr>
</tbody>
</table>
Table 4.2 (continued)

<table>
<thead>
<tr>
<th>Author(s) and reference</th>
<th>Predisposing condition</th>
<th>Fungal isolate</th>
<th>Medical antifungal therapy</th>
<th>Surgical therapy</th>
<th>Outcome (visual acuity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taravella, 1997 [264]</td>
<td>Pterygium excision</td>
<td><em>Pseudallescheria boydii</em></td>
<td>itraconazole, subconj. miconazole</td>
<td>biopsy</td>
<td>enucleation</td>
</tr>
<tr>
<td>Kumar, 1997 [48]</td>
<td>Pterygium excision/irradiation (3 × 8 Gy)</td>
<td><em>Scedosporium prolificans</em></td>
<td>itraconazole, natamycin 5%</td>
<td>debridement</td>
<td>‘successful outcome’</td>
</tr>
<tr>
<td></td>
<td>cataract surgery</td>
<td><em>Aspergillus fumigatus</em></td>
<td>oral itraconazole, econazole drops</td>
<td>debridement, lamellar graft</td>
<td>6/9</td>
</tr>
<tr>
<td></td>
<td>trabeculectomy</td>
<td><em>Aspergillus</em> sp.</td>
<td>oral itraconazole, clotrimazole 1%, amphotericin B intravitreal, subconj., topical</td>
<td>vitrectomy</td>
<td>light perception</td>
</tr>
<tr>
<td>Hsiao, 1998 [49]</td>
<td>Pterygium excision</td>
<td><em>Aspergillus</em></td>
<td>amphotericin B</td>
<td>debridement</td>
<td>enucleation</td>
</tr>
</tbody>
</table>

between 1951 and 1962, and only 22 cases in the interval between these 2 periods. Haggerty and Zimmerman [56] noted that only 3 cases of keratomycosis were recorded at the Armed Forces Institute of Pathology in Washington between 1933 and 1952, compared with 13 cases for the period 1952–1956. This represented an incidence of 1 case of mycosis/11,329 corneal infections in the first period, and 1 in 777 in the second. This rapid increase in fungal keratitides in the 1950s and 1960s has obviously not continued exponentially into the 1980s. Nevertheless, there were over 70 references in the literature for the period 1981–1990, and 68 for the period 1991–1997.

4.4.1

**Predisposing Factors**

4.4.1.1

**Corticosteroids**

The sudden increase in keratomycoses at the beginning of the 1950s has been attributed to the introduction of corticosteroids [7, 57–72]. This hypothesis was based on the results of animal studies, in which fungal infections
commonly occurred only after additional use of corticosteroids (see p. 162). Corticosteroids suppress the endogenous immune defence [73] and reduce the tolerance limit so that fungal diseases can become established [74, 75]. The term ‘tolerance limit’ represents the number of inoculated blastospores that the body can destroy without manifest signs of disease [76, 77]. The tolerance limit may be reduced by systemic conditions, e.g. diabetes, alcoholism, or malignant tumours, as well as by cytotoxic agents, whole-body irradiation, etc.

Corticosteroids are not known to have direct growth stimulating effect on fungi [75, 78, 79]. No evidence has been published to support the assumption [80] that corticosteroids have a direct effect on the virulence of fungi.

Overall, the use of corticosteroids must be regarded as an important risk factor for the occurrence of keratomycoses.

4.4.1.2 Antibiotics

Antibiotics have also been associated with the occurrence of fungal keratitis [56, 57, 81–85]. In vitro investigations have provided contradictory results. Although tetracyclines were found to have a growth promoting effect on C. albicans [67, 86], other investigators were unable to confirm this [87]. Tanaka [18] described a stimulant effect of tetracycline at concentrations below 0.01% and an inhibitory action at 0.25%. In vitro, doxycycline, neomycin and gentamicin reduced the MIC of imidazole derivatives against Candida spp. [88]. Prasad and Nema [89] also found marked in vitro activity of doxycycline against Aspergillus spp., Candida spp., Curvularia spp. and Penicillium spp. In animal experiments, Candida keratitis was exacerbated by tetracyclines [75, 90] and by polymyxin B [91], though when the experiments were repeated polymyxin B had the opposite effect [92].

The potential mode of a growth-promoting action of antibiotics on fungal growth has been described as a direct growth-stimulating effect; furthermore, the elimination of bacteria and the change in the bacterial balance was considered to create a ‘biological niche’ for the fungi to occupy [69, 75, 78, 79, 93, 94]. Such an effect of antibiotics has been completely rejected by others [95]. In in vitro experiments, numerous antibiotics (ampicillin, mezlocillin, cefotaxime, tetracycline, chloramphenicol, erythromycin, polymyxin B, sulphamethoxydiazine, etc.) were tested on C. albicans, T. glabrata and A. fumigatus [95]. Apart from tetracycline hydrochloride, the antibiotics exhibited no effect in experimental candidiasis in mice, leading to the conclusion that: ‘The sole factor of importance for the development of fungal infections is the condition of the patient who – for any of many reasons – is immunocompromised and thus needs antibiotics, and his impaired immune status also renders him liable to fungal infections.’
Table 4.3. Prevalence of foreign body injuries preceding keratomycosis

<table>
<thead>
<tr>
<th>Percentage of Cases</th>
<th>Authors</th>
<th>Year</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>39</td>
<td>Forster and Rebell [158]</td>
<td>1975</td>
<td>South Florida, USA</td>
</tr>
<tr>
<td>41</td>
<td>Zimmerman [110]</td>
<td>1963</td>
<td>USA</td>
</tr>
<tr>
<td>42</td>
<td>Shiota et al. [107]</td>
<td>1986</td>
<td>Japan</td>
</tr>
<tr>
<td>45</td>
<td>Naumann et al. [100]</td>
<td>1967</td>
<td>USA</td>
</tr>
<tr>
<td>45</td>
<td>Koul et al. [266]</td>
<td>1975</td>
<td>India</td>
</tr>
<tr>
<td>54</td>
<td>Dasgupta et al. [97]</td>
<td>1973</td>
<td>India</td>
</tr>
<tr>
<td>59</td>
<td>Zapater [152]</td>
<td>1980</td>
<td>Argentina</td>
</tr>
<tr>
<td>60</td>
<td>Liesegang and Forster [138]</td>
<td>1980</td>
<td>South Florida, USA</td>
</tr>
<tr>
<td>60</td>
<td>Jones et al. [164]</td>
<td>1969</td>
<td>South Florida, USA</td>
</tr>
<tr>
<td>60</td>
<td>Chaddah and Agarwal [96]</td>
<td>1978</td>
<td>India</td>
</tr>
<tr>
<td>63</td>
<td>Forster et al. [267]</td>
<td>1975</td>
<td>South Florida, USA</td>
</tr>
<tr>
<td>67</td>
<td>Newmark et al. [223]</td>
<td>1971</td>
<td>USA</td>
</tr>
<tr>
<td>68</td>
<td>Nema [101]</td>
<td>1979</td>
<td>India</td>
</tr>
<tr>
<td>70</td>
<td>Reddy et al. [104]</td>
<td>1982</td>
<td>India</td>
</tr>
<tr>
<td>71</td>
<td>Gugnani et al. [99]</td>
<td>1976</td>
<td>Nigeria</td>
</tr>
<tr>
<td>88</td>
<td>Dutta et al. [148]</td>
<td>1981</td>
<td>India</td>
</tr>
<tr>
<td>90</td>
<td>Salceda [113]</td>
<td>1973</td>
<td>Philippines</td>
</tr>
</tbody>
</table>

4.4.1.3

**Foreign Bodies**

Corneal foreign bodies have been reported to cause keratomycosis at frequencies varying from about 40 to 90% (table 4.3). The nature of the foreign body is a decisive factor. In many cases they are plant products, such as rice ears, which get into the eye during agricultural work [71, 96–110], but other foreign bodies can also provoke keratomycoses, such as metals, stone fragments, or insects [93, 100, 111–113]. The pathogens can either be introduced into the cornea by the foreign body itself [114], or they may damage the cornea and thus create an opening for the existent transient fungal flora [12, 94].

4.4.1.4

**Postoperative Keratomycosis**

Keratomycosis following keratoplasty is discussed in chapter 5.1.2 in the context of postoperative fungal endophthalmitis, as these cases generally extend to the deeper sections of the eye. Mention should be made here, however, of 1 case due to *C. parapsilosis* and 1 due to *A. fumigatus* after radial keratotomy [115–118].
Table 4.4. Preceding eye diseases of keratomycosis

<table>
<thead>
<tr>
<th>Eye disease</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herpes simplex</td>
<td>De Voe, 1971 [98]; Rosa, 1994 [72]</td>
</tr>
<tr>
<td>Dry eye</td>
<td>Brasseur, 1987 [58]</td>
</tr>
<tr>
<td>Trachoma</td>
<td>Verin, 1984 [108]</td>
</tr>
<tr>
<td>Ocular pemphigoid</td>
<td>Naumann, 1967 [208]; Ross, 1972 [65]</td>
</tr>
<tr>
<td>Radiation keratitis</td>
<td>Ross, 1972 [65]</td>
</tr>
<tr>
<td>Zoster ophthalmicus</td>
<td>Naumann, 1967 [100, 208]; Rosa, 1994</td>
</tr>
<tr>
<td>Lagophthalmos</td>
<td>Naumann, 1967 [100, 208]</td>
</tr>
<tr>
<td>Bullous keratopathy</td>
<td>Naumann, 1967 [100, 208]</td>
</tr>
</tbody>
</table>

4.4.1.5 Pre-Existing Eye Diseases

Reports of keratomycoses that have developed in eyes with pre-existing disorders are not uncommon (table 4.4). Four patients have been described with candida keratitis following topical anaesthetic abuse. Three of them had burn of the eye, chronic dry eyes, and corneal abrasion after dirt blew as preceding disease [119]. In a histopathological study including 73 cases of fungal keratitis, [100] found 25 eyes with corneal disorders (herpes zoster ophthalmicus, ocular pemphigoid, lagophthalmos, ulcer and other disorders) as well as 10 with glaucoma (including 7 with bullous keratopathy). As many of these eyes had been treated with corticosteroids or antibiotics for their original disease, it was no longer possible to determine the decisive factor in the development of the fungal infection. Spontaneous *C. albicans* keratitis has been reported in immunocompromised drug abusers [120].

4.4.1.6 Contact Lenses

Keratomycoses are commonly associated with contact lenses [72, 121–136]. This applies particularly to soft lenses of hydroxyethylmethacrylate, which can be infiltrated by fungi [125, 133]. After prolonged use and with poor hygiene, deposits of epithelial cells and foreign particles may accumulate on the surface of the contact lens and provide a nutrient medium for pathogens [129, 137]. In addition, a moist chamber results between the underside of the contact lens and the surface of the cornea, which provides improved conditions for growth, together with a diminished wipe effect of the lids and tears.

Fungi were detected in 22 of 312 patients with intolerance reactions after they had worn contact lenses for prolonged periods [125]. The contact lenses...
should be examined microscopically in such cases, as the conjunctival smear is usually negative. [132] obtained similar results in an investigation of 11 patients with fungal infections of their contact lenses; 7 of the contact lenses had been heat-disinfected in a medical practice and 3 had been treated with a liquid disinfectant. In this context it has to be borne in mind that because of their toxicity, preservatives are in most cases no longer added to these fluids. Several risk factors may be present together if soft contact lenses are worn on medical reasons with the concurrent use of corticosteroids and antibiotics in already damaged eyes for prolonged periods [124, 130, 131, 133, 138, 139]. Three cases have been reported of fungal corneal ulceration after photorefractive keratectomy with the use of disposable contact lenses [134].

The clinical reports of a contributory effect of contact lenses to the development of fungal infections, however, have not been supported by the results of animal experiments, in which they had neither a positive nor a negative effect on the eyes of rabbits [140].

4.4.1.7

Systemic Illness

Systemic illness generally predisposes to additional fungal diseases (see chapter 5.1.1.2), apparently because of reduced immune defences. Keratomycoses have been described in association with diabetes mellitus [69, 72, 81, 98, 100, 124, 141], immunological disorders [124, 142, 143], neoplasms [98, 144] and alcoholism [98]. A syndrome of multiple endocrine deficiency and chronic mucocutaneous candidiasis has been described, in which bilateral keratoconjunctivitis occurs [145, 146]. The keratitis, however, is not caused by candidiasis directly [147].

4.4.1.8

Age, Sex, Climate and Season

Keratomycoses occur in all age groups, though children tend to be less commonly affected [97, 101, 113, 141, 148, 149]. The majority of patients are male, which is attributed to more extensive outdoor activity and the associated greater exposure to foreign bodies [82, 97–100, 113, 149–152]. In contrast, an Indian study showed a predominance of female patients [101], presumably because Indian women are more often employed outside in agriculture.

Keratomycoses occur predominantly in warm, subtropical and damp climates. The incidence correlates with harvest time and the seasonal increase in temperature and humidity [102, 148, 149, 153–155]. However, a higher incidence in the cool, windy months has been found in south Florida [138, 141, 150]. An increase in the incidence of keratitis also seems to be associated with the cool, windy months in the temperate zone [156].
4.4.2

*Spectrum of Pathogens Causing Keratomycosis*

The literature conveys the impression that the prevalence of individual pathogens is largely dependent on geographical and climatic factors. *Fusarium* spp. are the pathogens most commonly isolated in South America [152, 157], Florida [72, 138, 158], Japan [159, 160], Nigeria [99] and South Africa [161]. *Aspergillus* spp. are more often involved than *Fusarium* spp. in India [97, 162, 163] and in Vietnam [108]. *Candida* spp. followed by *Aspergillus* spp. are the principal pathogens in the temperate zones, e.g. in San Francisco [69], the northern USA [153], the UK [164] and Germany [93]. The predominance of *Candida* spp. in keratomycosis in Central Europe reflects the frequency of organic and systemic infections in that region [165–169]. Table 4.5 lists the fungi that have been described as causes of keratomycosis.

4.4.3

*Clinical Aspects of Keratomycoses*

Fungal infections of the cornea start, at first relatively painlessly, about 1–4 weeks after a trauma. There is subepithelial infiltration, which is occasionally associated with recurrent erosion. The infiltration increases in intensity and extent even if high doses of antibiotics are given (because the condition is mistakenly attributed to bacteria). This is accompanied by violent inflammation in the anterior chamber with endothelial plaques and a hypopyon. The latter initially tends to present the flat, horizontal appearance associated with bacterial origin (fig. 4.1), often followed by a pyramid-like, convex form characteristic of a fungal infection (fig. 4.2, 4.4, 4.6) [170]. At this stage, the hypopyon is no longer fluid and mobile, but viscous and solid. This is presumably due to the hyphae forming a firm skeleton for fibrin and cellular elements. In some cases, inflammation of the anterior chamber with the hypopyon may be so pronounced that secondary glaucoma develops [171, 172]. At this time the patient will complain of severe pain, foreign body sensation and diminished vision. In addition, mixed or deep hyperemia and pseudoptosis will occur. The fungal ulcer developing from the infiltration may show a greater or lesser degree of epithelialization. The margins, however, are mostly covered by epithelium, as the pathogens work their way from there into the subepithelial tissues at the level of the corneal stroma. The ulcer is yellowish-grey in colour and is indistinctly margined.
Table 4.5. Fungi described as causes of keratomycosis

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Authors and year of description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aureobasidium sp.</td>
<td>Chander, 1994 [162]</td>
</tr>
<tr>
<td>Botryodiplodia spp.</td>
<td>Ishibashi and Matsumoto, 1984 [324], Valenten et al., 1975 [325], Srinivasan, 1997 [313]</td>
</tr>
</tbody>
</table>

Keratomycosis 79
### Table 4.5 (continued)

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Authors and year of description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cladosporium</strong> spp.</td>
<td>Chander, 1994 [162], Forster and Rebell, 1975 [158], Forster et al., 1975 [267], Ormerod, 1987 [288], Sundaram et al., 1989 [277], Upadhyay et al., 1991 [320], Srinivasan, 1997 [313]</td>
</tr>
<tr>
<td><strong>Colletotrichum</strong> sp.</td>
<td>Ritterband, 1997 [341]</td>
</tr>
<tr>
<td><strong>Cryptococcus</strong> spp.</td>
<td>Harris et al., 1988 [332, 269], Ritterband, 1997 [341]</td>
</tr>
<tr>
<td><strong>Cylindrocarpon</strong> sp.</td>
<td>Rosa, 1994 [72]</td>
</tr>
<tr>
<td>Fungus</td>
<td>Authors and year of description</td>
</tr>
<tr>
<td>--------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td><em>F. pedrosoi</em> (Chromoblastomyces)</td>
<td>Barton, 1997 [350]</td>
</tr>
<tr>
<td><em>Gibberella</em> sp.</td>
<td>Anderson and Chick, 1963 [290]</td>
</tr>
<tr>
<td><em>Melanconiales</em> (Colletotrichum atramentum)</td>
<td>Rosa, 1994 [72]</td>
</tr>
<tr>
<td><em>Microsporum</em> sp.</td>
<td>Kulshrestha et al., 1973 [303]</td>
</tr>
</tbody>
</table>

Keratomycosis 81
Table 4.5 (continued)

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Authors and year of description</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Phaeotrichomyces</em> sp.</td>
<td>Shukla et al., 1989 [366]</td>
</tr>
<tr>
<td><em>Phialophora</em> spp.</td>
<td>Forster and Rebell, 1975 [158], Forster et al., 1975 [267], Jones et al., 1969 [164], Upadhyay et al., 1991 [320], Wilson et al., 1966 [367], Hirst, 1995 [239]</td>
</tr>
<tr>
<td><em>Pseudallescheria boydii</em></td>
<td>Pautler, 1955 [279], Zapater, 1979 [278], Ruben, 1991 [368], Firdova, 1997 [369]</td>
</tr>
<tr>
<td>(Monosporium apiospermum)</td>
<td></td>
</tr>
<tr>
<td><em>Pythium insidiosum</em></td>
<td>Murdoch, 1997 [370]</td>
</tr>
<tr>
<td><em>Rhizopus</em> spp.</td>
<td>see <em>Mucoraceae</em></td>
</tr>
<tr>
<td><em>Trichosporon beigelli</em></td>
<td>Rosa, 1994 [72]</td>
</tr>
<tr>
<td><em>Wangiella</em> spp.</td>
<td>Pospisil et al., 1990 [375]</td>
</tr>
</tbody>
</table>

It is occasionally possible, with adequate magnification, to distinguish hyphae with feathery outward growth (fig. 4.3, 4.4). Another characteristic feature is the geographic form of the ulcer (fig. 4.5). Satellite phenomena are pathognomonic for fungal keratitis, but are not always present. Raised areas within the corneal infiltration are also typical [173] (fig. 4.6). In some cases a white ring is present [174], which may represent an interaction of fungal antigen and host antibody (so called Wessely ring).

The characteristics of keratomycosis are summarized in table 4.6. However, the signs may differ from those outlined above, and fungal keratitis may present a different appearance, especially if it is a complication of another keratopathy (fig. 4.8). Furthermore, not all the characteristics listed are always present at the same time. If they are seen, however, they should suggest the possibility of keratomycosis. *C. albicans* and *C. parapsilosis* may also cause...
Fig. 4.1. Case 1. *A. fumigatus* keratitis with flat, horizontal hypopyon.

Fig. 4.2. The same case as in figure 4.1, 3 weeks later. A pyramid-shaped, convex hypopyon is now visible.

an infectious crystalline keratopathy of the type associated with a bacterial infection [175–181].

If medical treatment is unsuccessful, a descemetocele and perforation may develop in the further course of the disease. Finally, fungal keratitis may lead to endophthalmitis and loss of the eye (see chapter 5.1.2.1.3).
Fig. 4.3. Case 2. Right eye: corneal infiltration without visible or stainable epithelial damage. Radially arranged hyphae protruding from the infiltration (arrow).

Fig. 4.4. Case 2. Left eye: Ulcer with feathery broadening hyphae (white arrow). Pyramid-shaped hypopyon (black arrow).

4.4.4
Further Diagnostic Measures in Keratomycosis

The confirmation of a clinically suspected diagnosis of keratomycosis depends on the demonstration of fungi. This requires the collection of material
that actually contains fungal elements. A positive result, however, is only rarely achieved from a specimen such as a conjunctival smear or a superficial sponge biopsy of the cornea, or from an impression product [182]. In order to include the pathogens lying under the epithelialized ulcer margins, it is necessary to remove, with the aid of a rigid spatula, sufficient corneal tissue (epithelium
Fig. 4.7. Case 3. Secondary fungal infection (by *Trichophyton rubrum*) complicating recurrent herpes simplex keratitis.

Table 4.6. Characteristics of keratomycosis

<table>
<thead>
<tr>
<th>Geographical configuration</th>
<th>Poorly margined feathery infiltration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Satellite phenomenon</td>
<td></td>
</tr>
<tr>
<td>Infiltrations commonly covered by epithelium</td>
<td></td>
</tr>
<tr>
<td>Prominent corneal areas, Kaufman and Wood, 1965 [173]</td>
<td></td>
</tr>
<tr>
<td>Viscous, pyramid-shaped, convex hypopyon, Behrens-Baumann, 1991 [170]</td>
<td></td>
</tr>
</tbody>
</table>

and superficial stroma [183]. Ophthalmologists are understandably reluctant to remove corneal substance from the ulcer, but from a diagnostic as well as a therapeutic viewpoint, this reluctance is misplaced (see chapter 4.4.6.1.1). Lemp et al. [184] recommend that where keratomycosis is suspected, several samples should be collected (twice daily for 3 days or until a positive specimen has been obtained). Sometimes even an abrasion with a sharp instrument does not lead to a diagnostic result, and a corneal biopsy (keratectomy) is required to obtain sufficient material for a diagnosis [185–187].

The specimens removed are placed in culture medium and transferred to a mycological laboratory (see chapter 1.8.2). Diagnosis by culturing takes 3–6 days, in the case of some fungi several weeks. It is thus useful to examine part of the specimens under a microscope in order to distinguish primarily between
fungal and bacterial pathogens. Furthermore, the diagnostic positive rate is higher than that achieved by culturing [160]. Suitable stains include a 10% potassium hydroxide solution [188] with ink in the proportions 9:1 [189], gram stain, and Giemsa stain. The best is said to be a modified methenamine-silver stain, which takes 1 h to prepare [190]. An even faster method (2 min) is to stain the fungi with Calcofluor 1% and Evans blue 1% and to observe them under a fluorescence microscope [189, 191–193]. Lactophenol cotton blue mounts of corneal scrapings are also recommended [194]. Confocal microscopy provides a new, noninvasive way of imaging the human cornea in vivo. Fungal hyphae are visualized as high-contrast filaments about 6 \mu m in diameter and 60–400 \mu m in length [195–197]. Another technique in identifying fungal elements may be the polymerase chain reaction [198].

In case of an emergency graft, it is useful to divide the corneal disc along the middle of the ulcer. One half can be passed on for microbiological diagnosis whilst the other is used for histopathological examination. In a study in which 61 corneal discs were thus examined, the histopathological examinations were positive in 77.04% and the culturing in 75.4% [199].

4.4.5

Histopathology of the Keratomycoses

The fungal elements can be visualized histologically by periodic acid-Schiff reagent, Gridley’s stain for fungi, or Gomori’s methenamine-silver stain [78, 100, 200–203]. A particularly useful method of visualizing fungi is with the use of fluorescein-conjugated lectin concanavalin A [192, 193]. By means of polymerase chain reactions, fungal DNA can be detected even in fixed tissue [204].

Although the fungi penetrate the cornea [110, 205, 206], this is not inevitably associated with necrosis of the overlying tissues [114]. The pathogens are consequently situated deep in the cornea [207] and are thus inaccessible for superficial diagnostic sponge biopsies [100, 208]. For the same reason, it is difficult for antifungal agents to reach fungal elements [209].

The ‘satellite phenomena’, a clinical characteristic of fungal infections, present a histological picture of microabscesses around fungal elements. They are situated in the periphery of the crater of the ulcer. The granulocytic infiltration is less marked and more focal than in bacterial keratitis [200]. The Wessely immune ring [173, 210], an occasionally observed clinical feature, corresponds histopathologically to a ring abscess [100]. In infectious crystalline keratopathy, the microorganisms produce a ruthenium red-staining glycocalyx, which may be visualized as a biofilm by electron microscopy [176]. The hyphae
may lie parallel to the corneal lamellae, but may also be in a perpendicular position [199]. The perpendicular direction has been interpreted as indicating prior treatment with corticosteroids. In rabbit models, however, this arrangement of the hyphae may be found also without drug treatment [209].

4.4.6
Treatment of Keratomycosis

Fungal keratitis should initially be treated with drugs, provided that the inflammatory process is not too far advanced. Surgical therapies are available as an alternative.

4.4.6.1
Medical Treatment

4.4.6.1.1
Antifungal Agents

Several agents are available for the treatment of keratomycosis, and these have been described in detail in chapter 2. As a rule, they are applied topically, as this results in higher tissue concentrations in the anterior parts of the eye than can be achieved by systemic administration Havener in 1983 [211], and furthermore the risk of systemic side-effects is low. Therefore, the prescription of oral antifungal agents [161, 212] should be considered only if the topical treatment has been unsuccessful, and endophthalmitis has developed.

A medical treatment regimen for keratomycosis is outlined in table 4.7. The choice of antifungal agent must take account of regional variations in the prevalence of fungi. As in the temperate zone candidal keratomycosis is the most common form (see chapter 4.4.2), amphotericin B is the standard antifungal agent [213, 214]. Drops for instillation are prepared as described in table 2.2. Amphotericin B with its high molecular weight of 924.11 daltons hardly penetrates the corneal epithelium, and a corneal abrasion should thus be carried out. This ‘debridement’ [102], which, depending on the result, should be repeatedly carried out, can serve at the same time to confirm the diagnosis. In Fusarium or Aspergillus keratomycosis, on the other hand, natamycin 5% is more effective than amphotericin B [164, 215].

Fluconazole has become established as an alternative to amphotericin B, due to its good tolerability and its effective penetration into the cornea and anterior chamber, even without abrasion [216]. It has been used successfully in candidal keratitis [217, 218], and has also been used orally in this indication [219].

---

4 Mycoses of the Anterior Segment of the Eye
Table 4.7. A medical treatment regimen for keratomycosis

| Topical (every hour) | fluconazole 0.2% drops (for *C. albicans*) (tables 2.9 and 2.10, p. 37, 38)  
| or natamycin 5% (for *Fusarium* spp.)  
| or amphotericin B 0.15-0.5% drops (table 2.2) after corneal debridement, provided that there is no extensive epithelial defect at night, a corresponding ointment or gel |
| Subconjunctival (if desired) | miconazole, 5–10 mg |
| Systemic | as a rule, pharmacokinetically not rational  
| if used, fluconazole, 2 × 200 mg  
| or itraconazole, 2 × 200 mg |

Miconazole 1%, flucytosine 1% and nystatin eye-drops may be used as second-line drugs. Collagen shields have been used as vehicles for medication [220]. Possibly, chlorhexidine gluconate and other antiseptics may be beneficial [221, 222].

4.4.6.1.2 Antifungal Agents plus Corticosteroids

It may seem surprising, in view of the fact that corticosteroids are thought to be predisposing factors for keratomycosis, that these agents are considered for medical treatment of the condition. However, the results of animal experiments (see chapter 7.4.3) suggest that low-dose dexamethasone (at a concentration of 0.01%) may have a beneficial effect [223]. Subconjunctival injections of 4 mg of dexamethasone every 2 days also proved more effective in reducing secondary inflammatory reactions in the cornea and anterior chamber and neovascularization in animal experiments than antifungal therapy alone [224]. These results are validated by clinical experience of the combination therapy. Roberts [225] found that use of corticosteroids in addition to antifungal therapy was not followed by any deterioration in the course of *Candida* keratitis; in fact, he described a definite reduction of iritis. Similar results were reported by O’Day et al. [226] in *Aspergillus* keratitis; it was not until 4 mg subconjunctival dexamethasone twice daily and hourly drops of prednisolone acetate were added to the antifungal treatment that the corneal oedema and intraocular inflammation were seen to subside.

Nevertheless care should be used in the additional administration of corticosteroids [227], particularly as some authors regard them as contraindi-
cated in fungal keratitis [228, 229]. They should only be given under strict monitoring, and in the later stages when the infiltrative process has been arrested. Considerable importance obviously attaches to the time factor and therefore to the stage of the keratitis (see chapter 7.4.3), which bears out the findings of [72] in 19 patients with fungal keratitis, who were treated with topical steroids for 24 days after a period of antifungal therapy averaging 14 days. Two of the 19 patients received topical corticosteroids within 1–3 days of starting a topical antifungal therapy, and clinical examinations showed their conditions to have worsened. Finally, it is essential that the patient is immunocompetent, as otherwise systemic dissemination of the fungi cannot be ruled out [226].

4.4.6.2

Surgical Treatment

The treatments formerly recommended for keratomycosis included keratoc-tectomy, lamellar keratoplasty [98, 230, 231], or a conjunctival graft [98]. In addition, cryotherapy was used in animal experiments [232]. As in the case of corneal ‘abrasion’ or ‘debridement’ [102], keratectomy may be useful for diagnostic purposes and to improve the pharmacokinetics of drug treatment, with the partial removal of the pathogens supporting the medical treatment [113]. A lamellar keratoplasty may also be justified on technical reasons if the focus is situated peripherally at the limbus [106]. As a surgical treatment, however, all of these methods appear to be of little benefit [233]. Either the pathogens are still in the superficial layers of the cornea, in which case medical treatment is likely to be successful and less invasive, or the infection has penetrated so deeply as to be no longer accessible to these methods.

In advanced cases where medical treatment cannot halt the process, it is preferable to carry out an emergency graft [1, 158, 187, 233–246]. In 34 of 125 patients (27%) with fungal keratitis, therapeutic penetrating keratoplasty was performed, typically within 4 weeks of presentation (74%), for medical treatment failure (56%), corneal perforation (32%) and recurrent keratitis while receiving medical therapy (12%) [72]. A repeat keratoplasty was required in 8 patients, 5 for graft failure and 3 for recurrent *F. oxysporum* keratitis. Keratoplasty has the advantage that it involves removal not only of the pathogenic organisms, but also of the leukocyte and fibrin accumulations that contribute to tissue damage. In addition, it prevents the vascularization that inevitably occurs in prolonged courses and worsens the prognosis of a subsequent, optical keratoplasty [247]. Good results have been obtained in other studies [246, 248], with up to 73% of grafts remaining clear after variable periods of follow-up (1 month to 9 years) and accelerated rehabilita-
tion. Sometimes large penetrating keratoplasties (recipient bed ≥9.5 mm) are necessary to preserve the structural integrity of the globe [249]. It is thus advisable not to wait too long before performing an emergency graft (Fig. 4.8).

However, when emergency grafts in microbial keratitis were compared with a group grafted subsequently for scarring after complete resolution of infection and inflammation, a clear difference in graft survival was evident, with a 51% 5-year survival rate for emergency grafts versus 90% for grafts performed in quiet eyes [245].

Topical and systemic corticosteroids should be used in an effort to reduce inflammation before surgery, when this is indicated, to reduce the incidence of postinflammatory sequelae and improve the prognosis for visual recovery [246]. Corticosteroids are also indicated postoperatively and do not cause recurrences if the fungus-infiltrated area has been surgically removed. As a safety measure, however, postoperative continuation of antifungal treatment is recommended. Failure to seal a perforation with cyanoacrylate adhesive in active keratitis is an absolute indication for penetrating keratoplasty, as irreversible synechial angle closure and secondary glaucoma will result if the anterior chamber remains flat, and there is a significant risk of spontaneous expulsive haemorrhage in the inflamed hypotonic eye [186].
4.4.7

Case Histories

A few examples of keratomycoses are presented below.

4.4.7.1

Case 1

A 40-year-old, generally healthy forester presented after a foreign-body injury of the right eye. He was treated with topical antibiotics (chloramphenicol and kanamycin) for several weeks. On admission he had an ulcer with hypopyon (fig. 4.1), which despite 3 weeks of antibiotic treatment increased in size (fig. 4.2). A conjunctival smear was negative, but *A. fumigatus* was isolated from a corneal abrasion. With the use of nystatin drops and ointment, with later use of natamycin ointment, the ulcer subsided after 53 days.

4.4.7.2

Case 2

A 60-year-old woman, who had elsewhere undergone extracapsular cataract extraction with posterior chamber lens in both eyes, presented with postoperative recurrent intraocular inflammations, which had been treated with antibiotics and corticosteroids. She was admitted to hospital 6 months after the extractions. The right eye showed corneal infiltration without inflammation of the anterior chamber (fig. 4.3). The left eye showed a corneal ulcer with radial infiltrations and hypopyon (fig. 4.4). *C. albicans* and *Staphylococcus epidermidis* were isolated from a smear. Bilateral treatment with topical amphotericin B and antibiotics (polymyxin, neomycin and bacitracin) reversed the infiltration in the right eye. As the left eye failed to improve within one week, penetrating keratoplasty was carried out (fig. 4.8), which was followed by subsidence of the intraocular inflammation.

---

Fig. 4.9. Case 4. Fourteen days after removal of the corneal stitches. Two discrete infiltrations without epithelial defect.
Fig. 4.10. Case 5. *Cryptococcus laurentii* corneal ulcer complicating herpes simplex keratitis.

Fig. 4.11. Same case as in figure 4.10. Marked regression of corneal vascularization after 14 months.
4.4.7.3  
Case 3  
A 10-year-old boy presented with recurrent herpes simplex keratitis in both eyes. *Trichophyton rubrum* was cultured from a corneal abrasion (fig. 4.7). Onychomycosis of the hands and feet was diagnosed at the same time. Treatment with amphotericin B eye-drops was followed by penetrating keratoplasty.

4.4.7.4  
Case 4  
A 41-year-old man had undergone penetrating keratoplasty 5 months previously for keratoconus, Amsler stage IV. Since then, he had used topical prednisolone acetate. After removal of the sutures, 2 discrete white infiltrates of the stroma were seen at the margin of the transplant (fig. 4.9). *C. albicans* was cultured from a conjunctival smear and from the faeces. The infiltrates disappeared within 19 days in response to nystatin eye-drops and natamycin ointment.

4.4.7.5  
Case 5  
A 33-year-old woman presented with a corneal ulcer and increasing vascularization following recurrent herpes simplex keratitis (fig. 4.10). Cryptococcus laurentii was cultured from a corneal abrasion and treatment started with nystatin eye drops (chapter 2, table 2.4) 10 times daily for 2 months following 4 times daily for 1 month. Subsequently, topical steroids were added, which resulted in regression of corneal vascularization (fig. 4.11).

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References


References
5.1
Pathogenesis and Clinical Features

Fungal endophthalmitis arises mainly via two pathogenetic pathways. Endogenous (metastatic) endophthalmitis commonly occurs as a result of sepsis, and must be distinguished from exogenously caused endophthalmitis, which can occur as a result of an operation or injury. Persistent keratomycosis may also lead to endophthalmitis.

5.1.1
Endogenous Fungal Endophthalmitis

5.1.1.1
Pathogens

The first description of endogenous endophthalmitis was by von Virchow [1] in 1856, but there was no microbial differentiation and the disease may also have had a bacterial cause. Both unilateral and bilateral ‘metastatic ophthalmic diseases’ have been described in the literature [2, 7]. The first description of endogenous fungal endophthalmitis was by Dimmer [2] and not by Miale [5] as mostly reported in the English-language literature. Stock [7] and Kreibig [4] had also published their cases earlier. The fungal species reported as causes of endogenous endophthalmitis are listed in table 5.1. It should be noted here that in the 1990s a change occurred in the causes of candidemia, with non-
C. albicans species increasing; this is ominous because of their in vitro resistance to most of the currently available antifungal agents [8].

5.1.1.2
Causes

The causes of endogenous endophthalmitis are varied. A review of the literature reveals the predisposing factors listed in table 5.2. Immunosuppres-
<table>
<thead>
<tr>
<th>Fungus</th>
<th>Authors and year of description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alternaria sp.</strong></td>
<td>Satpathy, 1997 [296]</td>
</tr>
<tr>
<td><strong>Blastomyces sp.</strong></td>
<td>Sinskey and Anderson, 1955 [315], Safneck, 1990 [316]</td>
</tr>
<tr>
<td><strong>C. immitis</strong></td>
<td>Luttrull et al., 1995 [267], Cunningham, 1998 [329]</td>
</tr>
</tbody>
</table>
Table 5.1 (continued)

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Authors and year of description</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Curvularia</em> sp.</td>
<td>Satpathy, 1997 [296]</td>
</tr>
<tr>
<td><em>Fusarium</em> sp.</td>
<td>Satpathy, 1997 [296]</td>
</tr>
<tr>
<td><em>Helminthosporium</em> sp.</td>
<td>Satpathy, 1997 [296]</td>
</tr>
<tr>
<td><em>Paecilomyces</em> sp.</td>
<td>Lam et al., 1999 [384]</td>
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<td><em>Penicillium</em> sp.</td>
<td>Swan et al., 1985 [345]</td>
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<tr>
<td><em>Petriellidium</em> sp.</td>
<td>Bohigian, 1986 [346]</td>
</tr>
<tr>
<td><em>Phialophora</em> sp.</td>
<td>Satpathy, 1997 [296]</td>
</tr>
<tr>
<td><em>Pseudoallescheria</em> spp.</td>
<td>Stern et al., 1986 [347], Heinsius, 1950 [3], von Herrenschwand, 1932 [348], Kreibing, 1940 [4], Nover, 1950 [6], Sañez et al., 1990 [316], Caya, 1988 [103], Pfeifer, 1991 [272]</td>
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<td><em>Rhizopus</em> sp.</td>
<td>Satpathy, 1997 [296]</td>
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<tr>
<td><em>Rhodotorula</em> sp.</td>
<td>Satpathy, 1997 [296]</td>
</tr>
<tr>
<td><em>Sporotrichum</em> sp.</td>
<td>Cassiday and Foerster, 1971 [349], Küper, 1962 [350]</td>
</tr>
<tr>
<td><em>Syncephalostrom</em> sp.</td>
<td>Satpathy, 1997 [296]</td>
</tr>
</tbody>
</table>

Table 5.2. Predisposing factors for fungal septicemia and endogenous endophthalmitis

<table>
<thead>
<tr>
<th>Factor</th>
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</thead>
<tbody>
<tr>
<td>Reduced immune defences, e.g. diabetes, chronic alcoholism, pancreatitis, malignancy</td>
</tr>
<tr>
<td>Hemodialysis, abdominal surgery</td>
</tr>
<tr>
<td>Parenteral hyperalimentation</td>
</tr>
<tr>
<td>Organ transplantation, immunosuppressive therapy</td>
</tr>
<tr>
<td>Indwelling intravenous catheter</td>
</tr>
<tr>
<td>Prolonged antibiotic therapy (?)</td>
</tr>
<tr>
<td>Intravenous drug abuse</td>
</tr>
<tr>
<td>AIDS</td>
</tr>
<tr>
<td>Prematurity</td>
</tr>
</tbody>
</table>

sion, either due to systemic disease or iatrogenically induced through immunosuppressive therapy, seems to be one of the main reasons for fungal septicemia, the prerequisite of fungal endophthalmitis. Such systemic diseases include malignancies, pancreatitis, diabetes mellitus, and alcoholism [9–18]. Iatrogenic factors include hemodialysis [19], systemic (especially gastrointestinal) surgery
and intravenous hyperalimentation [19–29] and transplants [30–37]. One case of *C. tropicalis* endophthalmitis as the only initial manifestation of pacemaker endocarditis has been described [38]. Three cases of invasive *Aspergillus* infections complicating coronary artery bypass grafting [39] and several cases of *C. albicans* endophthalmitis after lithotripsy of a kidney stone have also been reported [40–43].

Indwelling *intravenous catheters* are often implicated [15, 26, 29, 44–54]. In a series of culture-proven fungal endophthalmitis in 20 eyes of 18 patients, the most common association was long-term intravenous line placement (> 2 weeks), which was present in 12 patients (67%). After initial examination, only 2 patients had a systemic culture positive for a fungal organism (none had a positive blood culture) [21]. If a peripheral venous line remains in place for longer than 48 h in patients undergoing intensive care, a 0.1% incidence of sepsis must be expected, and this may rise to 1% with central venous catheters [45]. After *Staphylococcus aureus* and *S. epidermidis*, the next most common pathogenic organisms are fungi, which account for 10% of systemic infections. Arteriovenous fistulae used for hemodialysis are also a potential source [50, 55].

The administration of *systemic antibiotics* is also thought to favor fungal septicemia [50, 51, 56–58], though in vitro and in vivo experimental studies do not support this [59]. It must be remembered that patients with impaired immune defences receive antibiotics, and it is the underlying impairment that allows fungal sepsis to develop.

Another disease category to be considered is drug addiction [60–87]. General fungal diseases have also been described in association with *AIDS* [88–101], and include fungal endophthalmitis [88, 91, 102–106]. In 1 case of AIDS, *Cryptococcus* sp. was detected in an iris inflammatory mass [107], and 1 patient with limbal nodules was also found to have multifocal choroiditis [108]. Of 235 consecutive autopsies of patients with AIDS, 18 were found to have infectious choroiditis, including *C. neoformans, P. carinii, M. tuberculosis, H. capsulatum, Candida* spp., *A. fumigatus, Toxoplasma gondii* and *M. avium-intracellulare* [109].

The first case of *Candida* endophthalmitis in *premature infants* was noted in 1972 [110], and since then additional reports have been published [111–113]. The prevalence of retinal findings in systemic candidiasis in premature infants may reach 50% [114], but this was not confirmed in another study of 15 babies, in whom no retinal involvement was found despite repeated fundoscopic examination [115].

One case of *C. albicans* endophthalmitis has been reported after anabolic steroid abuse in an athlete [116], and a unique case of *A. flavus* endophthalmitis associated with periodontitis [117]. *C. albicans* endophthalmitis after induced abortion has been reported recently [118].
Fungal sepsis is likely to be associated with ocular involvement in 2.8–50% of cases [19, 23, 119, 120]. In a prospective study of 32 patients with positive blood cultures, chorioretinitis was observed in 28% [121], and in another study of 46 candidemic patients was observed in 13% of cases [122]. In a larger study of 108 patients, however, Candida chorioretinitis could only be found in 9% [123]. Fraser et al. [124] reviewed 105 patients with candidemia who underwent ophthalmoscopy and did not diagnose any cases of endophthalmitis. These authors suggest that this may be due to the lack of sequential evaluation by ophthalmologists. In a more recent report [125], a high rate of hematogenous candidal endophthalmitis (50%) was found in patients with postoperative candidemia. In that study, ophthalmoscopy was performed by the same ophthalmologist 5–8 days after the onset of symptoms of sepsis and was repeated after 5 days. Thus, Candida endophthalmitis may be more common than previously thought. Follow-up of patients by qualified personnel should help to identify this complication. In the most recent study of 107 patients, endophthalmitis was found in only 2.8% (3 patients) [119], which was ascribed to the fact that these patients received very early systemic therapy.

In contrast, endophthalmitis may also occasionally be the first sign of fungal septicemia [126, 127]. Of 10 patients with Candida endophthalmitis in an intensive care ward, the 80% mortality rate was clearly increased compared with an overall mortality of 17% for all patients in the surgical intensive care unit of that hospital [128]. It should also be noted that ocular lesions preceded symptomatic meningitis in 6 of 22 patients (27%) with CNS involvement [129].

In patients with the risk factors shown in table 5.2, therefore, regular examinations by an ophthalmologist should be undertaken [15, 127, 130]. This is particularly important in unconscious patients, who are unable to complain of visual symptoms. In addition, detection of antigen by means of a monoclonal antibody or class-specific antibody detection, including amplification procedures has been recommended for the diagnosis of systemic Candida infection [131].

5.1.1.3 Clinical Features

Initially, the eye is externally free of irritation and does not suggest inflammation. Even using a slit-lamp microscope, irritation of the anterior chamber can only be detected later. The patient does not initially complain of pain. After progression of endophthalmitis, blurred vision, photophobia and a red eye may develop (table 5.3). In addition, patients may complain of cobwebs, floaters, or a veil across their vision. Debilitated and extremely
ill patients may be unable to describe their visual symptoms or may be unaware of them.

The first signs of intraocular infection are white, creamy lesions at the posterior pole [47, 78, 132, 133], which are occasionally prominent (fig. 5.1–5.3). The lesions may be single or multiple. Both the retina [127, 134, 135] and the choroid [133] may be affected [29, 136]. The foci may be surrounded by hemorrhage and appear similar to Roth’s spots (fig. 5.1) [134, 137].

Cotton-wool spots have also been described [138]. In addition, the vitreous body is infiltrated, where snowball and string-of-pearls formations are characteristic (fig. 5.4–5.7) [127, 139–141]. Long-term observations are necessary, as in 6 cases of Candida endophthalmitis successfully treated with amphotericin B, choroidal neovascularization occurred within 2 weeks to 2 years [142]. There are 2 other case reports of choroidal neovascularization in Candida
Fig. 5.2. The same case as in figure 5.1, 7 days later, showing intensification of the infiltration into the retina and slow absorption of the hemorrhages.

Fig. 5.3. The same case as in figure 5.2, 4 weeks later, showing regression of the infiltration of the fovea.
endophthalmitis [385, 386]. Papilledema, retinal detachment and iritis with hypopyon may also occur [12]. Finally, episcleritis has been observed [78], and *Mucor* endophthalmitis has been wrongly diagnosed as Coats’ disease [143].

The above causes and clinical symptoms apply to endogenous endophthalmitis caused by a variety of different fungi, but especially by *Candida* spp. Infections by some fungi have additional specific features, which are described below.
5.1.1.3.1 Endogenous *Aspergillus* Endophthalmitis

The most common manifestation of aspergillosis is pneumonitis, and this occurs most commonly in immunocompromised hosts [144]. Because blood cultures are often negative for *Aspergillus* spp., the eye may be the only site
from which a positive culture is obtained. *Aspergillus* endocarditis has been reported as a particular risk factor for endophthalmitis [145–149]. This is controversial concerning orthotopic liver transplantation [150–153]. *Aspergillus* spp. are second only to *Candida* spp. as the etiological agent in endogenous endophthalmitis in drug abusers [71]. These healthy-appearing individuals present with a red, painful eye and decreased vision. Uveitis is a common initial diagnosis and is frequently treated with corticosteroids, resulting in exacerbation of the infection and delay in initiating appropriate treatment. The patient should be questioned about drug abuse and examined for evidence of multiple venepuncture sites. Almost all patients with *Aspergillus* endophthalmitis have acute anterior uveitis with ocular pain, redness and blurred vision [154]. In a series of 12 eyes with culture-proven endogenous *Aspergillus* endophthalmitis, 8 eyes revealed a central macular chorioretinal inflammatory lesion [155]. *Aspergillus* even involves the optic nerve [156]. Sandwich ELISA, which detects *Aspergillus* galactomannan, may be helpful in establishing an early diagnosis [157].

5.1.1.3.2

**Endogenous Cryptococcus Endophthalmitis**

When *Cryptococcus* sp. is the cause, there may be anterior segment inflammatory signs, including keratic precipitates and posterior synechiae [158]. The posterior segment lesions are often elevated and larger than *Candida* lesions. They may contain overlying retinal telangiectasia [158, 159]. The lesions may be confused with *Toxoplasma* retinochoroiditis [158, 160], ocular sarcoidosis, or tuberculosis. Histopathological pictures of optic nerve involvement have been shown by Friedenwald et al. [161].

*Cryptococcus* spp. are an important cause of morbidity and mortality in immunocompromised patients, especially in AIDS [100, 162]. The infection is usually acquired through inhalation. *Cryptococcus* sp. has been identified in an inflammatory mass in the iris of a patient with AIDS [107], and cryptococcal optic neuropathy has been described in further cases [163, 164]. In a patient with AIDS who had cryptococcal meningitis, sudden simultaneously bilateral blindness occurred. Both optic discs and retinas appeared normal. Lumbar puncture showed an opening pressure of 600 mm H₂O. At autopsy, fulminant necrosis of both optic nerves was found, with cryptococcal organisms throughout the basal meninges and in the sheaths of both optic nerves [165].

In a series of 80 patients seropositive for HIV and with cryptococcal infection, papilledema was observed in 26 patients (32.5%) [166]. Papilledema with visual impairment was found in 2 patients with cryptococcal meningitis, and, at autopsy, cryptococcal organisms were found in the optic nerve sheath but not in the optic nerve [167]. Of 6 patients with papilledema, 3 had loss
of visual acuity with multiple cryptococcal abscesses, while the other 3 with normal function had only minimal or no involvement of the visual pathways [168]. In another series of 36 patients with cryptococcal meningitis, papilledema was noted in 12 and extraocular muscle paresis in 5 cases [169]. Most patients with ocular involvement also have CNS infection [37, 158–160, 170–172].

In contrast, patients with cryptococcal meningitis may develop visual loss in the absence of other ocular lesions. There are 2 distinct patterns of visual loss: rapid visual loss within 12 h indicating direct invasion of the optic nerve by C. neoformans, and slow visual loss due to elevated CSF opening pressure [173].

In a patient intubated for 7 days and treated with intravenous methylprednisolone for status asthmaticus, an anterior chamber mass was identified on histopathology as A. fumigatus. Ocular and blood cultures were negative, as were the results of bone marrow biopsy, lung biopsy and anterior chamber paracentesis. Computed tomography showed a solitary lesion in the left lower pulmonary lobe and multiple brain lesions [174]. The patient responded to amphotericin B and flucytosine.

C. neoformans infection can be accurately and rapidly detected with a latex reagent for antigen detection [131].

5.1.2
Exogenous Fungal Endophthalmitis

5.1.2.1
Causes

5.1.2.1.1
Postoperative Fungal Endophthalmitis

Fungal infections following operative procedures on the eye were described early this century, when fungal endophthalmitis was reported after a retinal operation [175] and following cataract extraction [176]. In later publications, the infection was most often described following cataract surgery (table 5.4), even after sutureless small-incision phacoemulsification cataract surgery [177]. In a series of 15 and 13 cases of extracapsular cataract removal, the irrigation fluid was contaminated with C. parapsilosis, respectively [178, 179], and in another series of 13 cases with P. linaceus [180]. In sporadic infections, however, it is more likely that the pathogens originate from the conjunctival sac or the lid margins, particularly as fungi are found there even in healthy individuals (see chapter 4, p. 68). This mode of infection is also likely in the case of bacterial postoperative endophthalmitis [181].
Table 5.4. Fungal endophthalmitis after cataract surgery

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<td>Borne et al.</td>
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<td>Greetham and Makley</td>
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<td>Jones</td>
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<td>Kauffman et al.</td>
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<td>O'Day et al.</td>
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<tr>
<td>Weissgold</td>
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<td>Donor history</td>
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<td>Kincses et al., 1972</td>
<td>55</td>
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<td>83; a, b</td>
<td>25-year-old, immunosuppression-related myositis</td>
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<td>Larsen et al., 1978</td>
<td>76; a, b</td>
<td>31-year-old, car accident</td>
</tr>
<tr>
<td>Doughman et al., 1982</td>
<td>59; b</td>
<td>–</td>
</tr>
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<td></td>
<td>76; a, b</td>
<td>–</td>
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<tr>
<td>Levenson et al., 1984</td>
<td>29; keratokonus</td>
<td>–</td>
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<td>Stuart et al., 1985</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Insler et al., 1987</td>
<td>78; a, b</td>
<td>30-year-old, brain hemorrhage removed &gt; 3 days</td>
</tr>
<tr>
<td></td>
<td>66; a, b</td>
<td>30-year-old, brain hemorrhage removed &gt; 3 days</td>
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<tr>
<td>Weiss et al., 1987</td>
<td>61; triple procedure</td>
<td>34-year-old, trauma, removed at 40 min</td>
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<td>Fong et al., 1988</td>
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<td>Arocker-Mettinger et al., 1988</td>
<td>78; corneal dystrophy</td>
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<td>Perry et al., 1990</td>
<td>72; a, b</td>
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### Table 5.5 (continued)

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<th>Fungus</th>
<th>Treatment</th>
<th>Outcome</th>
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<td>22; keratoconus</td>
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<td>K sol 14 days</td>
<td><em>C. glabrata</em></td>
<td>amphotericin B, miconazole, natamycin, fluocytosine</td>
<td>TP clear</td>
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<tr>
<td></td>
<td>17; keratoconus</td>
<td>52-year-old, cardiac arrest</td>
<td>chondroitin sulphate medium 8 days</td>
<td><em>C. albicans</em></td>
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<td>TP clear</td>
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<tr>
<td></td>
<td>80; b</td>
<td>68-year-old, cardiac arrest</td>
<td>MCK 25 days</td>
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<td>amphotericin B, natamycin, miconazole</td>
<td>enucleation</td>
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<td>K sol (100)</td>
<td><em>C. glabrata</em></td>
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<td>Behrens-Baumann et al., 1991 [186]</td>
<td>76; a, b</td>
<td>54-year-old, alcoholic</td>
<td>MCK 22 h</td>
<td><em>C. tropicalis</em></td>
<td>amphotericin B, natamycin</td>
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<td>Kloess et al., 1993 [380]</td>
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<td>Dextral (100)</td>
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<td>eye bank</td>
<td>transport medium</td>
<td><em>C. glabrata</em></td>
<td>amphotericin B</td>
<td>TP clear</td>
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a = Aphakia or pseudophakia; b = bullous keratopathy; TP = transplant; MCK = McCarey-Kaufman medium; KP = keratoplasty.

Fungal infections following keratoplasty have been described repeatedly (table 5.5). This is understandable, as bacteria and viruses are also known to be transmitted [182–185]. True transmission from donor to recipient [186–188] must be distinguished, however, from perioperative infection (contamination of the preserving fluid and contamination by local pathogens). Preoperative infection must also be considered. In the case reported by Ross and Laibson [189] of a *C. albicans* infection following keratoplasty, the operation was performed on an eye that had suffered severe prior damage by descemetoceles, as was also found for the infection of transplants by *P. lilacinus* described by Gordon and Norton [190] and for 3 cases of *Fusarium* keratitis [191]. In the case described by Rao and Aquarella [192], a preoperative perforated ulcer was treated with a soft contact lens.

Contamination of the donor material may indeed be considerable. Of 70 transplants, the corneal margin and McCarey-Kaufman medium were contaminated in 14 cases; in two cases of contamination *C. parapsilosis* and *Aspergillus* sp. were found, respectively, the other were bacterial pathogens [193]. In a
more recent study of 9,250 organ-cultured corneas, 5% were discarded because of bacterial or fungal contamination [194].

An uncomplicated mitomycin C trabeculotomy with subsequent *A. niger* endophthalmitis was also recently reported [195].

5.1.2.1.2

**Fungal Endophthalmitis following Injury**

Fungal infection of the vitreous body following penetrating injury was reported a long time ago [196–199]. Since then, further trauma-induced mycoses have been described [200–207, 387]. Both plant material [161, 208–211] and metal foreign bodies [205, 208, 212–215] have resulted in intraocular fungal infection following penetration of the eyeball.

5.1.2.1.3

**Fungal Endophthalmitis following Keratomycosis or Scleritis**

In 1896, Schirmer [216] reported a ‘case of mould keratitis’ in which cord formation starting from a corneal ulcer was noted in the eyeball. Further observations have been published [191, 217–222]. A case of keratomycosis with endophthalmitis was reported following a photo-refractive keratectomy with the use of a disposable contact lens [223]. In each case of persistent keratomycosis, endophthalmitis may finally develop as pathogens penetrate into the anterior chamber and progress into the deep segments of the eye. Endophthalmitis, leading to enucleation, developed in a similar way from *A. niger* scleritis [224]. The reverse, i.e. keratomycosis following *Candida* endophthalmitis, has also been described [225, 226].

5.1.2.2

**Clinical Features and Diagnosis**

In comparison with acute bacterial infection, a more prolonged period relatively free of inflammation occurs postoperatively or posttrauma. This period is usually about 1–4 weeks [227, 228] but may last much longer [229]. Inflammation of the anterior chamber and visual impairment occur to a variable degree.

Hypopyon may disappear transiently and the fungal inflammation may simulate the *Propionibacterium acnes* syndrome [230]. Typically, there are gray-white infiltrates in the anterior part of the vitreous body and a fibrinous exudate in the anterior chamber becoming increasingly tougher.

In contrast to this typically insidious course of postoperative fungal infection, bacterial infection may occasionally be confused with intense irritation occurring shortly after trauma. In 2 cases of endophthalmitis caused by *C. pa-*
rapsilosis, the first clinical symptoms began as early as 3 days after extracapsular cataract extraction with posterior chamber lens [229].

Confirmation of the clinically suspected diagnosis of exogenous endophthalmitis is obtained by aspiration of the anterior chamber, repeated if necessary. In pseudophakia, the material from the anterior part of the vitreous body is positive more often than is an aspirate of the anterior chamber [180]. A microscopic study with gram, Giemsa or 10% potassium hydroxide staining can provide rapid clarification (see chapter 4, p. 87). Mycological differentiation may take 1 week or longer (see chapter 1, p. 23). A negative result is obtained in about 50% of samples and does not exclude a fungal infection [180]. On the other hand, negative bacterial cultures, the results of which are available within 2–3 days, suggest a fungal disease. Levels of D-arabinitol, a major metabolite of *Candida* spp., may be increased in the serum (normal value 4.4 ± 3.1 μmol/l) and vitreous body [231]. In addition, the *Candida* hemagglutination test may be useful as a screening test and for monitoring the course of the disease [232]. Moreover, polymerase chain reaction may aid in the detection of fungal DNA in small intraocular samples [233].

### 5.2 Treatment of Fungal Endophthalmitis

The treatment of fungal infections of the interior of the eye is oriented towards the etiology, individual initial findings, and the course of the disease. Although local therapy (drug and/or surgical) may be sufficient in exogenous endophthalmitis, endogenous causes usually require additional systemic administration of 1 or 2 antimycotic agents. Moreover, in endogenous fungal infection one should distinguish between choroiditis and endophthalmitis with regard to an adequate treatment [234].

#### 5.2.1 Drug Treatment

**Local Antimycotic Therapy**

Treatment with drops or subconjunctival application alone is not useful in endophthalmitis, as the antimycotic agents used do not penetrate sufficiently into the deep vitreous body. Only in cases in which the infection is limited to the anterior section of the eye may superficial therapy be useful [235, 236]. Intracameral administration [186, 237] in intracapsular aphakia or pseudo-
phakia, or intravitreal injection of an antimycotic agent, is currently the most effective treatment of endophthalmitis. The main disadvantage (that of performing an intraocular injection) is balanced by the advantage of achieving a high concentration of the drug. This is particularly the case for amphotericin B, which despite the availability of the new azoles, is the antimycotic of choice in many cases. As this drug may have pronounced side effects when given systemically, it should be applied topically whenever possible. The preparation of amphotericin B solution for intraocular injection is described in chapter 2, table 2.2 (see p. 29). With the development of new agents with more favorable pharmacokinetics and improved fungicidal action, as well as fewer systemic side effects, oral or parenteral administration alone may be effective.

Amphotericin B is usually given in a dose of 5 µg to a maximum of 10 µg, injected into the middle of the vitreous body, together with vitrectomy [63, 67, 78, 160, 205, 208, 210, 213, 238, 240]. The opening of the cannula should not be directed towards the retina [241]. The amphotericin B injection can be repeated if necessary [191]. Intraocular amphotericin B has also been shown to be useful in a rabbit model. In unmodified phakic eyes, Candida-infected eyes, aphakic eyes and aphakic vitrectomized eyes of rabbits, the half-lives of drug clearance after a single 10-µg intravitreal injection were 9.1, 8.6, 4.7 and 1.4 days, respectively [242]. In a case of endogenous endophthalmitis caused by A. terreus, only intravitreal and subconjunctival amphotericin B were applied after vitrectomy; the treatment was successful without systemic administration of the antifungal agent [243].

Alternatively, 40 µg of miconazole has been recommended on the basis of experimental data in animals [244], or 25 µg intravitreally and 10 mg subconjunctivally given clinically [245]. Miconazole should be given particularly in P. lilacinus, as resistance of this fungus to amphotericin B can occur [180, 240]. As the therapeutic range of substances administered by the intravitreal route is limited, it is recommended that the relevant injection volume is prepared by the pharmacist rather than by the ophthalmic surgeon [246].

5.2.1.2

Systemic Antimycotic Therapy

In some patients with endogenous Candida endophthalmitis, vitrectomy with intravitreal injection of amphotericin B but without parenteral therapy was successful [238, 243]. However, systemic antimycotic treatment is recommended in endogenous endophthalmitis, because eye involvement usually indicates that further organs are also affected by the sepsis [12, 15, 19, 247]. In contrast, local treatment alone (operative and drug therapy) may be sufficient in exogenous mycosis.
Amphotericin B has been regarded as the most important antymycotic agent for systemic use [248–251]. Treatment should be administered by an experienced infectious diseases specialist. After administration of a test dose, the dosage is increased daily up to 1 mg/kg body weight/day. Renal function in particular must be monitored (for general information on amphotericin B, see chapter 2, p. 25). After administration of 0.6 mg/kg body weight, the concentration in the vitreous body of 2 patients was 0.1 and 0.23 µg/ml and was therefore within the MIC range for the infecting fungus [252], though in another study it was only 0.04–0.17 µg/ml [253].

As an alternative to amphotericin B, systemic flucytosine, 150 mg/kg body weight/day, is often used, with or without simultaneous vitrectomy [63, 78, 88, 130, 158, 210, 237, 238, 254–257]. A high intravitreal concentration (22.2 µg/ml) could still be measured 18 h after oral administration of flucytosine, 1.5 g [253]. Flucytosine is also prescribed in combination with amphotericin B, as these 2 agents are synergistic [258], and thus the amphotericin B dose may be reduced to 0.5 mg/kg body weight/day. The possibility of development of resistance, even in Candida spp., must, however, be borne in mind [178, 191], and this limits the value of this substance (for general information on flucytosine, see chapter 2, p. 31).

Fluconazole appears to be particularly suitable because of its favorable pharmacokinetics and few side effects [259, 260]. In C. albicans infections, fluconazole is now used as first-line therapy [261, 262] (see chapter 2, p. 36). Candidal endophthalmitis was cured in 15 of 16 eyes (94%), which included 5 infections complicated by vitreitis; successful treatment required the administration of oral fluconazole, 100–200 mg/day for 2 months [263]. In a case of postoperative endophthalmitis caused by C. parapsilosis, the pathogens could not be eliminated despite 4 weeks of oral treatment with ketoconazole, 100 mg twice daily, and 2 doses of intravitreal amphotericin B, 5 µg; after subsequent oral administration of fluconazole, 100 mg twice daily over 4 months, the pathogens could no longer be isolated, and 1 year later the eye was free from irritation with visual acuity of 20/25 [264]. Other favorable results with few side effects have been reported [28, 43, 86, 265–271]. Following oral fluconazole, 400 mg/day, the concentrations of the agent were 15 µg/ml in the vitreous cavity and 19 µg/ml in plasma [268]. In 2 cases of P. boydii endophthalmitis, fluconazole produced no additional effect after miconazole on the progression of the infection in 1 case, and following amphotericin B in the other case [272]. In this report, however, the dose of fluconazole (200 mg/day), was rather low. In a series of 6 patients with Candida endophthalmitis, all eyes were cured by vitrectomy and systemic fluconazole [273]. A report has recently appeared of a case of systemic lupus erythematosus and cryptococcal meningitis with bilateral superior oblique paresis, bilateral optic nerve head swelling and in-
creased intracranial pressure that was treated with oral fluconazole, acetazolamid and dexamethasone, in addition to repeated lumbar punctures to reduce intracranial pressure. This treatment produced a favorable outcome, with recovery of visual acuity from no light perception to 20/20 and normal ocular motility [274].

Oral ketoconazole, 400–600 mg/day, has also been used as an alternative to amphotericin B [191, 238, 257, 275–278]. A concentration of 0.71 µg/ml was found in the aqueous humor and 0.35 µg/ml in the vitreous body 6 h after oral administration of ketoconazole, 600 mg [279]. A disadvantage of this drug is that in vitro resistance of Candida spp. to amphotericin B has developed when both ketoconazole and amphotericin were present simultaneously [280] (for general information on ketoconazole, see chapter 3, p. 34).

Miconazole, 20–30 mg/kg body weight/day, has also been recommended [16, 63, 140, 213, 281, 282]. The concentration in the vitreous body is about 75% of the peak serum concentration [283], however, this agent appears to be less effective than amphotericin B [140]. It is, nevertheless, preferable against P. lilacinus [191, 240] (for general information on miconazole, see chapter 2, p. 33).

On the basis of animal experiments (see chapter 7) the injection of intravitreal dexamethasone in addition to antifungal agents has been proposed [284] in order to lessen the adverse inflammatory defence mechanisms. This may be helpful; however, steroids should be applied at least systemically to depress the leukocyte pool in the peripheral systemic vascular system [285].

Based on the available literature, the treatment scheme given in table 5.7 is currently recommended for fungal endophthalmitis, particularly that caused by C. albicans. In cases in which the fungal pathogen has been identified, the antimycotic agent should be selected in accordance with table 2.13 (see chapter 2, p. 40).

5.2.1.3
Surgical Treatment
Numerous reports contain mention of vitrectomy for the treatment of intraocular fungal infection (table 5.6). As in cases of bacterial infection, vitrectomy should not be delayed too long in severe cases of fungal endophthalmitis [139, 191, 237, 257, 286–289].

Pars plana vitrectomy has several advantages. First, sufficient material can be obtained for microbiological confirmation of the diagnosis [290]. The first 40 ml of the aspirate of the vitreous body should be taken up in a sterile syringe, centrifuged and the supernatant examined [205]. Such an examination may be more productive than without centrifugation [60]. Diagnostic vitrectomy is the best technique for culture [291]. Second, a large number of the
Table 5.6. Pars plana vitrectomy for treatment of fungal endophthalmitis

<table>
<thead>
<tr>
<th>Author</th>
<th>Year of report</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affelt et al.</td>
<td>1987</td>
<td>208</td>
</tr>
<tr>
<td>Aguilar et al.</td>
<td>1979</td>
<td>60</td>
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<td>Brod et al.</td>
<td>1990</td>
<td>238</td>
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<tr>
<td>Christmas</td>
<td>1996</td>
<td>273</td>
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<tr>
<td>Doft et al.</td>
<td>1980</td>
<td>63</td>
</tr>
<tr>
<td>Forster</td>
<td>1974</td>
<td>382</td>
</tr>
<tr>
<td>Furia et al.</td>
<td>1984</td>
<td>286</td>
</tr>
<tr>
<td>Gallo et al.</td>
<td>1985</td>
<td>65</td>
</tr>
<tr>
<td>Gilbert and Novak</td>
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<td>237</td>
</tr>
<tr>
<td>Gross</td>
<td>1992</td>
<td>243</td>
</tr>
<tr>
<td>Hammer et al.</td>
<td>1983</td>
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<td>Heinemann et al.</td>
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<tr>
<td>Henderly et al.</td>
<td>1987</td>
<td>171</td>
</tr>
<tr>
<td>Kroll et al.</td>
<td>1984</td>
<td>287</td>
</tr>
<tr>
<td>Lance et al.</td>
<td>1988</td>
<td>67</td>
</tr>
<tr>
<td>Malton et al.</td>
<td>1987</td>
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<td>Miller et al.</td>
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<td>Peyman et al.</td>
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<td>Pulido et al.</td>
<td>1990</td>
<td>364</td>
</tr>
<tr>
<td>Snip and Michels</td>
<td>1976</td>
<td>288</td>
</tr>
<tr>
<td>Sorrell et al.</td>
<td>1984</td>
<td>78</td>
</tr>
<tr>
<td>Stransky</td>
<td>1981</td>
<td>366</td>
</tr>
<tr>
<td>Tavakolian et al.</td>
<td>1981</td>
<td>289</td>
</tr>
</tbody>
</table>

Pathogens are simply removed mechanically, so that the host’s natural defences are better able to overcome the remaining pathogens. Third, the undesired abscess of fibrin, macrophages and toxins is removed. Finally, an antimicrobial drug can be placed inside the vitreous cavity at the end of the operation, or even used perioperatively in the irrigation fluid. An intraocular lens does not necessarily have to be removed [191], but central capsule excision is recommended [229]. The fear that pathogenic organisms spread more easily following vitrectomy [111, 255] may be balanced by the fact that the same is true for any antifungal agent used.

On the other hand, vitrectomy is an additional source of irritation and bleeding risk for the already inflamed eye, and in mild cases and those with little
Table 5.7. Drug treatment regimen of first choice for fungal endophthalmitis, particularly caused by *C. albicans*: for cases caused by other pathogens, see Chapter 2, table 13 (p. 40)

**Endophthalmitis in the anterior section**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topical (hourly)</td>
<td>fluconazole 0.2% (chapter 2, tables 2.11 and 2.12, p. 37, 38) for <em>C. albicans</em> or natamycin 5% for <em>Fusarium</em> sp. or amphotericin B 0.15–0.5% (chapter 2, tables 2.3 and 2.4, p. 29, 31) after debridement, if no extensive epithelial defect; appropriate ointment or gel at night</td>
</tr>
<tr>
<td>Intracameral</td>
<td>amphotericin B, 7.5 µg every 2 days</td>
</tr>
<tr>
<td>Systemic</td>
<td>with intracameral treatment not necessary; if required see below</td>
</tr>
</tbody>
</table>

**Endophthalmitis in the posterior section**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topical</td>
<td>not recommended on pharmacokinetic causes; if required see above</td>
</tr>
<tr>
<td>Intravitreal</td>
<td>amphotericin B, 7.5 µg, repeat if necessary</td>
</tr>
<tr>
<td>Systemic (daily)</td>
<td>fluconazole, 2 × 200 mg, for <em>C. albicans</em> or itraconazole, 2 × 200 mg, for <em>Candida non-albicans, Aspergillus</em> spp., <em>Cryptococcus</em> spp. or amphotericin B (test dose, increase to a maximum of 1 mg/kg body weight), possibly in combination with flucytosine, 150 mg/kg body weight, then reduce the amphotericin B dose to 0.5 mg/kg body weight</td>
</tr>
</tbody>
</table>

Progression drug treatment alone may be successful [74, 254–256, 292–295]. In this context, some authors have not distinguished between choroiditis and endophthalmitis [234]. Spontaneous remission of fungal chorioretinitis has also been described [20, 78]. Thus, the deciding factors for vitrectomy must be the individual's initial state and course. Recently, a bilateral endogenous *Candida* endophthalmitis following gastrointestinal operation and use of an indwelling catheter for 10 days has been published demonstrating the value of surgical intervention [54]. Both eyes showed a prominent inflammatory reaction in the anterior chamber. The vitreous was equally infiltrated, visual acuity 5/200 in each eye. Following pars plana vitrectomy on the right eye, both eyes received 5 µg amphotericin B intravitreally. The culture of the vitrectomy fluid yielded *C. albicans*. Postoperatively the patient was treated systemically with oral fluconazole (200 mg/day). During the first postoperative days,
a marked inflammatory reaction was successfully treated with topical and periocular steroids. Visual acuity improved dramatically in the right eye but remained unchanged in the left eye. Intravitreal injection of amphotericin B was repeated in both eyes. Vitrectomy was scheduled for the left eye, but refused by the patient. After a 9-month follow-up, visual acuity in the right eye was 20/60 despite cystoid macula edema associated with epiretinal membrane. In contrast, the unvitrectomized left eye was functionally lost with complete retinal detachment.

If the endophthalmitis begins from keratomycosis, a penetrating keratoplasty will usually be necessary (see chapter 4, p. 90).

References


References


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176 Verhoe, F.H., Mycosis of the choroid following cataract extraction, and metastatic choroiditis of the other eye, produced the clinical picture of sympathetic uveitis. Arch. Ophthal., 1924. 53: p. 517-530.


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Chapter 6

Histoplasmosis

6.1

Systemic Histoplasmosis

6.1.1

Epidemiology and Clinical Features

Systemic histoplasmosis occurs predominantly in North America, where it is endemic in the Mississippi Valley and in Arkansas, Kentucky, Missouri and Tennessee [1, 2]. The disease may also be found, however, in Central and South America and in parts of Africa and the Far East [3]. A warmer climate appears to play a role in determining this distribution. The disease occurs in these regions mainly along the larger river basins. It is rare in Europe, and if found has usually been imported from endemic areas [4–6].

In 1906, Darling [7] described for the first time 3 cases of fatal systemic histoplasmosis. He suspected a protozoal disease on the basis of his autopsy examination. He called these structures *Histoplasma* because they resembled ‘plasmo’-dium-like organisms within ‘histo’-cytes. The fungal nature of the pathogenic organism was subsequently proved in 1934 [8]. Despite its name, *H. capsulatum* is an unencapsulated organism.

Before the 1940s, histoplasmosis was considered a rare disease in the USA, but during this decade routine radiological examination of soldiers in the 1940s indicated a much higher prevalence. In the USA in the 1960s, an estimated 30 million inhabitants were thought to be infected with *H. capsulatum*, and it was estimated that about 500,000 new infections and 800 deaths occurred per year [1].

The fungus is present in the superficial layers of the soil and is thought to be associated with bat excrement. Pigeons and poultry are not thought to be carriers [9]. The route of entry into the human body is nearly always the airway. Differentiation is made between acute pulmonary histoplasmosis and chronic pulmonary histoplasmosis, and also between a disseminated form and a benign asymptomatic infection [9]. The acute pulmonary form shows the
nonspecific symptoms of an acute lung inflammation and is self-limiting within a few weeks. Therefore it generally has a good prognosis. In contrast, chronic pulmonary histoplasmosis leads to cavity formation and is a progressive disease. The disseminated form predominantly affects the very young or the very old with reduced resistance, and most organs may be affected. This also occurs in AIDS patients. [2, 10–15]. Over 90% of cases of systemic histoplasmosis lead to the benign, asymptomatic form, which is often not noticed and for which no primary infection can often be recalled.

6.1.2
Diagnosis

6.1.2.1
Histoplasmin Skin Testing

The most important diagnostic test is the histoplasmin skin test. Histoplasmin is an antigen derived from the mycelial phase of the fungus. The test is performed in the same way as the tuberculosis skin test. A positive reaction is shown as a large area of induration of at least 5 mm (diameter) within 48–72 h after injection of 0.1 ml intracutaneously on the volar side of the forearm [9]. Other authors use 2 mm as the critical size and thus obtain a larger number of positive reactions [16]. A positive reaction to this skin test cannot be obtained until 2–4 weeks after Histoplasma infection. The sensitivity is lifelong, except in patients with immunosuppression arising, for example, from use of corticosteroids, severe disease, or advanced age.

The skin test is only positive in 80% of cases of chronic pulmonary histoplasmosis and in 50% of cases of disseminated histoplasmosis [9]. Even in endemic regions, the skin test was no more frequently positive in patients with ocular histoplasmosis syndrome than in a control group [17], probably because a large proportion of the population in such regions are systemically infected without developing ocular symptoms. In a study of 1,417 adults, the overall prevalence was 52.8%, but was higher in men (59.9%) than in women (44.3%); more positive skin tests were obtained in the black population (61.4%) than in the white population (50.7%) [17]. Of this study group, 22 individuals had peripheral symptoms (histo-spots) of ocular histoplasmosis syndrome, and of these 14 responded positively to histoplasmin.

In another study, despite typical ocular histoplasmosis syndrome, false-negative skin results were found in 11% [18]. In a histologically proven case of disseminated histoplasmosis in a 14-year-old boy who died of histoplasmosis, the skin test was also negative [19]. The percentage of positive reactions can be increased to 83% by using the booster effect of a second skin
test performed after 3 weeks [16, 20, 21]. This approach is particularly useful in elderly individuals with reduced sensitivity, and shows a clearly enhanced immune response in patients with ocular histoplasmosis syndrome with maculopathy compared with patients who have only peripheral histo-spots [22].

Hemorrhage in the region of the posterior pole of the eye may occur after histoplasmin skin testing [23], and has been reported in 7% of 57 patients [18]. On the other hand, patients in whom a skin test is performed belong to a selected group where the risk of macular hemorrhage is already increased [24].

6.1.2.2 Other Tests

The complement fixation reaction is not generally recommended for diagnosis of histoplasmosis [9], as it is only positive in 16–68% of cases. Despite negative skin tests, however, it may also be increased in proven histoplasmosis [19, 25]. Even when the complement fixation reaction gives a negative result, counter-current immunoelectrophoresis may be positive [6]. A radioimmunoassay, in which antigens may be detected in the urine and serum, has also been recommended [26]. The in vitro stimulation of lymphocytes by *H. capsulatum* is thought to be diagnostically superior to the complement fixation reaction [22, 27], and has the advantage that macular hemorrhage, possibly brought about by the skin test, is avoided.

HLA-B7 is markedly increased in ocular histoplasmosis syndrome with maculopathy compared with healthy individuals [28–31], but not in patients without maculopathy and peripheral lesions (histo-spots). HLA-DRw2 was increased in 21 of 26 patients (81%) with maculopathy and 8 of 13 patients (62%) with peripheral ocular histoplasmosis syndrome compared with 28% of healthy individuals [31]. Similar patients in Mexico, however, did not show these differences [32]. In another series of 4 cases in England, a detailed immunological assessment (including lymphocyte immunophenotyping, flow cytometric analyses, HLA typing and T-cell receptor variable region expression) was carried out in patients and a control group [33]. Analysis of T-cell receptor variable region expression revealed no significant preferential expression. HLA typing also failed to reveal any links. All lymphocyte markers analysed were unremarkable, with the exception of CD38, which was significantly raised compared with controls (p < 0.01). This finding was confirmed by the use of 2 different CD38-specific monoclonal antibodies. The raised CD38 in these cases was shown to be persistent when the patients were retested after an interval of several months. Significantly, this may correlate with poor T-cell function, as in common variable immunodeficiency, making these patients more susceptible to various stimuli [33].
Miliary calcifications can be found radiologically in the late stages after pulmonary involvement. Computed tomography [34] and bronchoscopy [35] are also used in diagnosis. Finally, histoplasmosis is proved by identification of *H. capsulatum* by histological and microbiological criteria. When taking biopsy samples, e.g. from lymph nodes, only a portion of it should therefore undergo fixation, and the remainder should be sent for microbiological examination [6].

### 6.2 Ocular Histoplasmosis [21]

Ocular histoplasmosis syndrome is a constellation of clinical findings with atrophic, punched-out chorioretinal scars (histo-spots), peripapillary scarring, and absence of inflammation. A small percentage (0-4.5%) of the patients also develop choroidal neovascularization [9, 36-38].

The importance of ocular histoplasmosis syndrome in the 1960s and 1970s is indicated by the observation that in the endemic regions of the USA, 1/1,000 adults developed maculopathy as part of this disease, and without treatment, half of them were legally classified as blind [9]. In Tennessee in particular, with the highest incidence of *Histoplasma* infection in the USA, 2.8% of new applicants for Aid to the Blind had ocular histoplasmosis syndrome [39].

The prognosis has since improved, and in a 5-year follow-up of 516 patients, the risk of legal blindness was low, even for patients who had bilateral involvement [40]; 81% retained a visual acuity of 20/20 in at least 1 eye and 20% retained this visual acuity in both eyes. Of 252 cases and controls examined in 1970, 216 were still alive in 1985; of these, 202 (94%) were interviewed, 197 (91%) underwent visual acuity measurements, and 173 (80%) were examined by a study ophthalmologist [38]. Both in 1970 and in 1985, cases with disciform macular lesions of ocular histoplasmosis had a higher prevalence of both unilateral and bilateral visual impairment and blindness. Although the prevalence of visual impairment and blindness in 1985 was similar among controls and cases of ocular histoplasmosis without disciform lesions, in 1994 this group of cases had about twice the prevalence of visual impairment as that of controls. The 95% confidence intervals on estimates of relative risks were broad, however, and included unity. No new disciform lesions attributable to ocular histoplasmosis were found in 28 eyes of 18 cases free of these in 1970 or among 148 controls [38].

For many years this syndrome has been referred to as ‘presumed’ ocular histoplasmosis, because Koch’s criteria for ocular histoplasmosis were not
fulfilled in humans. However, in a primate model of ocular histoplasmosis syndrome [41–43], intravascular injection of live yeast-phase *H. capsulatum* led to the development of typical scars [41]. It has recently been demonstrated that antigenic challenge could result in a reactivation of the inflammatory component of the choroidal scars [43]. This is probably the most compelling evidence for the relationship between *H. capsulatum* infection and ocular histoplasmosis syndrome, and justifies abandoning the term ‘presumed’ [44], though this is not accepted in general [45].

After a histoplasmosis epidemic, about 6–7% of affected individuals develop ocular histoplasmosis syndrome [46]. This relatively rare involvement of the eyes explains why no retinal changes could be found in 134 histologically or microbiologically proven cases [47].

After the first description by Krause and Hopkins [23] in 1951, Woods and Wahlen [48], in 1960, unequivocally established ocular histoplasmosis syndrome as an independent disease entity. This provided the impetus for numerous experimental (see chapter 7, p. 187) and clinical studies [9, 17, 18, 37, 46, 49–51]. The fungus has been described in the eyes of patients in numerous histopathological publications [25, 47, 49, 52–63]. In this context, a disseminated form (endophthalmitis) [25, 59, 61, 62] can be differentiated from a solitary chorioretinal granuloma [19, 54, 61]. The latter may be up to 5 mm in diameter, which may suggest a tumor. In most patients with ocular histoplasmosis syndrome, however, only chorioretinal scars with occasional lymphocytic infiltration can be found histopathologically. The retinal pigmented epithelium is missing from the center of the scar and is often hypertrophied at the edge, while Bruch’s membrane may show defects. These defects may also be seen in maculopathy with subretinal vessel proliferation [64]. *H. capsulatum* has been identified in the optic nerve sheath in a patient with AIDS [65].

The pathogen probably spreads from the primary focus in the lung to the choroid, where it produces peripheral ‘histo-spots’ that initially cause no problems. These punched out lesions are prominent as round or oval scars (fig. 6.1), which affect the retinal pigmented epithelium, and to a greater or lesser extent the choroid, so that the color may appear between yellowish and white. Occasionally, a choroid vessel passes through. Histo-spots are at the posterior pole, reaching to the mid-periphery, and show a variably large diameter. There is sometimes hyperpigmentation at the edge of the scar. Histo-spots may also be arranged in a line in the periphery (fig. 6.2), described as a ‘linear streak of the equator’ [9, 49, 66–68] or ‘peripheral streak lesion’ [69]. By photographic monitoring of the progression, it was established that new histo-spots occurred (26%), some increased in size (46%), and that the foci became narrower and to some extent invisible (45%) without the patient noticing anything [70]. These findings were confirmed in 11 of 81 individuals followed up for a period of 7 years [71].
is in agreement with experimental findings in primates [41, 43]. Adenocarcinoma of the retinal pigment epithelium arising from a juxtapapillary histoplasmosis scar has been reported in one patient [72]. Acute histoplasmosis choroiditis has recently been reported in 2 immunocompetent brothers [73], whose serial follow-up examinations will be extremely important for our understanding of the pathogenesis of this syndrome.

Finally, vision suddenly deteriorates, probably after several years. Mental stress was reported by 25% of individuals at the time that this occurred [37]. In 162 patients, the mean age at deterioration was 40 years (range, 16–73 years), and in those with involvement of the other eye 44 years (range, 23–66 years) [74]. Subretinal vessel proliferation is found in the macular region, which can be clearly visualized by fluorescence angiography (fig. 6.3, 6.4), usually with hemorrhage at the edge. Serous or hemorrhagic retinal detachment is found above the neovascularization membrane. A well-circumscribed granuloma containing eosinophils has been identified in this membrane [75]. Inflammatory changes cannot be detected in either the posterior or anterior eye segment. Peripapillary chorioretinal scars (fig. 6.5) have been described, with a prevalence ranging from 16% [51] to 85% [76]. The peripapillary disc scarring consists of choroidal and retinal pigment epithelium atrophy, with a line of pigment at the disc margin of the scar, in contrast to some normal eyes with optic nerve ‘crescents’ that have pigment at the outer border of the scar [77]. In addition, there may be papilledema [78, 79]. Before central subretinal
Fig. 6.2. Linear hyperpigmentation and depigmentation in the periphery (so-called linear streak of the equator or peripheral streak lesion).

Fig. 6.3. Fluorescence angiogram of the other eye of the patient in figure 6.2 with subretinal vessel proliferation (early phase).
Fig. 6.4. Fluorescence angiogram of the other eye of the patient in figure 6.2 with subretinal vessel proliferation (late phase).

Fig. 6.5. Central, hemorrhagic chorioretinopathy. Peripapillary, chorioretinal scar. Other eye of the patient in figure 6.2.
neovascularization develops, minimal choroidal inflammation (minimal recurrence) is thought to occur de novo or at the edge of a scar, initially leading to metamorphopsia [80], which then develops into a hole in Bruch's membrane with subsequent neovascularization. Alternatively, however, spontaneous remission may occur, and a pigmented limbus around the central focus has been said to be a good prognostic indicator of this [81]. The pigmented limbus consists of pigment-loaded macrophages or represents proliferation of the retinal pigment epithelium [82].

6.3

Treatment of Histoplasmosis

6.3.1

Treatment of Systemic Histoplasmosis

Amphotericin B is available for the treatment of systemic histoplasmosis [9, 83, 84]. The side effects must be considered, however, and consist predominantly of nephrotoxicity (see chapter 2, p. 27). Thus, the agent is not used in acute pulmonary histoplasmosis with a favorable prognosis, but only in the disseminated and chronic pulmonary progressive forms. A combination of amphotericin B and rifampicin or rifamycin derivatives is thought to be better, as the dose of the single components, and therefore the side effects can be reduced [85].

Of the newer antimycotic agents, ketoconazole does not appear to have any effect [86]. Fluconazole shows better in vitro activity against *H. capsulatum* compared with amphotericin B [87]. Itraconazole is regarded as the treatment of choice [88] (see chapter 2, p. 36).

6.3.2

Treatment of Ocular Histoplasmosis

6.3.2.1

Drug Treatment

Antimycotic therapy of ocular histoplasmosis is not indicated [9] in cases with atrophic scars with or without neovascularization. Apart from the side effects, this approach is not valid because ocular histoplasmosis syndrome is a consequence of primary infection, and the affected individuals are generally healthy. As increased stress is reported in association with the development of visual deterioration [37, 89], a reduction in stress has been recommended [9].

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Even without hypertensive stress, however, bleeding and detachment of the retina with reduced vision probably occurs at some time after macular neovascularization. Moreover, the development of neovascularization is not affected by stress reduction, nor by Valsalva’s maneuver or platelet aggregation inhibitors. Subcutaneous interferon alpha, $3 \times 10^6$ U/m² 4 times daily (mean total dose, 204 MU) did not lead to regression of choroidal neovascularization [90].

The administration of corticosteroids may be appropriate for treatment of minimal recurrences [9, 80]. Using Amsler charts, patients may notice early metamorphopsia, and minimal choroiditis may be suppressed with an intensive course of systemic cortisone. These patients with reactivation of inflammatory lesions may receive additional itraconazole systemically. Perhaps these patients represent a subset of patients with OHS [91]. Previous studies have shown a genetic association in patients with OHS. Both HLA-DRw and HLA-B7 have been found in a high percentage of patients with OHS. In addition, HLA-B7 appeared to be related to the presence of disciform scars. Several, but not all, of these patients have an additional chronic fungal infection of the foot or a history of recurrent vaginal yeast infections. One could speculate that these patients have some immunologic or genetic inability to eradicate fungal infections and that the peripheral fungal infection may somehow trigger an immune response leading to the reactivation of ocular inflammation. This was the rationale in initially treating these patients with itraconazole [91].

6.3.2.2 Photocoagulation and Surgical Treatment

Although some studies found no difference in outcome between coagulation treatment of neovascularization and a spontaneous course [92–94], photocoagulation is approved by many authors [74, 95–99]. The subretinal membrane, however, appears to be larger than expected from fluorescence angiography, and either recurrences are frequent, or with sufficient coagulation, damage with a reduction in vision occurs [100]. A meaningful prospective study with regard to the long-term evidence of efficacy is difficult in cases with subfoveal localization of the neovascularization [101]. The situation in the case of juxtafoveal or extrafoveal choroidal neovascularization should be assessed differently [102, 103]. In a study of 117 patients, the site of recurrence was extrafoveal in 16%, juxtafoveal in 18%, and subfoveal in 66% [104]; 16 eyes were treated with laser photocoagulation, 17 eyes underwent repeat submacular surgery, and 18 eyes were observed. The visual outcome for patients with recurrences amenable to laser treatment was better than for patients who were observed or who underwent surgery.

In a study of 231 patients, 46% had spontaneously suffered considerable reduction in vision at 18 months compared with 13% of cases treated with
argon laser [95]. The entry criteria for this study included subretinal neovascularization within 200–2500 µm of the foveal vessel-free zone, with visual acuity of 0.2 or better. In a retrospective study of 101 eyes with 5–16 years of follow-up, visual acuity of 20/40 or better was observed in 71% of eyes with treated extrafoveal choroidal neovascularization and in 68% with treated juxtafoveal choroidal neovascularization [105]. Recurrent choroidal neovascularization was observed in 23% of treated eyes during a mean follow-up of 9.6 years.

The other eye of a patient with ocular histoplasmosis with maculopathy should be carefully monitored [106]. As 23% of these eyes with histo-spots in the region of the macula will develop neovascularization within a mean of 4 years (range, 1 week to 36 years) [74], regular monitoring by the patient using the Amsler chart is recommended.

The subretinal vessel membrane was removed for the first time in 2 patients in 1991 and led to an improvement in vision from 1/20 to 0.5 and 1.0 over 3 months and 7 months [107]. The results are encouraging, but long-term follow-up is not yet available [108–110, 146], including follow-up related to the development of postoperative choriocapillaris atrophy [111]. Operative results are more favorable, however, compared with age-related macular degeneration [112–114], because the new vessels arising in the choroid in postoperative patients usually grow within the subsensory retinal space and not in the subpigment epithelial space, as occurs in patients with age-related macular degeneration [115]. Postoperatively, there may even be a clear improvement in visual acuity [112, 116]. The sagittal arrangement of the neovascular membranes is apparently important [117], rather than the pathological-anatomical structure of the membrane [118]. Ultrastructural findings of idiopathic subfoveal membranes in ocular histoplasmosis syndrome are similar to those in age-related macular degeneration, with the exception of the presence of basal laminar (linear) deposits only in membranes from eyes with age-related macular degeneration [119]. Within these membranes, transforming growth factor beta 1 and basic fibroblast growth factor are present within the major cell types, which suggests a possible pathogenetic role in the development of the neovascular complex [120].

6.4 Differential Diagnosis of Ocular Histoplasmosis

A number of other diseases show some symptoms of ocular histoplasmosis and should be considered in the differential diagnosis. These include the diseases described by Nozik and Dorsch [121] and by Dreyer and Gass [122], which, however, like diffuse unilateral subacute neuroretinitis (DUSN) [71] and acute zonal occult outer retinopathy (AZOOR) [123–125], produce in-
flammatory symptoms. The same is true for the cases observed by Doran and Hamilton [126] and Palestine et al. [127]. In contrast to ocular histoplasmosis syndrome, multifocal choroiditis and panuveitis also show active vitritis with chorioretinal lesions during the clinical course [128].

Diffuse subretinal fibrosis is very much more pronounced than ocular histoplasmosis [127, 129]. While the foci in acute posterior multifocal placoid pigment epitheliopathy (APMPPE) [130] can be differentiated ophthalmologically and by fluorescence angiography from histo-spots, the foci in ‘birdshot’ [131] and vitiliginous chorioretinopathy [132] are much less clearly defined. Among the findings reported by Watzke et al. [133] and Morgan and Schatz [134] in 21 myopic female patients with punctate inner choroidopathy (PIC), some are reminiscent of ocular histoplasmosis, while others can be clearly distinguished from it. Typical symptoms of punctate inner choroidopathy are photopsia, central or paracentral scotomas, and peripheral field loss. Central and peripheral retinochoroidopathy also occur in cases of trichinosis with the histo-spots tending to be somewhat smaller [135]. Finally, coccidioidomycosis can lead to chorioretinitis that is similar to ocular histoplasmosis [136], though inflammatory signs (paravascular sheathing, vitritis) and much heavier pigmentation are found.

In Europe, cases have been described in which the retina showed the typical signs of ocular histoplasmosis but in which the skin test was negative, and the patients had also never stayed in endemic regions [66, 137, 138, 147]. In 6 of 7 patients from the author’s study, the skin test was negative even after the booster, so that histoplasmosis appeared to be ruled out; in 2 patients, however, chronic reactivation of Epstein-Barr virus was observed [137]. It is thus possible that different organisms produce similar disease pictures [66, 137, 139–142]. Linear streaks of the equator also occur without ocular histoplasmosis [143, 144] (see our case in fig. 6.2). A histopathological study using the polymerase chain reaction with the appropriate primers for \textit{H. capsulatum} and other organisms is required for such cases [145].

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Chapter 7

Experimental Findings in the Eye

The treatment of ocular fungal diseases is prolonged and often unsatisfactory, though new antimycotic agents are continually being developed. Many experimental studies have therefore been performed in order to define the optimal treatment plan. In order to do this, it is first necessary to develop a model in which the various substances and methods may be tested.

7.1
Experimental Models of Keratomycosis

The numerous models of keratomycosis described in the literature have usually been developed in rabbits, with a few in rats [1] and mice [2]. Forster and Rebell [3] used owl monkeys and found no advantage compared with rabbits. The morphology of the rabbit eye is relatively similar to that of the human eye, and this species may thus be regarded as a suitable experimental animal. However, the rabbit eye does show some differences from the human eye which should be remembered: (1) there is no true Bowman's membrane [4] or a superficial, subepithelial layer is regarded as a basal membrane of around 1 μm thickness [5]. This is not thought to have any significant effect on the permeability characteristics of the cornea [5]; (2) the medially situated palpebra tertia; (3) Harder’s gland (glandula palpebrae tertiae profunda) posterior to the palpebra tertia in the orbit, which forms a lipid-rich and alkaline secretion [4, 6]; (4) different dimensions (table 7.1).

7.1.1
Development of Different Models of Infection with Live Pathogens

7.1.1.1
Inoculation Technique and Immunosuppression

As early as the last century, studies were undertaken to produce fungal infection in rabbits [7, 8]. This is difficult, however, because the immunological
Table 7.1. Corneal dimensions of the fully grown rabbit eye [4, 5, 185] and the adult human eye [186]

<table>
<thead>
<tr>
<th></th>
<th>Rabbit</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness, central/peripheral, mm</td>
<td>0.37/0.45</td>
<td>0.5/0.74</td>
</tr>
<tr>
<td>Diameter, horizontal/vertical, mm</td>
<td>15.6/13.8</td>
<td>11.7/10.6</td>
</tr>
<tr>
<td>Radius of curvature, mm</td>
<td>7.3</td>
<td>7.8 (anterior corneal surface)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.6 (posterior corneal surface)</td>
</tr>
<tr>
<td>Area of cornea, cm²</td>
<td>1.55–2.03</td>
<td>1.04</td>
</tr>
<tr>
<td>Fresh weight, mg</td>
<td>ca. 160</td>
<td>about 180</td>
</tr>
<tr>
<td>Height of epithelium, μm</td>
<td>30–40</td>
<td>50–100</td>
</tr>
<tr>
<td>Thickness of Descemet's membrane (μm)</td>
<td>7–22/11–45</td>
<td>5–78–10</td>
</tr>
<tr>
<td>central/peripheral</td>
<td>(7–22/11–45</td>
<td>(5–78–10</td>
</tr>
<tr>
<td>Temperature of surface/eyeball, ºC</td>
<td>32/38</td>
<td>31–34/about 36.5</td>
</tr>
</tbody>
</table>

Defences of the rabbit are apparently sufficient to prevent infection, and simply placing drops of a fungal suspension does not generally lead to manifest infection in an undamaged eye [9–11]. Following epithelial abrasion or scarification of the epithelium and superficial stroma also no infection will occur [8], or only minimal keratitis [12, 13]. In most models, therefore, intracorneal injection of the fungal suspension has been used [14–19]. Some references only suggest that this technique was used [20].

As in many cases manifest fungal infection does not occur even with this inoculation technique, many investigators have initiated immunosuppression of the animal by giving corticosteroids locally or systemically [1, 14, 21–27]. A summary is given in table 7.2. Fractionated cobalt whole-body radiation [28, 29], administration of antilymphocyte serum [25] and alloxan-induced diabetes [30] have also been used for immunosuppression.

This immunosuppression represents artificial interference in the defence mechanisms of the animal and should be avoided in order to maintain natural conditions as far as possible. Only by doing this conclusions relating to the pathogenic situation in humans can be drawn. Therapeutic studies with additional immunosuppression are therefore not useful [31].

7.1.1.2

Strains Used for Infection

The different fungal strains that have been used for experimental infection are listed in table 7.2. This demonstrates that *C. albicans* and *Aspergillus* spp.
Table 7.2. Summary of the fungal species used to date with various inoculation techniques to produce experimental keratomycosis

<table>
<thead>
<tr>
<th>Superficial inoculation</th>
<th>Without corticosteroids</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Allescheria boydii</em></td>
<td><em>Allescheria boydii</em></td>
</tr>
<tr>
<td>Ley, 1956 [47]</td>
<td>Ley, 1956 [47]</td>
</tr>
<tr>
<td>Rheins et al., 1966 [41]</td>
<td>Rheins et al., 1966 [41]</td>
</tr>
<tr>
<td><em>Aspergillus</em> spp.</td>
<td><em>Aspergillus</em> spp.</td>
</tr>
<tr>
<td>Hasany et al., 1973 [33]</td>
<td>Hasany et al., 1973 [33]</td>
</tr>
<tr>
<td>Ley, 1956 [47]</td>
<td>Ley, 1956 [47]</td>
</tr>
<tr>
<td>Rheins et al., 1966 [41]</td>
<td>Rheins et al., 1966 [41]</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td><em>C. albicans</em></td>
</tr>
<tr>
<td>Berson et al., 1965 [21]</td>
<td>Berson et al., 1967 [22]</td>
</tr>
<tr>
<td>Berson et al., 1967 [22]</td>
<td>Hasany et al., 1973 [33]</td>
</tr>
<tr>
<td>Hasany et al., 1973 [33]</td>
<td>Hoffmann and Schmitz, 1963 [43]</td>
</tr>
<tr>
<td>Rheins et al., 1965 [40]</td>
<td>Richards et al., 1969 [114]</td>
</tr>
<tr>
<td>Stern et al., 1979 [26]</td>
<td></td>
</tr>
<tr>
<td><em>C. tropicalis and C. pseudotropicalis</em></td>
<td><em>C. tropicalis and C. pseudotropicalis</em></td>
</tr>
<tr>
<td><em>Cephalosporium</em> spp.</td>
<td><em>Cephalosporium</em> spp.</td>
</tr>
<tr>
<td>Ley, 1956 [47]</td>
<td></td>
</tr>
<tr>
<td><em>Geotrichum</em> sp.</td>
<td><em>Geotrichum</em> sp.</td>
</tr>
<tr>
<td>Ley, 1956 [47]</td>
<td>Ley, 1956 [47]</td>
</tr>
<tr>
<td><em>Pityrosporum ovale</em></td>
<td><em>Pityrosporum ovale</em></td>
</tr>
<tr>
<td>Chowhuvech et al., 1973 [9]</td>
<td></td>
</tr>
<tr>
<td><em>Sporotrichum schenckii</em> and</td>
<td></td>
</tr>
<tr>
<td><em>Scopulariopsis brevicaulis</em></td>
<td></td>
</tr>
<tr>
<td>Rheins et al., 1966 [41]</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intracorneal inoculation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus</em> spp.</td>
<td><em>Aspergillus</em> spp.</td>
</tr>
<tr>
<td>With corticosteroids</td>
<td>Without corticosteroids</td>
</tr>
<tr>
<td>----------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>Hasany et al., 1973 [33]</td>
<td>François and Rijsselaere 1974 [46]</td>
</tr>
<tr>
<td>Ivandic, 1973 [34]</td>
<td>Ley, 1956 [47]</td>
</tr>
<tr>
<td>Newmark et al., 1971 [129]</td>
<td>Singh et al., 1974 [134]</td>
</tr>
</tbody>
</table>

**C. albicans**

| Hasany et al., 1973 [33] | Hasany et al., 1973 [33] |
| Ishibashi and Matsumoto, 1984 [38] | Ivandic, 1973 [34] |
| O’Day et al., 1984 [189] | Behrens-Baumann et al., 1987 [45] |

**C. krusei**

| Tandon, 1984 [27] |

**C. tropicalis and C. pseudotropicalis**

| Graf, 1963 [10] |

| O’Day, 1991 [187] |

**Cephalosporium spp.**

| Burda and Fischer, 1959 [1] |

**Lasiodiplodia spp.**

| Forster et al., 1975 [3] |

**F. solani**

| Forster et al., 1975 [3] |

| François and Rijsselaere, 1974 [46] |

| Ishibashi, 1979 [190] |

| O’Day, 1991 [133] |

| Fiscella, 1997 [126] |

| N. pseudotropicalis |

| Fiscella, 1997 [126] |

| Newmark et al., 1971 [129] |

| Singh et al., 1974 [134] |

| O’Day, 1992 [188] |

**Lasiodiplodia spp.**

| Forster et al., 1975 [3] |
Fig. 7.1. Experimental keratomycosis with *C. albicans* DSM 70010, a strain that shows marked filamentous growth. Marked corneal ulceration. Seventh day after inoculation, no antimycotic treatment.

have been used most often. In many models, apart from the name, no further information on the characteristics or origin of the infecting strain is given [23, 24, 26, 32–35]. Many authors have used pathogenic isolates from human swabs [2, 12, 14, 17, 18, 25, 27, 29, 36–42], which may not necessarily have the same effect in rabbits and may require immunosuppression of the host. Furthermore, while the fungal strains are named as a species, they are not characterized further, so that the studies must not be regarded as reproducible. Details of the strain with the name of the relevant microbiological laboratory have only been given in some instances [12, 20–22, 43–45].

It is surprising that although various approaches to immunosuppression have been attempted, little attention has been paid to the virulence of the fungal strain in developing a model of keratombysis. It has been known for some time that different strains of a fungal species may have different virulence [1, 46, 47]. O’Day et al. [12] were able to produce manifest keratitis with only 2 of the 5 strains of *C. albicans* studied. The author’s group has also found that different results may be obtained using different subgroups of the same species [45]. In the author’s model, *C. albicans* DSM 70010 (DSM = Deutsche Sammlung für Mikroorganismen = German Collection of Microorganisms) is injected intracorneally as the infecting strain, which reliably produces severe infection with synchronous progression (fig. 7.1). The reason for the high frequency and reproducibility of infection despite the absence of immunosup-
Fig. 7.2. Experimental keratomycosis with \textit{C. albicans} CBS 2730, a strain with low filament formation, showing an infiltrate but no ulceration. Seventh day after inoculation, no antifungal treatment.

Expression is apparently related to the high virulence of the strain used. The strain shows marked filamentous growth with the formation of pseudohyphae [48], which is probably an important factor for invasiveness and virulence [49–51], as filamentous forms are better than blastospores at withstanding the body’s defences [52–54]. According to Davies and Denning [52], hyphae of \textit{C. albicans} longer than about 200\,\mu m nearly always resist phagocytosis by polymorphonuclear cells. Blastospores, in contrast, are for the most part phagocytosed. Furthermore, mycelial forms are believed to have a lesser chemotactic effect than blastospores [53] and form a metabolic product that inhibits contact between the hyphae and the neutrophil granulocytes [54]. The author’s comparator strain, CBS 2730 (CBS = Centraal Bureau voor Schimmelcultures), a strain of \textit{C. albicans} with low filament formation, reliably produced an infiltrate but never a florid ulcer (fig. 7.2). The serotype class [55, 56] is in itself not decisive for virulence; both strains DSM 700101 and CBS 2730 of \textit{C. albicans} belong to serotype A. These findings are in good agreement with the results achieved by [57] in a quantitative model of candidal keratitis in rabbits; among strains of \textit{C. albicans} susceptible to amphotericin B, there appeared to be a variation in the degree of susceptibility in vivo that correlated with the MIC.

The infecting strain should be applied together with the 24-hour culture medium glucose broth, as the infection then has a more intense course [45]. Infection is also achieved with live yeast in fresh glucose broth or in 0.9\%
sodium chloride, but with a delay of 2–3 days and a lesser inflammatory reaction. The filamentous growth of \textit{C. albicans}, which is apparently decisive for the invasiveness and virulence, may be stimulated by various factors [58, 59]. Serum has a strong stimulatory effect [60], though the active component has not yet been identified [61].

The aqueous humor also has a stimulatory effect on filament formation of \textit{C. albicans} [48]. Strain DSM 70010 clearly responds, whereas the less virulent CBS 2370 remains exclusively in the blastospore phase and shows filamentous growth only after the addition of serum [48]. Thus, there is a clear need to build up fungicidal concentrations of an antimycotic agent quickly, not only in the cornea but also in the aqueous humor. Progression of corneal infection in the anterior chamber may initially be silent [62, 63], and in some strains, therefore, rapid progression of infection to endophthalmitis may be expected as a result of the filament-stimulating effect of the aqueous humor.

7.1.1.3 Antibiotics and Bacterial Superinfection

A growth-stimulating effect of bacitracin and streptomycin, but not of erythromycin and tetracycline, on \textit{C. albicans} has been described in vitro [64]. Stern [65] studied antibiotic combinations with amphotericin B in vitro and found synergism (a 4-fold reduction in the MIC) for rifampicin, and antagonism (a 4-fold increase in the MIC) for tetracycline in 14% of organisms. There was also synergism for the combination of natamycin (pimaricin) and rifampicin as well as gentamicin against \textit{F. solani}. In vivo, tetracycline was not found to have an effect on the course of keratomycosis [66] while Hoffmann [67] described a negative effect. Terramycin [47], neomycin [40] and the combination of bacitracin, aureomycin and polymyxin B [68] appeared to have an unfavorable effect. In contrast, polymyxin B alone was beneficial in keratomycosis [40]. Overall, the data in the literature are contradictory on this issue. In recent years the general opinion has increased that antibiotics do not have any direct effect on mycoses (see chapter 4, p. 74). While on the one hand a questionable growth-influencing effect of some antibiotics on \textit{C. albicans} might suggest that these substances should not be used in experimental keratomycosis, on the other hand bacterial superinfection must really be avoided. Both Burda and Fisher [1] and Kunze [17] reported that in their studies of keratomycosis, the ulcers observed may also have been caused by bacteria. Berson et al. [22] did not use rabbit eyes that showed organisms on the conjunctival swab before the start of the study. Organisms are present in the conjunctival sac of inflammation-free eyes both in rabbits [69] and in humans [70], and bacterial superinfection in keratomycosis cannot thus be excluded. In fact, Uter [69] found colonies of cocci on histology in 2 of 8 fungally infected eyes. Thus, antibac-
terial prophylaxis appears to be necessary in experimental models, to avoid the possibility of mixed infection leading to incorrect assessments, particularly in the testing of antimycotic agents. Even if an antimycotic agent were effective, the infection would progress clinically as a result of the bacterial component and thus wrongly suggest a lack of efficacy of the tested antimycotic.

7.1.1.4

**Immune Status of Experimental Animals**

The immune status of rabbits with regard to prior infection with *C. albicans* does not appear to have a recognizable effect on the course of subsequent infection. Graf [32] did not find an altered course following reinfection of the cornea after either intravenous or intracorneal initial infection. An immune response does, however, take place. Uter [69] found an increase in anti-*Candida* antibodies of around 2 titre levels in the hemagglutination test [71].

7.1.2

**Keratomycosis Models Using Fungal Extracts**

Not only live yeasts, but also dead yeasts or their components may have a toxic effect, and under the appropriate study conditions may be pyrogenetic and even fatal [72]. Intracorneal injection of an extract of *Cephalosporium* sp., isolated from a clinical case of keratitis, caused severe corneal ulceration in the experimental animal within 2–4 h, which did not, however, lead to perforation [73]. Corresponding studies with extracts of *F. moniliforme* produced similar results [74]. In both studies, proteases of the fungi were thought to be responsible for the corneal destruction. *Candida* spp. are also known to produce proteases [75, 76]. A histological study to assess the possible success of treatment of a test substance is therefore less meaningful, because no distinction is made between living and dead fungal elements [3]. Recultivation of yeasts from the cornea is much more important, which allows clear microbiological assessment of the results of treatment, in addition to the clinical findings [77, 78].

7.1.3

**A Reproducible Model of Keratomycosis**

A review of the literature on experimental keratomycosis leads to the conclusion that the requirements listed in table 7.3 should be fulfilled in a model of infection in order to obtain comparable results. Only by doing this...
Table 7.3. Requirements for an infection model for keratomycosis

- No immunosuppression of the experimental animals, e.g. by cortisone, antilymphocyte serum, whole-body irradiation
- Characterization of the infecting strain used, so that reproducibility is guaranteed
- High incidence of synchronously progressing, sufficiently severe infection
- Sufficiently long, florid stage of infection before the start of reparative defect healing
- Prevention of primary or later onset mixed infection by bacteria

Table 7.4. Reproducible model of keratomycosis [Behrens-Baumann et al., 1987–1990] [45, 77, 78, 81, 95, 131]

- Pigmented rabbit with antibody titre to Candida = 1:20 (hemagglutination test)
- Antibiotic prophylaxis with gentamicin 0.5% without preservative
- Defined, virulent C. albicans strain (DMS 70010) injected intracorneally (2.5 × 10⁵ cells in 10 µl)
- Infiltrates on second day, ulcer after 5–8 days

Table 7.5. Treatment and course of infection in the reproducible model of keratomycosis Behrens-Baumann et al., 1987–1990 [45, 77, 78, 81, 95, 131]

- Start of treatment after 48 h
- 10 drops/day at hourly intervals
- Duration of treatment 3 weeks
- Recultivation at the end of treatment
- Regular photographic documentation

Reproducibility can be guaranteed and a clinically relevant study of antimycotic agents be possible. These requirements have been fulfilled with the development of a new model [45]. Using this model it is possible to reproducibly achieve a corneal ulcer without immunosuppression of the rabbit (table 7.4 and fig. 7.1). After about 2 weeks, this leads to descemetoceles or perforation, or enters a reparative stage. C. albicans DSM 70010 (which shows marked filamentous growth [48]) is injected intracorneally as the infecting strain (2.5 × 10⁵ cells).

In this model, commencement of treatment within 48 h after inoculation is recommended, as at this time infection has become firmly established; this period can also be realistically extrapolated to the human situation. Table 7.5 summarizes the treatment and course of keratomycosis in this model. In efficacy studies of antimycotic agents, recultivation of the infecting strain after 3 weeks has proved to be particularly worthwhile.
Table 7.6. Molecular weights of some antimycotic agents after Windholz et al. [84]

<table>
<thead>
<tr>
<th>Antimycotic Agent</th>
<th>Molecular Weight (Daltons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td>924.10</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>344.84</td>
</tr>
<tr>
<td>Econazole</td>
<td>381.68</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>306.30</td>
</tr>
<tr>
<td>Fluocytosine (5-fluorocytosine)</td>
<td>129.09</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>531.44</td>
</tr>
<tr>
<td>Miconazole</td>
<td>416.12</td>
</tr>
<tr>
<td>Nystatin</td>
<td>926.11</td>
</tr>
<tr>
<td>Natamycin (pimaricin)</td>
<td>665.75</td>
</tr>
</tbody>
</table>

7.2

**Bioavailability of Antimycotic Agents**

Pharmacokinetic studies of topical antimycotic agents have until now been performed mainly in rabbits. The absence of a true Bowman's membrane in the rabbit eye is not thought to have a significant effect on the permeability of the cornea [5]. At worst, too high a concentration of the substance being studied would be expected in the cornea or aqueous humor. The epithelium of the cornea appears to be the essential barrier to the bioavailability of antimycotic agents in the corneal stroma [79–81] and to penetration into the aqueous humor [81]. A corneal abrasion therefore considerably increases the concentration, so that the clinical course of the experimental keratomycosis is clearly influenced.

Lack of permeability through the corneal epithelium is apparently due to the molecular weight of many antimycotic agents. Above a molecular weight of about 500 daltons, substances penetrate the cornea either to only a small extent or not at all [82], as the force of friction increasingly reduces diffusion [83]. Consideration of table 7.6, which gives the molecular weights of the important antimycotic agents after Windholz et al. [84], indicates why debridement [85] is important. Below a molecular weight of 200 daltons friction does not influence diffusion, which is more dependent on lipid solubility [86], or in other words, the ability to cross the corneal epithelium and endothelium [82, 87]. The formulation may also contribute to the bioavailability of a substance [83]. Nonionized, lipophilic molecules, which are easily soluble in oily vehicles, have only a small tendency to leave this phase and
to cross into an aqueous medium [88], and the coefficient of solubility is thus more important than the difference in concentration. Drugs that are in equilibrium between a nonionized and an ionized state penetrate best of all and thus can make use of the layered structure of the cornea (sandwich characteristic) [87, 89, 90]. These pharmacological relationships explain the discrepancy that is often found between good in vitro characteristics of a drug and poor in vivo results.

7.2.1
Amphotericin B

The bioavailability of amphotericin B in the cornea and aqueous humor of rabbit eyes has been studied using the serial dilution test [81]. Amphotericin B 0.5% was administered as drops 10 times at intervals of 1 h to both eyes after the right eye was abraded and the other eye was left with its epithelium intact. In the eyes with intact epithelium, the active ingredient could not be identified in either the cornea or the aqueous humor. In the eyes with corneal abrasions, amphotericin B was found in the aqueous humor at a concentration above the lower limit of detection (0.06 \( \mu \)g/ml). The corneas of these eyes showed a qualitative inhibitory effect on fungus. This is in agreement with the results of a study by O’Day et al. [91], in which amphotericin B 0.15% was used. Only after removal of the epithelium could amphotericin B be identified in the aqueous humor. This clearly demonstrates the limiting effect of the epithelium on penetration of the drug.

In a previous study, amphotericin B concentrations above the lower limit of detection of 0.6 \( \mu \)g/ml could not be found in the presence of either intact or abraded epithelium [18]. Intravenous injection of 1 mg/kg body weight in rabbits with experimental uveitis produced a concentration of 0.13 \( \mu \)g/ml in the aqueous humor after 24 h [92]. Subconjunctival administration of 150 \( \mu \)g produced only traces of amphotericin B in the aqueous humor. In an in vitro model in calf eyes, penetration of amphotericin B was not improved by iontophoresis [93].

Ocular clearance of amphotericin B after direct intravitreal injection has been studied in a rabbit model. Using high-pressure liquid chromatography to assess drug level, the half-lives of drug disappearance from unmodified phakic eyes, Candida-infected eyes, aphakic eyes and aphakic vitrectomized eyes after single 10-\( \mu \)g intravitreal injections were 9.1, 8.6, 4.7 and 1.4 days, respectively. The disappearance slope for vitrectomized eyes was significantly different from all nonvitrectomized eyes (p<0.001). The rapid disappearance of amphotericin B from vitrectomized eyes must be
considered in the clinical management of patients with fungal endophthalmitis [94].

7.2.2

*Bifonazole and Clotrimazole*

With a detection limit of 0.5 μg/ml, bifonazole could not be found in the cornea or in the aqueous humor, independently of whether the epithelium had been removed or not [69].

Clotrimazole applied as a 1% solution in castor oil was only found natively in the cornea but not in the aqueous humor in eyes without debridement [95]. After corneal abrasion the drug could be identified in the aqueous humor in 3 of 6 eyes. Growth of *C. albicans* was inhibited by a 1:2 dilution of the corneal homogenate in 5 of 6 of these debrided eyes.

7.2.3

*Natamycin (Pimaricin)*

Natamycin 5% in Methocel could be measured in the aqueous humor only after removal of the corneal epithelium; in unabraded corneas it could be found in 4 of 6 eyes natively and after debridement in all eyes in at a quarter of the original concentration. Natamycin 2.5 and 1% did not penetrate either the cornea or into the aqueous humor [77]. Iontophoresis did not improve penetration [93].

7.2.4

*Fluconazole*

In a study with fluconazole 0.2% eye-drops, a high concentration of fluconazole was found in the anterior segments of the eye using the serial dilution test. In the aqueous humor of rabbits it was found in a dilution of 1:2, despite an intact epithelium. After abrasion it was found in a dilution of 1:8. In the cornea, detection was possible in a dilution of 1:2 and 1:4 with and without a corneal abrasion, respectively [78]. In a more precise study with fluconazole 0.2%, peak corneal levels were reached almost immediately (after 5 min) in the corneas (debrided $8.2 \pm 1.2 \, \mu g/g$; nondebrided $1.6 \pm 0.6 \, \mu g/g$; means ± SEM) and 15 min after application in the aqueous humor (debrided $9.4 \pm 2.3 \, \mu g/ml$; nondebrided $1.6 \pm 0.6 \, \mu g/ml$; means ± SEM) [96] identical with
the report of Cheng et al. [97]. Semilogarithmic plots provided an estimate of the half-life ($t_{1/2}$) in the debrided eyes of 15 min and in the nondebrided eyes of 30 min. A loading dose of $1 \times 2 \ \mu l \ \text{drop/min}$ for 5 min yielded levels of $59.9 \pm 11.3 \ \mu g/g$ (mean ± SEM) in the debrided corneas and $32.4 \times 1.9 \ \mu g/ml$ (mean ± SEM) in the corresponding aqueous humor. A regimen consisting of this loading dose followed by $1 \times 20 \ \mu l \ \text{drop/h}$ for 6 h produced a level of $45.9 \pm 3.5 \ \mu g/g$ (mean ± SEM) in the debrided corneas and $8.8 \pm 1.7 \ \mu g/ml$ (mean ± SEM) in the corresponding aqueous humor. The same regimen yielded values of $3.1 \pm 0.2 \ \mu g/g$ in the nondebrided corneas and $1.3 \pm 0.2 \ \mu g/ml$ (mean ± SEM) in the aqueous humor.

After oral administration of fluconazole, 20 mg/kg body weight, penetration into the entire eye was better than that of ketoconazole, miconazole, or itraconazole [98]. After fluconazole, 25 mg/kg/day for 14 days, a high concentration was detected in the vitreous humor (peak, $15.7 \pm 5.3 \ \mu g/g$; trough, $9.4 \pm 1.9 \ \mu g/g$) and in the choroid (peak, $17.1 \pm 5.6 \ \mu g/g$; trough, $9.8 \pm 1.9 \ \mu g/g$) of the rabbit eye [99]. The mean tissue:plasma concentration ratio was 0.5 (peak) and 1.6 (trough) in the vitreous humor and 0.5 (peak) and 1.7 (trough) in the choroid. As approximately 90% of fluconazole circulates in plasma as the unbound drug, its apparent distribution into extracellular and intracellular free water would account for most of its large volume of distribution. Concentrations of fluconazole in tissue declined more slowly over time than did concentrations of fluconazole in plasma. Thus, tissue:plasma fluconazole ratios were higher at the time of trough concentrations in plasma than at the time of peak concentrations in plasma. These data suggest that the presence of fluconazole in tissues may continue to provide antifungal cover even at trough levels in plasma. The penetration of fluconazole into the choroid and vitreous humor explains the important role for fluconazole in the treatment of Candida endophthalmitis [99].

7.2.5

**Miconazole, Ketoconazole and Itraconazole**

Miconazole was administered to rabbits intravenously (30 mg/kg), subconjunctivally (10 mg) and as drops (10 mg/ml) [100]. After intravenous infusion, no antимyotic agent could be found in the cornea and vitreous body, though it was present in the aqueous humor at a concentration of $1.59 \pm 0.71 \ \mu g/ml$ after 4 h. Subconjunctival injection of miconazole produced levels of $7.7 \pm 0.92 \ \mu g/g$ dry weight in cornea with epithelium after 4 h, reaching a concentration of $35.86 \pm 7.23 \ \mu g/g$ after corneal abrasion. In the aqueous humor, a concentration of $10.22 \pm 3.80 \ \mu g/ml$ could be detected after 4 h, but
in the vitreous body no drug could be found after 2 h. Topical administration (8 drops every 15 min) resulted in the highest concentration in corneas with defective epithelium (93.35 ± 9.14 μg/g dry weight of corneal tissue), compared with only 10.0 ± 1.0 μg/g dry weight when the epithelium was intact. Also with topical administration, the concentration reached in the aqueous humor was only 0.56 ± 0.19 μg/ml with intact epithelium and 4.61 ± 0.35 μg/l after debridement. As expected, miconazole could not be found in the vitreous body even after intensive drop application.

Intraocular penetration of ketoconazole has been studied in rabbits. The concentrations of ketoconazole 1% in the cornea 1 h after topical drug administration with or without complete corneal epithelial debridement were 44.0 ± 10.1 and 1391.5 ± 130.0 μg/g, respectively. Drug levels in the vitreous were not detectable after topical or subconjunctival drug administration, but were improved slightly by prior epithelial debridement (8.3 and 0.12 μg/ml after 1 h, respectively). Orally administered ketoconazole resulted in high corneal concentrations (45.0 ± 7.6 μg/g after 1 h) that were still substantial 24 h later (55.0 ± 7.0 μg/g); levels in the aqueous humor were low [101].

In a more recent study the effect of vehicle on corneal penetration of trititated ketoconazole and itraconazole has been studied in the rabbit [102]. In the case of ketoconazole, balanced salt solution was significantly worse than boric acid or polyvinyl alcohol, achieving less than one third of the concentration. In the case of itraconazole, balanced salt solution was better than basic acid.

7.3

Experimental Toxicity of Antimycotic Agents

7.3.1

Corneal Toxicity

Foster et al. [103] performed epithelial abrasion in rabbit eyes and then gave drops containing 1% of antimycotic agents. Amphotericin B was the least tolerated antimycotic, followed by ketoconazole, and produced epithelial disturbances. Miconazole and fluocytosine were the best tolerated. Clotrimazole 1% in polyethylene glycol and in Cremophor produced considerable epithelial disturbances; in ointment form the substance did not have this side effect [104]. Injection of amphotericin B, 125 mg and 250 mg, into the anterior chamber produced considerable corneal edema and clouding with chemosis, though after injection of 35 μg these disturbances receded within 4 days without sequelae [105].
7.3.2

Retinal Toxicity

Axelrod et al. [106] injected amphotericin B, 5–10 μg, intravitreally and found no clinical or histological damage. After 4 weeks the electoretinogram was normal. In contrast, Souri and Green [107] found retinal detachment and necrosis histologically even with 1 μg; they suspected damage by the solvent desoxycholate. Raichand et al. [108] established the maximum nontoxic intravitreous dose as 75 μg/ml for amphotericin B methylester, and recommended 10 μg/ml for vitrectomy infusion. In a model of endophthalmitis, Ellison [109] injected natamycin intravitreally; whereas a dose of 25 μg was ineffective but did not cause any impairment of the electoretinogram, 50 μg caused severe retinal damage and iridoplegia, and 100 μg caused loss of sight in the eye with extinguished electoretinogram.

Of the new antimycotic agents, fluconazole has less toxicity; up to an intravitreal dose of 100 μg/0.1 ml no toxic effect could be found either clinically or histologically or using the electoretinogram [110]. It must be borne in mind that the vitreous space in rabbits is about 1.4 ml smaller than the volume in humans (4.0 ml). Intravitreal miconazole, at concentrations of 10–80 μg, caused retinal necrosis in some rabbit eyes, but not in the eyes of owl monkeys, in which an electoretinogram was also performed [111]. These authors recommend an intravitreal dose of 40 μg, compared with the 50 μg recommended by Kawasaki et al. [112]. Flucytosine, 0.1 ml containing various doses from 10 to 1,000 μg, was injected intravitreally into the eyes of rabbits [113]; 100 μg did not produce retinal damage either on light or electron microscopy or on the electoretinogram.

7.4

Treatment of Experimental Keratomycosis

7.4.1

Timing of Treatment

The period of time between infection and the start of treatment decisively affects the success of treatment. The earlier the treatment starts, the more effective it is. After superficial inoculation of *C. albicans*, amphotericin B was highly effective at a concentration of 0.5% [114] and 0.075% [12]. Treatment was started, however, immediately or after 30 min. Delaying the start of treatment until 2 h considerably reduced its efficacy [114]. After intracorneal injection of *C. albicans*, amphotericin B 0.5% led to regression of infection.
immediately or 24 h after inoculation [18]. When the start of treatment was delayed by 48 and 72 h, half and all eyes, respectively, developed keratomycosis like the untreated control eyes. The reduced success with a later start of treatment was ascribed to the different phases of growth of the fungi [115]. It is also clear, however, that the drug does not reach the pathogens as well when they have already penetrated deeply into the cornea compared to those studies with fungal elements still in the superficial layers.

Starting treatment within 24 h appears less sensible, therefore, because the infection must first be manifest in the tissue. If treatment is given too early, this is equivalent to an in vitro experiment, in which animals are unnecessary and the results deceptively encouraging. A later start of treatment, moreover, is closer to everyday clinical practice. However, delay of more than 48 h is generally inappropriate. Infection may then be so advanced that possible differences between the treatment and control group may no longer be recognizable.

7.4.2

Effect of Antimycotic Agents on Experimental Keratomycosis

Most of these studies have been performed in rabbits.

7.4.2.1

Amphotericin B

Amphotericin B 0.5%, was tested in a reproducible model with a defined \textit{C. albicans} strain, injected intracorneally, without immunosuppression [81]. As expected from the pharmacokinetics (see 7.2.1), this agent was only effective after repeated corneal abrasion (fig. 7.3). After removal of the epithelium, the clinical (descemetoceles or perforation, hypopyon) and microbiological (recultivation of \textit{Candida} after 3 weeks of therapy) efficacy was significantly better than in eyes without corneal abrasion (p<0.001; \chi^2 test). O’Day et al. [80] studied the abrasion effect using a nondefined strain of \textit{C. albicans} and the same inoculation technique (period of treatment, 5 days). The epithelium was clearly shown to be a barrier to amphotericin B 0.15 and 0.075%. The effect with 1% drops was very good after abrasion, but only poor without abrasion (start of treatment 1 h after inoculation) [116]. In another study using the \textit{C. albicans} model with treatment beginning as early as 30 min, amphotericin B was most effective, followed by natamycin 5% and then fluocytosine 1% and miconazole 1%; ketoconazole 1% was the least effective [12]. Using the same model, the efficacy of amphotericin B was increased with collagen shields [117].
Montana and Sery [18] and Ellison et al. [23] also studied the efficacy of topical amphotericin B after intracorneal injection of *C. albicans*. Both groups, however, used immunosuppression with corticosteroids and no corneal abrasion. It was thus not possible to achieve a significant therapeutic success, though an inhibitory effect on the progression of the ulcers was observed [23]. The results were not improved by intravenous or subconjunctival application of amphotericin B [18].

Richards et al. [114], Stern et al. [26] and O’Day et al. [12, 79] studied the efficacy of local amphotericin B in superficial infection. At 48 h, 3 and 4 days, however, the periods of treatment and observation were too short to draw definite conclusions. In addition, isolates of *C. albicans* have been described that are resistant in vitro (MIC) and in vivo [57]. In a *C. albicans* model that was not described further, the effect of amphotericin B 0.1% (1 mg/ml) was improved with local hyperthermia up to 52 °C [118]; these authors postulated that increased permeability of the cell membrane as a result of the hyperthermia produced the greater efficacy of the antimycotic.

In an *A. fumigatus* model with characterization of the strain of fungus but an imprecisely described inoculation technique, amphotericin B was studied at 2 concentrations [20]. Eyes treated with 0.5% drops showed better results than those treated with 0.2% (2 mg/ml). In this model, treatment started after 24 h, but the duration is not stated.
7.4.2.2  

**Bifonazole and Clotrimazole**

Bifonazole [119, 120] was studied using the model described in chapter 7.1.3 [95]. Bifonazole 1% in castor oil led to a lower rate of perforation than in the control group, but did not prevent the formation of significant corneal scars with neovascularization.

Clotrimazole was studied using a model of keratomycosis with *C. albicans* (in the English summary: *fumigatus*) after whole-body radiation [28, 29]. Neither systemic nor local therapy with a 30% suspension in glycerine influenced the clinical findings. Clotrimazole 1% in castor oil was studied in the reproducible model in chapter 7.1.3 [95]. Compared with the control group, clotrimazole significantly reduced the recovery of yeasts on recultivation at the end of the study, the complications such as descemetoceles and perforation, and the incidence of hypopyon. The keratitis, however, still led to considerable scarring with neovascularization. Clotrimazole 1% was effective in a superficial inoculation model (microtrepanation) with a strain of *C. albicans* that was not characterized in more detail [39].

7.4.2.3  

**Natamycin (Pimaricin)**

Natamycin 1 and 2.5%, in the model described in chapter 7.1.3, was without any effect on the course of infection [77]. In another study, natamycin 1% also showed no effect [34], or was effective only when treatment was started as early as 4 h or immediately after infection [2, 114]. Natamycin 5%, in contrast, was effective [20, 23], even with subconjunctival administration (250 μg) in an *A. fumigatus* model [121]. In an abrasion model developed by O’Day et al. [80] natamycin 5% was effective only after removal of epithelium.

7.4.2.4  

**Ketoconazole, Miconazole and Fluconazole**

Local ketoconazole 1% was not effective in the abrasion model [12]. In contrast, ketoconazole 2% 3 times daily for 3 weeks was described as being effective in another model [37]. In this model, dexamethasone was applied subconjunctivally for 5 days before intracorneal injection of *Candida* sp. After 21 days no yeasts could be recultivated; however, the fungi were also only recovered in 2 of 20 eyes of the control group, so the virulence of the infecting strain obtained from a patient appears low. No precise details on complications, e.g. perforation or hypopyon, are given, but simply the mean values of all graduation points shown. In the same model, ketoconazole was studied after oral administration, 100 mg/day for 3 weeks, and was found to be effective.
Intracorneal *A. fumigatus* keratomycosis was successfully treated with topical ketoconazole only after the addition of natamycin 5% [123], while using another inoculation technique (microtrepanization) ketoconazole 1% oily drops alone were sufficient [124].

Miconazole, 90 mg/day for 3 weeks intravenously, was also studied in the model described above [37, 125]. The results were in accordance with those obtained for ketoconazole. Positive results have also been described following subconjunctival injection of miconazole, 0.6 mg twice daily for 21 days [36]. Miconazole drops, 10 mg/ml, were effective in an *A. fumigatus* model in 6 of 10 animals and in 5 of 10 animals after subconjunctival administration [20]. Miconazole 1% drops produced more rapid healing in the microtrepanization model compared with clotrimazole drops [39].

Fluconazole produced the best results of the antifungal agents tested in the reproducible model (see chapter 7.1.3) [78]. The results with respect to all criteria (incidence of hypopyon, recultivation of yeasts at the study conclusion, descemetocoeles and perforation) were highly significantly better with fluconazole than in the control group (p<0.01). This also applied to eyes without corneal abrasion, though even in this study debridement was shown to improve the results (fig. 7.4) [78].
7.4.2.5

Miscellaneous Agents

Polyhexamethylene biguanide (PHMB) 0.02% was tested topically in a rabbit model of Fusarium keratomycosis. No clinical differences to the control group could be found [126]. Microbiologically, however, there was a significant difference (p = 0.06) between treated eyes and control eyes of 182.5 ± 314.44 colony-forming units (CFU)/ml (mean value); 7 of 12 eyes (58%) in the PHMB group exhibited no growth, compared with 2 of 12 eyes (17%) in the control group, and 1 of 12 eyes (8%) showed more than 100 CFU in the PHMB group compared with 7 of 12 eyes (58%) in the control group. The duration of treatment, however, was only 6 days.

7.4.3

Corticosteroids in the Treatment of Experimental Keratomycosis with Antimycotic Agents

Corticosteroids are administered in addition to antimycotic agents to reduce nonspecific inflammatory processes [2, 127, 128], including corneal edema and intraocular irritation with fibrin and synechiae formation. This approach is supported experimentally by Newmark et al. [129], who injected A. fumigatus intracornally in rabbit eyes and then treated with natamycin and dexamethasone drops at a variable dose 4 times daily. The group with a low dexamethasone concentration (0.01%) showed the least degree of inflammation without an increase in infection, which was seen only when the corticosteroid concentration increased. A further argument for the use of corticosteroids is to reduce the neovascularization that occurs with persistent corneal inflammation. Although it has been disputed [130], new vessel formation also occurs with experimental keratomycosis [131]. This neovascularization can be significantly (p = 0.05) reduced by dexamethasone, 4 mg subconjunctivally every 2 days, without the infection being negatively influenced, though higher concentrations have an unfavorable effect on the course of infection [131].

On the other hand, corticosteroids have led to a worsening of fungal infection in numerous animal studies [10, 33, 43, 44, 46, 132, 133]. In these studies, however, steroids were given before or immediately after inoculation of the pathogens, which results in primary induced immunosuppression with a consequent more intense spread of fungus.

The time of corticosteroid administration is apparently decisive. If it is given 2 days before infection there is a maximal negative effect, whereas the effect when administered 1 day following infection is only slight; if cortisone is not applied until 7 days after infection, this does not lead to significant
deterioration [132]. This study using a systemic Candida infection model in mice is possibly the key to evaluating combination therapy. However systematic studies of the time factor, and thus the stage of infection, are still lacking, in the keratomycosis model. The positive findings of Newmark et al. [129] described above are in accord with this; these authors started treatment only 1 day after infection. Currently, our study group is conducting an appropriate animal experiment, and preliminary findings confirm the hypothesis that the time factor is important [Behrens-Baumann, 1999, unpubl. data].

The dose of corticosteroid also appears to be important. Dexamethasone, 4 mg subconjunctivally every 2 days, did not negatively influence the course of infection in the reproducible keratomycosis model, whereas this was the case at higher concentrations [131]. In another Candida keratomycosis model, prednisolone acetate 1% reduced the efficacy of natamycin 5%, flucytosine 1%, and micronazole 1%; however, the dose of 0.5 or 0.15% prednisolone acetate did not influence the efficacy of amphotericin B 0.5% [116]. On the other hand, the effect of corticosteroids may be different, between C. albicans and A. fumigatus keratomycosis [31].

The problem of combination therapy in keratomycosis must therefore be viewed in a differentiated manner; particular consideration should be given to the stage of inflammation (time factor) and the dose.

7.4.4 
Operative Treatment of Experimental Keratomycosis

In order to compare lamellar and penetrating keratoplasty, keratomycosis caused by A. fumigatus was produced after subconjunctival cortisone pretreatment [134]. Either lamellar or penetrating keratoplasty was performed after 6–8 days and in different rabbits, after the appearance of hypopyon. All eyes treated by lamellar keratoplasty were lost to reinfection, while 8 of 10 eyes with penetrating keratoplasty could be preserved.

In another rabbit model, Candida sp., Fusarium sp. and Aspergillus sp. were each injected into a corneal lamellar pocket and immunosuppression was initiated by local corticosteroids [135]. Therapeutic lamellar keratectomy in this area was later shown to be advantageous.

In a further A. fumigatus model, ulcer regression was achieved within 23 days with cryotherapy (control, 65 days) [136]; the ulcers treated with nystatin, 100,000 IU, healed within 10 days, however, and the authors concluded that a combination of cryotherapy and antifungal may be the best.

Lamellar keratectomy using the excimer laser has been investigated in the treatment of experimental keratomycosis [42]. Undefined strains of C. albicans
(clinical isolates) were injected intracorneally, and immunosuppression initiated with local corticosteroids. Some eyes underwent superficial inoculation after removal of epithelium. After 2 days, the infected superficial corneal layer was removed using an argon excimer laser (193 nm). The deeper lying clear corneal layers healed well in all 11 eyes. The advantage of lamellar keratectomy using the excimer laser in the authors’ opinion was the technically simpler handling procedure compared with a surgical procedure. In order to test the fungicidal effect, a krypton excimer laser (248 nm) was also used [42]. The fungal cultures remained positive, however, and corneal opacities developed. The energy of the ArF excimer laser itself appears to be fungicidal [137, 138].

7.5

**Experimental Fungal Endophthalmitis**

Following the first description by von Virchow [139] of capillary embolism of the retina in bacterial sepsis, Weber [140] carried out the corresponding experiments in cats. Later, fungi were also used and were either injected intravenously (endogenously) or exogenously into the eye.

7.5.1

**Experimental Endogenous Fungal Endophthalmitis**

7.5.1.1

**Experimental Models**

As early as 1902, Cohn [141] observed conjunctivitis and node-shaped iritis after intravenous injection of yeasts in rabbits. Similar results were achieved by Stock [142], who in addition described bright gray foci at the ocular fundus. After intravenous injection of yeasts, opacities of the vitreous and cornea were described in dogs [143], [144] generated micro-abscesses of the choroid and sclera by injecting *Actinomyces*, at that time regarded as a fungus, into the carotid artery. He identified the abscesses histologically and was able to culture the pathogens from them.

Fundamental, standardized studies were conducted at the start of the 1960s, primarily by Hoffmann and coworkers [145–148]. In these studies, defined strains of *C. albicans* were injected into the auricular vein of rabbits. After observation of the clinical course, a histological study was performed. The study period was between 15 min and 2 months. After 1–2 days, there were commonly ‘grey-white and somewhat indistinctly delimited small foci’
at the ocular fundus. Over the following days these became confluent, forming a ‘string-of-pearls’ pattern. ‘Satellite small foci’ were observed. At a higher inoculum of the infecting strain, vitreous body abscesses developed. Isolated blastospores could be found histologically in the choroid vessels as early as 15 min after injection [145], after 30 min they could be identified in the choriocapillaries and after 1 h started to ‘migrate in a broad front into the retina’. Many blastospores were observed, particularly in the retinal pigment epithelium. After 6 h, defence mechanisms in the form of polymorphonuclear leucocytes could be found for the first time in the choroid vessels, and after 10 h, infiltrates could be found in the choriocapillaris. Pseudomycelia in the retina were only found after 12 h after the pathogens had crossed Bruch’s membrane. There they underwent a massive increase in numbers, while the number in the choroid decreased further after 2 days [145]. These comprehensive studies generated the conclusion that in rabbits the fungi reach the retina not via the retinal vessels, but via the choroid. This is advantageous, because the humoral and tissue defence mechanisms of the choroid can eliminate some of the organisms, while the retina, being a special sensory tissue, is unable to do this. In the retina the pathogenic organisms can spread rapidly, apparently finding better conditions than in the choroid, where granuloma are formed early to repel the pathogens.

Although fungi could also be detected histologically in the anterior sections of the eye, culture studies of anterior chamber punctates were unsuccessful [145]. Thus, the finding of a sterile aqueous humor does not rule out pathogens in the deeper parts of the eye. Similarly, fungi could later no longer be detected in the choroid granuloma, which initially contained organisms. The absence of organisms in inflammatory processes involving the retina does not, therefore, mean that the problem was not caused by a pathogenic organism.

Similar studies with *C. albicans* were performed by Edwards et al. [149] in 80 rabbits; they confirmed movement of the pathogens from the choroid through Bruch’s membrane into the retina, and from there into the eyeball. Positive cultures of other organs, particularly the kidneys, were obtained in 95% of animals with positive cultures of the chorioretina; conversely, 68% of rabbits with positive cultures of other organs also showed positive eye cultures. No histopathological changes could be found in the anterior chamber and iris, and were only found on 2 occasions in the ciliary body. Such changes were more often detectable in a postequatorial site.

In another series using the same rabbit model, *Candida* non-*albicans* species did not infect the eyes, suggesting different pathogenicity of various *Candida* spp. [150]. Endogenous endophthalmitis caused by *A. fumigatus* has been produced in a similar manner with and without immunosuppression.
In addition, *C. neoformans* endophthalmitis could be established in rabbits [152], and in cats and mice [153].

7.5.1.2

**Therapeutic Studies in the Endogenous Endophthalmitis Model**

In studies with intravenous amphotericin B, ketoconazole and miconazole in the *Candida* endophthalmitis model, amphotericin B was the most effective in both prophylaxis and cure, followed by ketoconazole; miconazole was unable to prevent the development of endophthalmitis when it was injected 24 h after inoculation of the pathogens [154].

A single intravitreal injection of amphotericin B, 5 µg, produced slow healing in endogenous *Candida* endophthalmitis, while the control eyes developed a retinal detachment [155]. Experimental *Candida* endophthalmitis has also been treated with vitrectomy, with amphotericin B methyl ester, 10 µg/ml, added to the infusion [108]. Systemic ketoconazole was more effective than miconazole in *Candida* endophthalmitis when treatment was started after 1 week [156]. When fluconazole, ketoconazole and itraconazole were studied in the same model, fluconazole showed the best pharmacokinetics; all three azoles were effective when treatment was started within 24 h, and ketoconazole was also effective even when treatment was not started until 7 days after inoculation [157].

The effect of intravenous fluconazole on endogenous *Candida* endophthalmitis in rabbits was investigated in preventive and therapeutic experiments [158]. In the preventive series, rabbits were injected intravenously with fluconazole, 5 mg/kg body weight, at 30 min, 1 day and 2 days after intravenous inoculation with *C. albicans* spores. The control group received no medication. None of the treated rabbits developed ocular lesions, and no *Candida* spores were isolated from the treated eyes. In contrast, all control rabbits developed bilateral chorioretinitis and *C. albicans* was invariably isolated from the control eyes. In the therapeutic series, intravenous fluconazole, 5 mg/kg body weight, was administered at 3–6 days after inoculation. All rabbits developed chorioretinitis, and *Candida* spores were isolated from all eyes. The results of this study thus indicate that intravenous fluconazole is more effective in preventive use than in therapeutic use against endogenous *Candida* endophthalmitis in rabbits [158].

Another study compared fluconazole and amphotericin B for the treatment of disseminated candidiasis and endophthalmitis in rabbits, and found amphotericin B to be superior in this model [159]. After 17 days of therapy, the fungal colony counts of the choroid-retina were decreased to a significantly greater extent by fluconazole than by the saline control; however, after 24 days this treatment effect was lost.
7.5.2
Experimental Exogenous Fungal Endophthalmitis

In contrast to the endogenous route of infection, with the bloodstream as the carrier, fungi have been administered directly into the eye by several investigators. Grawitz [160] and Stoewer [8] injected *C. albicans* and Nobbe [161] injected *A. fumigatus* into the vitreous body to produce inflammation similar to ‘retinitis albuminica’, as Stoewer writes. He also performed control studies with saline and destroyed yeasts. Fungi (*Achorion quinckeanaum*, the old synonym for *Trichophyton mentagrophytes*) were even injected into the lens of guinea pigs [162], after 5 days ‘clear growth of mycelial fibres’ was noted.

Fine and Zimmerman [163] injected *A. fumigatus* into the vitreous body of rabbits and treated with nystatin, 200 IU in 0.1 ml intravitreally; this dose was well tolerated and for 24 h was above the MIC for this strain. In the same model, natamycin 25 µg given intravitreally was ineffective, though 50 µg was effective with respect to endophthalmitis but led to severe retinal damage with electroretinographic disturbances [109]. After injection of *Volutella* sp. into the anterior chamber, severe endophthalmitis developed, which was treated with amphotericin B [105].

The efficacy of oral fluconazole, alone or in combination with oral flucytosine, has been investigated in the treatment of *Candida* endophthalmitis using a rabbit model [164]. Albino rabbits were infected with an intravitreal inoculation of 1,000 CFU of susceptible *C. albicans* and randomized 5 days later to receive treatment with oral fluconazole alone, 80 mg/kg body weight/day, a combination of oral fluconazole and oral flucytosine, 100 mg/kg body weight/12 h, or no treatment. The treatment effect was assessed at 2 and 4 weeks after therapy by funduscopy, quantitative vitreous culture and histopathology. Intravitreal levels of fluconazole 2–24 h after the first dose were more than 10 times the MIC of the drug for *C. albicans*. Among rabbits treated with fluconazole for 2 weeks, 67% had more than 90% reduction in their fungal load (p < 0.05) and 33% were sterile. After 4 weeks, all had greater than 99% reduction in fungal load (p < 0.05) and 75% were sterile (p = 0.01). This treatment effect was unchanged 4 weeks after discontinuation of fluconazole. Among rabbits treated with fluconazole and flucytosine for 2 weeks, 67% died during therapy. Among the surviving rabbits, 75% had more than 90% reduction in fungal load (p < 0.05) and 25% were sterile. The authors concluded that oral fluconazole may be useful for treatment of *Candida* endophthalmitis, and that addition of flucytosine was associated with high toxicity and minimal additional antifungal effect in this rabbit model.

The influence of corticosteroids or antibiotics on fungal infections of the eye was investigated in rabbits by Prenner 1963 [165]. *Alternaria* spp., *Penicil-
*lium* spp. or *Neurospora sitophila* (isolated from human endophthalmitis) were injected into the vitreous body and into the anterior chamber; *Penicillium* gave rise to mild and *Alternaria* to no signs of inflammation, while *Neurospora* produced intense endophthalmitis. In a second series, the animals were given high intramuscular doses of corticosteroids and tetracycline; these drugs had no effect on the course of inflammation.

The effect of intravitreal dexamethasone in exogenous *C. albicans* endophthalmitis in rabbits has been investigated [166]. On clinical grading the fourth day after infection, the vitreous of eyes in the 2 drug-treated groups was significantly clearer than that of eyes in the control group. By the seventh day after infection, eyes treated with amphotericin B plus dexamethasone had significantly (*p* > 0.0017) clearer vitreous than had eyes receiving only amphotericin B. Quantitative culture results were negative in both treatment groups, and histopathological examination confirmed the clinical grading. Contrary to current belief, there was no evidence that the addition of corticosteroids impaired antifungal activity or enhanced fungal proliferation.

### 7.6 Experimental Histoplasmosis

In 1949, Day [167] produced experimental ocular histoplasmosis for the first time. Fifteen rabbits were injected with 0.05–0.2 ml of a suspension of 125,000–3 million spores/ml into the anterior chamber of an eye. All eyes developed granulomatous iritis, which persisted for 6 weeks. At this time the skin test was positive. Eight of these animals received an injection of spores to the other eye a few weeks after the first injection. None of these previously irritation-free eyes showed clinical symptoms of inflammation. Five rabbits were injected intravenously with spores. After 4 administrations at intervals of a few weeks, 1 animal developed fibrinous iridocyclitis.

No infection developed in pigeons after intracameral injection of yeast when the birds were kept at room temperature, but granulomatous iritis developed at 13 °C [168], when only the temperature in the anterior sections of the eyes, but not the rectal temperature, was reduced. In a study to produce endocarditis in dogs intravenous injection of *H. capsulatum* was followed by the development of iridocyclitis, scleritis and granulomatus choroiditis [169].

A broad series of experimental investigations was then undertaken by Smith and Singer [170–172]. Initially, anterior chamber infection was produced in rabbits, similar to the method of Day [167], but this time using yeasts. After intravitreal injection of yeast, rabbits developed severe choroiditis and peripheral foci, which were similar to presumed ocular histoplasmosis syn-
drome. The retina was not affected by low inocula (yeasts of *H. capsulatum* in sodium chloride, 1:100,000), but infiltrates with inflammatory cells and numerous *Histoplasma* organisms were found in the choroid on histopathology. Within 4 weeks, the animals had a positive histoplasmin skin test. Similar studies were then undertaken in primates [173].

On the basis of these experiments with intravitreal and intravenous injections, the affinity of *H. capsulatum* for the choroid was identified. Choroiditis corresponding to that of humans, however, did not occur via the bloodstream. Further studies also did not achieve success [174–179]. Finally, peripheral choroidal foci were produced by Wong [180], followed by Smith et al. [181] in primates and also in the posterior pole. The latter study was particularly important as, in contrast to rabbits, primates have a macula and the choroidal round foci at the posterior pole may be more likely to lead to the characteristic hemorrhagic maculopathy. However, such subretinal neovascularization was not produced.

It was shown, however, that the number of pathogenic organisms in the inoculum is important: a large inoculum results in undesired severe intraocular inflammation, whereas a small inoculum produces the typical choroidal foci. These occasionally escape ophthalmological observation in the follow-up period [181] and therefore explain apparently newly developed foci [182]. Intrarterial injection of spores of *H. capsulatum* [181] is also thought to correspond better to human infection than the injection of conides. The latter are thought to develop into spores in the human lung and only then to affect other organs.

Using the nonhuman primate model [181, 183], experimental ocular histoplasmosis has been shown to result in chronic lesions that resemble typical histo-spots or choroidal scars, but which contain infiltrates of lymphocytes for as long as 10 years after intracarotid injection of live *H. capsulatum*. Using this model, Palvolgyi et al. [184] attempted to reactivate these late choroidal lesions via intracarotid challenge with specific antigen (heat-killed *H. capsulatum*). No clinical changes suggestive of reactivation of the lesions were observed following this antigenic challenge. Immunopathological analysis of choroidal lesions at 1, 3 and 7 days after antigenic challenge, however, revealed significant increases in both the numbers of inflammatory cells and the relative percentages of helper/inducer lymphocyte and macrophage populations. These results demonstrate that, following antigenic challenge, a cellular change consistent with type IV delayed hypersensitivity can be observed in previously active, but clinically quiescent, histoplasmosis lesions. In the light of the many parallels between the primate experimental model and human ocular histoplasmosis, these findings suggest that in humans, significant subclinical immunopathological activity may occur in the choroid of affected individuals. It is possible that repeated episodes of subclinical reactivation may induce or enhance chronic
choroiditis, and over many years, ultimately produce slow progressive damage to the Bruch’s membrane/retinal pigment epithelium complex, resulting in clinically ‘active’ macular disease and, in selected cases, subretinal neovascularization [184].

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