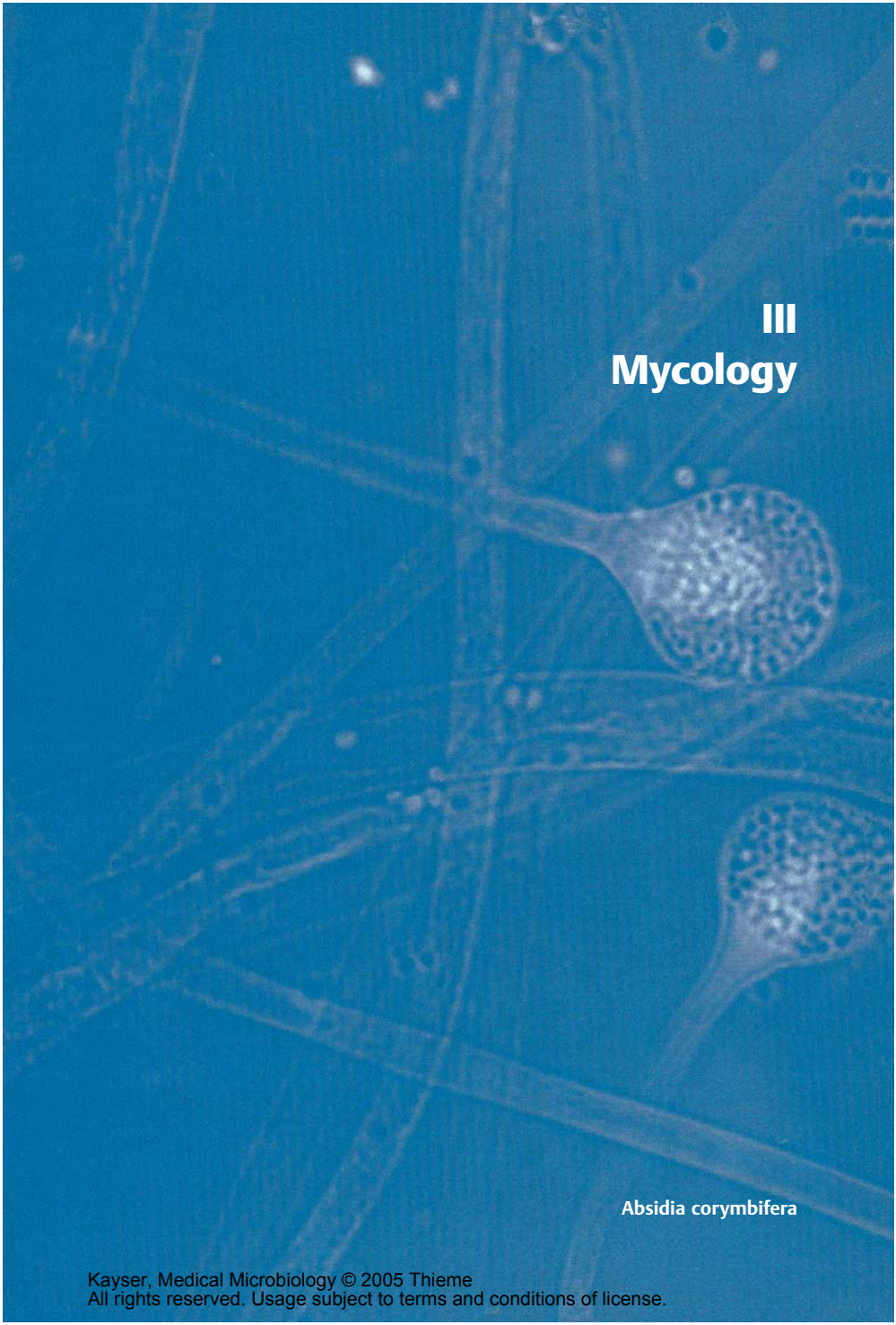


III
Mycology

Absidia corymbifera

5 General Mycology

F. H. Kayser

General Characteristics of Fungi

Fungi are eukaryotic microorganisms (domain eucarya) that occur ubiquitously in nature. Only about 200 of the thousands of species have been identified as human pathogens, and among these known pathogenic species fewer than a dozen are responsible for more than 90% of all human fungal infections.

The basic morphological element of filamentous fungi is the hypha and a web of intertwined hyphae is called a mycelium. The basic form of a unicellular fungus is the yeast cell. Dimorphic fungi usually assume the form of yeasts in the parasitic stage and the form of mycelia in the saprophytic stage. The cell walls of fungi consist of nearly 90% carbohydrate (chitin, glucans, mannans) and fungal membranes are rich in sterol types not found in other biological membranes (e.g., ergosterol). Filamentous fungi reproduce either **asexually** (mitosis), by hyphal growth and tip extension, or with the help of asexual spores. Yeasts reproduce by a process of budding. **Sexual** reproduction (meiosis) on the other hand, produces sexual spores. **Fungi imperfecti** or deuteromycetes are the designation for a type of fungi in which the fructification forms are either unknown or missing entirely.

Definition and Taxonomy

Fungi are microorganisms in the domain eucarya (see. p. 5). They show less differentiation than plants, but a higher degree of organization than the prokaryotes bacteria (Table 5.**1**). The kingdom of the fungi (*Mycota*) comprises over 50 000 different species, only about 200 of which have been identified as human pathogens. Only about a dozen of these "pathogenic" species cause 90% of all human mycoses. Many mycotic infections are relatively harmless, for instance the dermatomycoses. In recent years, however, the increasing numbers of patients with various kinds of immune defects have resulted in more life-threatening mycoses.

Table 5.1 Some Differences between Fungi and Bacteria

Properties	Fungi	Bacteria
Nucleus	Eukaryotic; nuclear membrane; more than one chromosome; mitosis	Prokaryotic; no membrane; nucleoid; only one "chromosome"
Cytoplasm	Mitochondria; endoplasmic reticulum; 80S ribosomes	No mitochondria; no endoplasmic reticulum; 70S ribosomes
Cytoplasmic membrane	Sterols (ergosterol)	No sterols
Cell wall	Glucans, mannans, chitin, chitosan	Murein, teichoic acids (Gram-positive), proteins
Metabolism	Heterotrophic; mostly aerobes; no photosynthesis	Heterotrophic; obligate aerobes and anaerobes, facultative anaerobes
Size, mean diameter	Yeast cells: 3–5–10 µm. Molds: indefinable	1–5 µm
Dimorphism	In some species	None

The taxonomy of the fungi is essentially based on their morphology. In medical mycology, fungi are classified according to practical aspects as dermatophytes, yeasts, molds, and dimorphic fungi. Molds grow in filamentous structures, yeasts as single cells and dermatophytes cause infections of the keratinized tissues (skin, hair, nails, etc.). Dimorphic fungi can appear in both of the two forms, as yeast cells or as mycelia (see the following pages).

Fungi are carbon heterotrophs. The saprobic or saprophytic fungi take carbon compounds from dead organic material whereas biotrophic fungi (parasites or symbionts) require living host organisms. Some fungi can exist in both saprophytic and biotrophic forms.

Morphology

Two morphological forms of fungi are observed (Fig. 5.1):

- **Hypha:** this is the basic element of filamentous fungi with a branched, tubular structure, 2–10 µm in width.

Basic Morphological Elements of Fungi

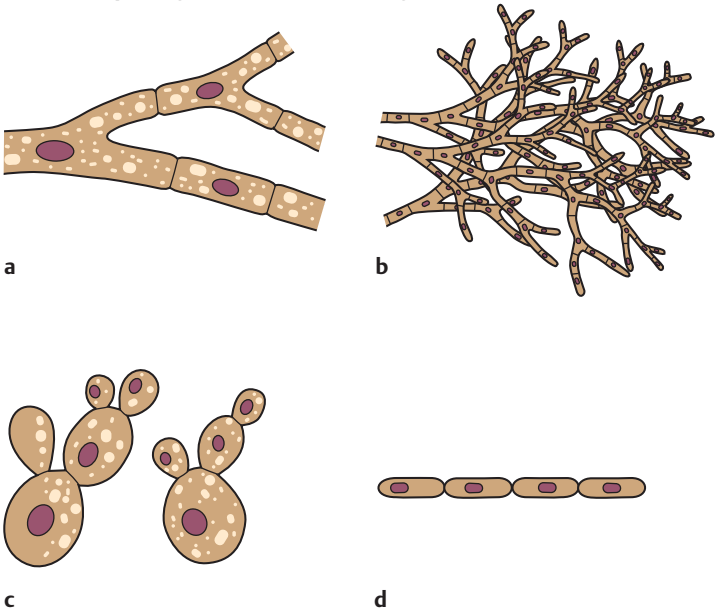


Fig. 5.**1** There are two basic morphological forms: hypha and yeast.
a Hypha, septate, or nonseptate.
b Mycelium: web of branched hyphae.
c Yeast form, budding (diameter of individual cell 3–5 µm).
d Pseudomycelium.

- **Mycelium:** this is the web or matlike structure of hyphae. Substrate mycelia (specialized for nutrition) penetrate into the nutrient substrate, whereas aerial mycelia (for asexual propagation) develop above the nutrient medium.
- **Fungal thallus:** this is the entirety of the mycelia and is also called the fungal body or colony.
- **Yeast:** the basic element of the unicellular fungi. It is round to oval and 3–10 µm in diameter. Several elongated yeast cells chained together and resembling true hyphae are called pseudohyphae.
- **Dimorphism:** some fungal species can develop either the yeast or the mycelium form depending on the environmental conditions, a property called dimorphism. Dimorphic pathogenic fungi take the form of yeast cells in the parasitic stage and appear as mycelia in the saprophytic stage.

Metabolism

All fungi are carbon heterotrophs, which means they are dependent on exogenous nutrient substrates as sources of organic carbon, and with a few exceptions, fungi are obligate aerobes. Many species are capable of maintaining metabolic activity in the most basic of nutrient mediums. The known metabolic types of fungi include thermophilic, psychrophilic, acidophilic, and halophilic species. The metabolic capabilities of fungi are exploited in the food industry (e.g., in the production of bread, wine, beer, cheese, or single-cell proteins) and in the pharmaceutical industry (e.g., in the production of antibiotic substances, enzymes, citric acid, etc.). The metabolic activity of fungi can also be a damaging factor. Fungal infestation can destroy foods, wooden structures, textiles, etc. Fungi also cause numerous plant diseases, in particular diseases of crops.

Reproduction in Fungi

Asexual reproduction. This category includes the vegetative propagation of hyphae and yeasts as well as vegetative fructification, i.e., formation of asexual spores.

- **Hyphae** elongate in a zone just short of the tip in which the cell wall is particularly elastic. This apical growth process can also include formation of swellings that develop into lateral hyphae, which can in turn also branch out.

- **Yeasts** reproduce by budding. This process begins with an outgrowth on the mother cell wall that develops into a daughter cell or blastoconidium. The isthmus between the two is finally cut off by formation of a septum. Some yeasts propagate in both the yeast and hypha forms (Fig. 6.**2**, p. 362).

- **Vegetative fructification.** A type of propagative form, the **asexual spores**, is formed in this process. These structures show considerable resistance to exogenous noxae and help fungi spread in the natural environment. Asexual spores come in a number of morphological types: **conidia, sporangiospores, arthrospores, and blastospores**. These forms rarely develop during the parasitic stages in hosts, but they are observed in cultures. The morphology of the asexual spores of fungi is an important identification characteristic.

Sexual fructification. Sexual reproduction in **fungi perfecti** (eumycetes) follows essentially the same patterns as in the higher eukaryotes. The nuclei of two haploid partners fuse to form a diploid zygote. The diploid nucleus then undergoes meiosis to form the haploid nuclei, finally resulting in the haploid

sexual spores: **zygospores, ascospores, and basidiospores**. Sexual spores are only rarely produced in the types of fungi that parasitize human tissues.

Sexual reproduction structures are either unknown or not present in many species of pathogenic fungi, known as **fungi imperfecti** (deuteromycetes).

General Aspects of Fungal Disease

■ Besides fungal allergies (e.g., extrinsic allergic alveolitis) and mycotoxicoses (aflatoxicosis), fungal infections are by far the most frequent fungal diseases. Mycoses are classified clinically as follows:

- **Primary mycoses** (coccidioidomycosis, histoplasmosis, blastomycoses).
- **Opportunistic mycoses** (surface and deep yeast mycoses, aspergillosis, mucormycoses, phaeohyphomycoses, hyalohyphomycoses, cryptococcoses; penicilliosis, pneumocystosis).
- **Subcutaneous mycoses** (sporotrichosis, chromoblastomycosis, Madura foot (mycetoma).
- **Cutaneous mycoses** (pityriasis versicolor, dermatomycoses).

Little is known about fungal pathogenicity factors. The natural resistance of the macroorganism to fungal infection is based mainly on effective phagocytosis whereas specific resistance is generally through cellular immunity. Opportunistic mycoses develop mainly in patients with immune deficiencies (e.g., in neutropenia). Laboratory diagnostic methods for fungal infections mostly include microscopy and culturing, in order to detect the pathogens directly, and identification of specific antibodies. Therapeutics for treatment of mycoses include polyenes (above all amphotericin B), azoles (e.g., itraconazole, fluconazole, voriconazole), allylamines, antimetabolites (e.g., 5-fluorocytosine), and echinocandins (e.g., caspofungin). Antimycotics are often administered in combination. ■

Fungal Allergies and Fungal Toxicoses

Mycogenic Allergies

The spores of ubiquitous fungi continuously enter the respiratory tract with inspirated air. These spores contain potent allergens to which susceptible individuals may manifest strong hypersensitivity reactions. Depending on the localization of the reaction, it may assume the form of allergic rhinitis, bron-

chial asthma, or allergic alveolitis. Many of these allergic reactions are certified occupational diseases, i.e., "farmer's lung," "woodworker's lung," and other types of extrinsic allergic alveolitis.

Mycotoxicoses

Some fungi produce mycotoxins, the best known of which are the aflatoxins produced by the *Aspergillus* species. These toxins are ingested with the food stuffs on which the fungi have been growing. Aflatoxin B1 may contribute to primary hepatic carcinoma, a disease observed frequently in Africa and Southeast Asia.

Mycoses

Data on the general incidence of mycotic infections can only be approximate, since there is no requirement that they be reported to the health authorities. It can be assumed that **cutaneous mycoses** are among the most frequent infections worldwide. **Primary** and **opportunistic mycoses** are, on the other hand, relatively rare. Opportunistic mycoses have been on the increase in recent years and decades, reflecting the fact that clinical manifestations are only observed in hosts whose immune disposition allows them to develop. Increasing numbers of patients with immune defects and a high frequency of invasive and aggressive medical therapies are the factors contributing to the increasing significance of mycoses. Table 5.**2** provides a summary view of the most important human mycoses. The categorization of the infections used here disregards taxonomic considerations to concentrate on practical clinical aspects.

Host-pathogen interactions

The factors that determine the onset, clinical picture, severity, and outcome of a mycosis include interactions between fungal pathogenicity factors and host immune defense mechanisms. Compared with the situation in the field of bacteriology, it must be said that we still know little about the underlying causes and mechanisms of fungal pathogenicity.

Humans show high levels of nonspecific resistance to most fungi based on mechanical, humoral, and cellular factors (see Table 1.**6**, p. 22). Among these factors, phagocytosis by neutrophilic granulocytes and macrophages is the most important. Intensive contact with fungi results in the acquisition of spe-

Table 5.2 Overview of the Most Important Mycoses in Humans

Disease	Etiology	Remarks
Primary mycoses (do not occur endemic in Europe)		
Coccidioidomycosis	*Coccidioides immitis*	Pulmonary mycosis. Inhalation of spores. Southwestern US and South America
Histoplasmosis	*Histoplasma capsulatum*	Pulmonary mycosis. Inhalation of spores. Dissemination into RES. America, Asia, Africa
North American Blastomycoses	*Blastomyces dermatitidis*	Primary pulmonary mycosis. Secondary dissemination (dermal). North America, Africa
South American Blastomycoses	*Paracoccidioides brasiliensis*	Primary pulmonary mycosis. Secondary dissemination
Opportunistic mycoses		
Candidiasis (soor)	*Candida albicans*, other *Candida* sp.	Endogenous infection. Primary infection of mucosa and skin with secondary dissemination
Aspergillosis	*Aspergillus fumigatus* (90%); other *Aspergillus* sp.	Aspergilloses of the respiratory tract, endophthalmitis; aspergillosis of CNS; septic aspergillosis
Cryptococcosis	*Cryptococcus neoformans* (yeast; thick capsule)	Aerogenic infection. Pulmonary cryptococcosis. Secondary dissemination into CNS
Mucormycoses (zygomycoses)	*Mucor* spp.; *Rhizopus* spp.; *Absidia* spp.; *Cuninghamella* spp., and others	Rhinocerebral, pulmonary, gastrointestinal, cutaneous mucormycosis
Phaeohyphomycoses (caused by "dematious" or "black" fungi)	Over 100 species discovered to date, e.g., *Curvularia* spp.; *Bipolaris* spp.; *Alternaria* spp. Melanin integrated in cell wall	Subcutaneous infections, paranasal sinus infections, infections of the CNS, sepsis also possible
Pneumocystosis	*Pneumocystis carinii*	Defective cellular immunity

Table 5.2 *Continued: Overview of the Most Important Mycoses in Humans*

Disease	Etiology	Remarks
Hyalohyphomycoses (caused by colorless [hyaline] molds)	More than 40 species discovered to date, e.g., *Fusarium* spp.; *Scedosporium* spp.; *Paecilomyces lilacinus*	Infections of cornea and eye, pneumonia, osteomyelitis, arthritis, soft tissue infections, sepsis also possible
Yeast mycoses (except candidiasis)	*Torulopsis glabrata; Trichosporon beigelii; Rhodotorula* spp.; *Malassezia furfur*, and others	Infections of various organs in immunosuppressed patients. Sepsis also possible. *Malassezia furfur* in catheter sepsis in neonates and in intravenous feeding with lipids
Penicilliosis	*Penicillium marneffei*	Most frequent opportunistic infection in AIDS patients in Southeast Asia. Primary infection focus in lungs
Subcutaneous mycoses		
Sporotrichosis	*Sporothrix schenckii*	Dimorphic fungus, ulcerous lesions on extremities
Chromoblastomycosis	*Phialophora verrucosa Fonsecea pedrosoi Cladosporium carrionii*, etc.	Black molds. Wartlike pigmented lesions on extremities. Tropical disease
Madura foot (mycetoma)	*Madurella mycetomi Scedosporium apiospermum*, etc.	Subcutaneous abscesses on feet or hands. Can also be caused by bacteria (see p. 273). In tropics and subtropics
Cutaneous mycoses		
Pityriasis (or tinea versicolor)	*Malassezia furfur*	Surface infection; relatively harmless; pathogen is dependent on an outside source of fatty acids
Dermatomycoses Tinea pedis, T. cruris, T. capitis, T. barbae, T. unguinum, T. corporis	*Trichophyton* spp. *Microsporum* spp. *Epidermophyton* spp.	All dermatophytes are filamentous fungi (hyphomycetes). Anthropophilic, zoophilic, geophilic species. Always transmitted by direct or indirect contact

cific immunity, especially the cellular type. The role of humoral immunity in specific immune defense is secondary.

Diagnosis

The primary concern here is identification of the pathogen.

- **Microscopy.** Native preparation: briefly heat material under coverslip with 10% KOH. Stained preparation: stain with methylene blue, lactophenol blue, periodic acid-Schiff (PAS), ink, etc.
- **Culturing.** This is possible on universal and selective mediums. Sabouraud dextrose agar can contain selective agents (e.g., chloramphenicol and cycloheximide), this medium has an acid pH of 5.6. The main identifying structures are morphological, in particular the asexual and, if present, sexual reproductive structures. Biochemical tests are used mainly to identify yeasts and are generally not as important in mycology as they are in bacteriology.
- **Serology.** By the identification of antibodies to special fungal antigens in patient's serum. The Interpretation of serological findings is quite difficult in fungal infections.
- **Antigen detection.** By finding of specific antigens in the diagnostic material by direct means using known antibodies, possible in some fungal infections (e.g., cryptococcosis).
- **Cutaneous test.** Cutaneous (allergy) tests with specific fungal antigens can be useful in diagnosing a number of fungal infections.
- **Nucleic acid detection.** Combined with amplification, such tests are useful for rapid detection of mycotic diseases in immunocompromised patients.

Therapy

A limited number of anti-infective agents are available for specific treatment of fungal infections:

- **Polyenes.** These agents bind to membrane sterols and destroy the membrane structure:
  - Amphotericin B. Used In systemic mycoses. Fungicidal activity with frequent side effects. There are conventional galenic form and (new) various lipid forms.
  - Nystatin, natamycin. Only for topical use in mucosal mycoses.

■ **Azoles.** These agents disrupt ergosterol biosynthesis. Their effect is mainly fungistatic with possible gastrointestinal side effects. Hepatic functional parameters should be monitored during therapy:
- Ketoconazole. One of the first azoles. No longer used because of side effects.
- Fluconazole. Oral or intravenous application. For the treatment of surface and systemic mycoses and cryptococcal meningitis in AIDS patients.
- Itraconazole. Oral and intravenous application. Use in systemic and cutaneous mycoses and also for the treatment of aspergillosis.
- Voriconazole. Oral and intravenous application. Good activity against *Candida* and *Aspergillus*. No acitivity against *Mucorales*.

■ **Antimetabolites.** 5-Fluorocytosine. Interferes with DNA synthesis (base analog). Given by oral application in candidiasis, aspergillosis, and cryptococcosis. It is necessary to monitor the course of therapy for the development of resistance. The toxicity of amphotericin B is reduced in combination with 5-fluorocytosine.

■ **Allylamines.** Terbinafine. By oral and topical application to treat dermatomycoses. Inhibition of ergosterol biosynthesis.

■ **Echinocandins. Caspofungin** has been approved as a salvage therapy in refractory aspergillosis. It is useful also in oropharyngeal and esophageal candidiasis. Inhibition of the biosynthesis of glucan of the cell wall.

■ **Griseofulvin.** This is an older antibiotic used in treatment of dermatomycoses. By oral application, therapy must often be continued for months.

6 Fungi as Human Pathogens

Primary Mycoses

■ Primary systemic mycoses include histoplasmosis (*Histoplasma capsulatum*), North American blastomycosis (*Blastomyces dermatitidis*), coccidioidomycosis (*Coccidioides immitis*), and South American blastomycosis (*Paracoccidioides brasiliensis*). The natural habitat of these pathogens is the soil. Their spores are inhaled with dust, get into the lungs, and cause a primary pulmonary mycosis. Starting from foci in the lungs, the organisms can then be transported, hematogenously or lymphogenously, to other organs including the skin, where they cause granulomatous, purulent infection foci. Laboratory diagnostics aim at direct detection of the pathogens under the microscope and in cultures as well as identification of antibodies. The therapeutics used to treat these infections are amphotericin B and azoles. All of the primary systemic mycoses are endemic to certain geographic areas, in some cases quite limited in extent. Central Europe is not affected by these diseases. They are not communicable among humans. ■

Histoplasma capsulatum (Histoplasmosis)

Histoplasma capsulatum is the pathogen responsible for histoplasmosis, an intracellular mycosis of the reticuloendothelial system. The sexual stage or form of this fungus is called *Emmonsiella capsulata*.

Morphology and culture. *H. capsulatum* is a dimorphic fungus. As an infectious pathogen in human tissues it always forms yeast cells (Fig. 6.**1**). The small individual cells are often localized inside macrophages and have a diameter of 2–3 μm.

Giemsa and gram staining do not "take" on the cell walls of *H. capsulatum*, for which reason the cells often appear to be surrounded by an empty areola, which was incorrectly taken to be a capsule, resulting in the designation *H. capsulatum*. This species can be grown on the nutrient mediums normally used for fungal cultures. *H. capsulatum* grows as a mycelium in two to three weeks on Sabouraud agar at a temperature of 20–30 °C.

Histoplasma capsulatum

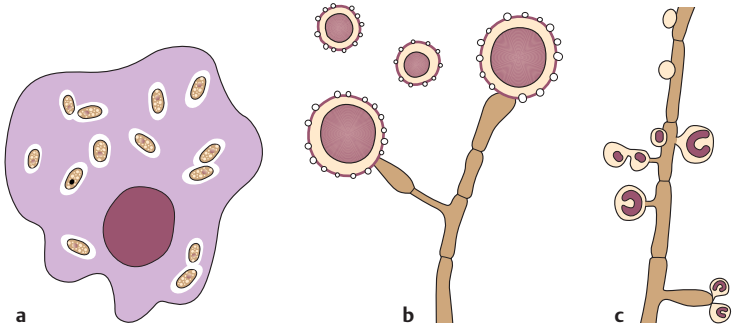


Fig. 6.1 **a** Yeast cells in macrophage.
b Macroconidia (7–15 µm).
c Microconidia (2–5 µm).

Pathogenesis and clinical picture. The natural habitat of *H. capsulatum* is the soil. Spores (conidia) are inhaled into the respiratory tract, are taken up by alveolar macrophages, and become yeast cells that reproduce by budding. Small granulomatous inflammatory foci develop. The pathogens can disseminate hematogenously from these primary infection foci. The reticuloendothelial system (RES) is hit particularly hard. Lymphadenopathies develop and the spleen and liver are affected. Over 90% of infections remain clinically silent. The clinical picture depends heavily on any predisposing host factors and the infective dose. A histoplasmosis can also run its course as a respiratory infection only. Disseminated histoplasmoses are also observed in AIDS patients.

Diagnosis. Suitable material for diagnostic analysis is provided by bronchial secretion, urine, or scrapings from infection foci. For microscopic examination, Giemsa or Wright staining is applied and yeast cells are looked for inside the macrophages and polymorphonuclear leukocytes. Cultures on blood or Sabouraud agar must be incubated for several weeks. Antibodies are detected using the complement fixation test and agar gel precipitation. The diagnostic value of positive or negative findings in a histoplasmin scratch test is doubtful.

Therapy. Treatment with amphotericin B is only indicated in severe infections, especially the disseminated form.

Epidemiology and prevention. Histoplasmosis is endemic to the midwestern USA, Central and South America, Indonesia, and Africa. With few exceptions,

Western Europe is free of the disease. The pathogen is not communicable among humans. No special prophylactic measures are taken.

Coccidioides immitis (Coccidioidomycosis)

Morphology and culture. *C. immitis* is an atypical dimorphic fungus. In cultures, this fungus always grows in the mycelial form; in body tissues, however, it neither buds nor produces mycelia. What is found in vivo are spherical structures (spherules) with thick walls and a diameter of 15–60 μm, each filled with up to 100 spherical-to-oval endospores.

C. immitis is readily cultivated on the usual fungus nutrient mediums. After five days of incubation, a white, wooly (fuzzy) mycelial colony is observed. One of the morphological characteristics of the mycelium is the asexual arthrospores seen as separate entities among the hyphae.

Pathogenesis and clinical picture. The infection results from inhalation of dust containing arthrospores. Primary coccidioidomycosis is always localized in the lungs, whereby the level of manifestation varies from silent infections (60 % of infected persons) to severe pneumonia. Five percent of those infected develop a chronic cavernous lung condition. In fewer than 1 %, hematogenous dissemination produces granulomatous lesions in skin, bones, joints, and meninges.

Diagnosis. The available tools are pathogen detection in sputum, pus, cerebrospinal fluid or biopsies, and antibody identification. The spherules can be seen under the microscope in fresh material. The fungus can be readily cultured on Sabouraud agar at 25 °C. The resulting arthrospores are highly infectious and must be handled very carefully. Antibodies can be detected using the complement fixation test, gel precipitation or latex agglutination. A coccidioidin skin test measuring any cellular allergy to components of the fungus is used as an initial orientation test if an infection is suspected.

Therapy. Amphotericin B can be used to treat the disseminated forms. An oral azole derivative will serve as an alternative, or for use, in clinically less severe forms.

Epidemiology and prevention. Coccidioidomycosis is endemic to desert areas of California, Arizona, Texas, New Mexico, and Utah and is only rarely observed elsewhere. The source of infection is the fungus-rich soil. Animals can also be infected. This disease is not transmitted among humans or from animals to humans.

Blastomyces dermatitidis (North American Blastomycosis)

Blastomyces dermatitidis is a dimorphic fungus that causes a chronic granulomatous infection. The pathogens occur naturally in the soil and are transmitted to humans by inhalation.

The primary blastomycosis infection is pulmonary. Secondary hematogenous spread can lead to involvement of other organs including the skin. **Laboratory diagnostic** methods include microscopy and culturing to identify the fungus in sputum, skin lesion pus, or biopsy material. Antibody detection using the complement fixation test or agar gel precipitation is of limited diagnostic value. Amphotericin B is the **therapeutic agent** of choice. Untreated blastomycoses almost always have a lethal outcome.

Blastomycosis occurs mainly in the Mississippi Valley as well as in the eastern and northern USA. Infections are also relatively frequent in animals, especially dogs. Susceptible persons cannot, however, be infected by infected animals or humans. There are no prophylactic measures.

Paracoccidioides brasiliensis (South American Blastomycosis)

Paracoccidioides brasiliensis (syn. *Blastomyces brasiliensis*) is a dimorphic fungus that, in living tissues, produces thick-walled yeast cells of 10–30 µm in diameter, most of which have several buds. When cultivated (25 °C), the fungus grows in the mycelial form.

The natural habitat of *P. brasiliensis* is probably the soil. Human infections are caused by inhalation of spore-laden dust. Primary purulent and/or granulomatous infection foci are found in the lung. Starting from these foci, the fungus can disseminate hematogenously or lymphogenously into the skin, mucosa, or lymphoid organs. A disseminated paracoccidioidomycosis progresses gradually and ends lethally unless treated. The **therapeutic agents** of choice are azole derivatives (e.g., itraconazole), amphotericin B, and sulfonamides. Therapy can prevent the disease from progressing, although no cases are known in which the disease is eliminated over the longer term. **Laboratory diagnostics** are based on detection of the pathogen under the microscope and in cultures as well as on antibody detection with the complement fixation test or gel precipitation.

Paracoccidioidomycosis is observed mainly among farmers in rural parts of South America.

Opportunistic Mycoses (OM)

Opportunistic mycoses (OM) that affect skin and mucosa as well as internal organs are caused by both yeast and molds. A precondition for development of such infections is a pronounced weakness in the host's immune defenses. Candidiasis is an endogenous infection. Other OMs are exogenous infections caused by fungi that naturally inhabit the soil or plants. These environmental fungi usually invade via the respiratory tract. The most important are aspergillosis, cryptococcosis, and the mucormycoses. Besides *Candida* and other yeasts, phaeohyphomycetes and hyalohyphomycetes, which are only very mildly pathogenic, can also cause systemic infections. All OMs have a primary infection focus, usually in the upper or lower respiratory tract. From this focus, the pathogens can disseminate hematogenously and/or lymphogenously to infect additional organs. Infection foci should be removed surgically if feasible. Antimycotic agents are used in chemotherapy. In infected immunocompromised patients, the prognosis is usually poor.

Candida (Soor)

At least 70 % of all human *Candida* infections are caused by *C. albicans*, the rest by *C. parapsilosis*, *C. tropicalis*, *C. guillermondii*, *C. kruzei*, and a few other rare *Candida* species.

Candida albicans

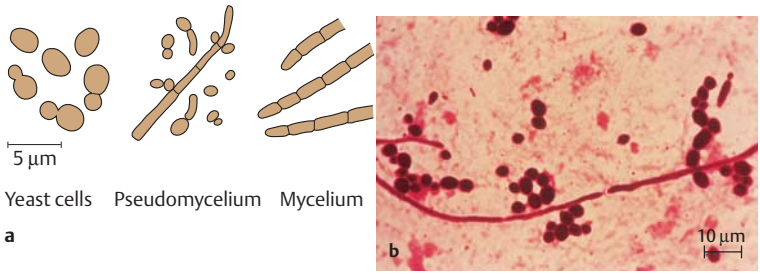


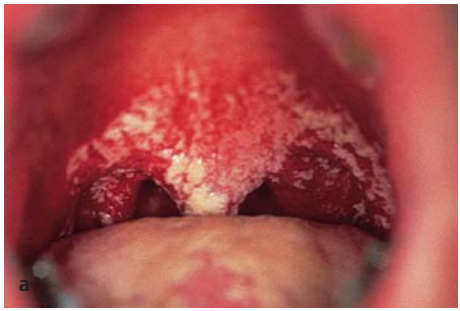
Fig. 6.**2** *Candida albicans.*
a Morphological forms.
b Gram staining of sputum: Gram-positive yeast cells and hyphae. Clinical diagnosis: candidiasis of the respiratory tract.

Morphology and culture. Gram staining of primary preparations reveals *C. albicans* to be a Gram-positive, budding, oval yeast with a diameter of approximately 5 μm. Gram-positive pseudohyphae are observed frequently and septate mycelia occasionally (Fig. 6.**2**).

C. albicans can be grown on the usual culture mediums. After 48 hours of incubation on agar mediums, round, whitish, somewhat rough-surfaced colonies form. They are differentiated from other yeasts based on morphological and biochemical characteristics.

Pathogenesis and clinical pictures. *Candida* is a normal inhabitant of human and animal mucosa (commensal). *Candida* infections must therefore be considered endogenous. Candodoses usually develop in persons whose immunity is compromised, most frequently in the presence of disturbed cellular immunity. The mucosa are affected most often, less frequently the outer skin and inner organs (deep candidiasis). In oral cavity infections, a white, stubbornly adherent coating is seen on the cheek mucosa and tongue. Patho-

Clinical Forms of Candidosis



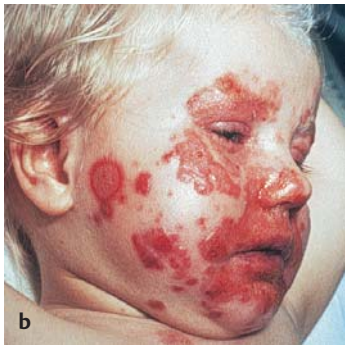


Fig. 6.**3** **a** Oral soor; surface infection of cheek mucosa and tongue by *Candida albicans* in an AIDS patient.
b Chronic mucocutaneous candidiasis in a child with a cellular immunodeficiency syndrome.

morphologically similar to oral soor is vulvovaginitis. Diabetes, pregnancy, progesterone therapy, and intensive antibiotic treatment that eliminate the normal bacterial flora are among the predisposing factors. Skin is mainly infected on the moist, warm parts of the body. *Candida* can spread to cause secondary infections of the lungs, kidneys, and other organs. Candidial endocarditis and endophthalmitis are observed in drug addicts. Chronic mucocutaneous candidiasis is observed as a sequel to damage of the cellular immune system (Fig. 6.**3**).

Diagnosis. This involves microscopic examination of preparations of different materials, both native and Gram-stained. *Candida* grows on many standard nutrient mediums, particularly well on Sabouraud agar. Typical yeast colonies are identified under the microscope and based on specific metabolic evidence.

Detection of *Candida*-specific antigens in serum (e.g., free mannan) is possible using an agglutination reaction with latex particles to which monoclonal antibodies are bound. Various methods are used to identify antibodies in deep candidiasis (agglutination, gel precipitation, enzymatic immunoassays, immunoelectrophoresis).

Therapy. Nystatin and azoles can be used in topical therapy. In cases of deep candidiasis, amphotericin B is still the agent of choice, often administered together with 5-fluorocytosine. Echinocandins (e.g., caspofungin) can be used in severe oropharyngeal and esophageal candidiasis.

Epidemiology and prevention. *Candida* infections are, with the exception of candidiasis in newborn children, endogenous infections.

Aspergillus (Aspergillosis)

Aspergilloses are most frequently caused by *Aspergillus fumigatus* and *A. flavus*. *A. niger*, *A. nidulans*, and *A. terreus* are found less often. Aspergilli are ubiquitous in nature. They are found in large numbers on rotting plants.

Morphology and culture. *Aspergillus* is recognized in tissue preparations, exudates and sputum by the filamentous, septate hyphae, which are approximately 3–4 μm wide with Y-shaped branchings (Fig. 6.**4**).

Aspergillus grows rapidly, in mycelial form, on many of the mediums commonly used in clinical microbiology. Sabouraud agar is suitable for selective culturing.

Pathogenesis and clinical pictures. The main portal of entry for this pathogen is the bronchial system, but the organism can also invade the body through injuries in the skin or mucosa. The following localizations are known for aspergilloses:

Aspergillus fumigatus

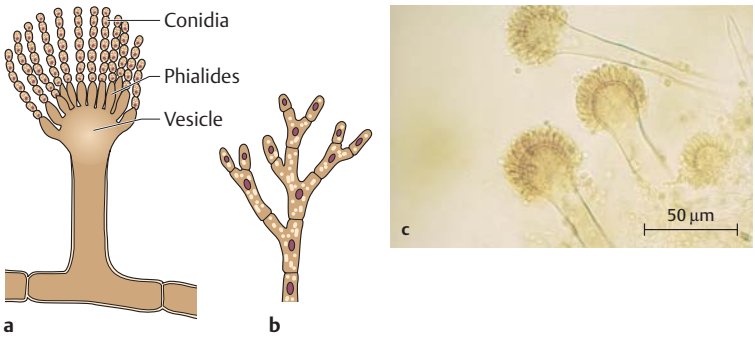


Fig. 6.4 **a** Conidiophore with conidia (2–5 µm).
b Y-branched, septate hyphae (1.5–8 µm).
c Native preparation; the conidia have fallen off.

Aspergillosis of the respiratory tract. An aspergilloma is a circumscribed "fungus ball" that usually grows in a certain space (e.g., a cavern). Another pulmonary aspergillosis is a chronic, necrotizing pneumonia. Acute, invasive pulmonary aspergillosis is seen in patients suffering from neutropenia or AIDS or following organ transplants and has a poor prognosis. Another aspergillosis of the respiratory tract is tracheobronchitis. Of all fungi, aspergilli are most frequently responsible for various forms of sinusitis. In persons with atopic allergies, asthma may be caused by an allergic aspergillus alveolitis.

Other aspergilloses. Endophthalmitis can develop two to three weeks after surgery or an eye injury and the usual outcome is loss of the eye. Cerebral aspergillosis develops after hematogenous dissemination. Less often, *Aspergillus* spp. cause endocarditis, myocarditis, and osteomyelitis.

Diagnosis. Since *Aspergillus* is a frequent contaminant of diagnostic materials, diagnosis based on direct pathogen detection is difficult. Finding the typically branched hyphae in the primary preparation and repeated culture growth of *Aspergillus* make the diagnosis probable. If the branched hyphae are found in tissue biopsies stained with methenamine silver stain, the diagnosis can be considered confirmed.

Using latex particles coated with monoclonal antibodies, *Aspergillus*-specific antigen (*Aspergillus* galactomannan) can be detected in blood serum in an agglutination reaction. Antibodies in systemic aspergilloses are best detected by immunodiffusion and ELISA. PCR-based methods detect *Aspergillus-DNA*.

Therapy. High-dose amphotericin B, administered in time, is the agent of choice. Azoles can also be used. The echinocandin caspofungin has been approved in the treatment of refractory aspergillosis as salvage therapy. Surgical removal of local infection foci (e.g., aspergilloma) is appropriate.

Cryptococcus neoformans (Cryptococcosis)

Morphology and culture. *C. neoformans* is an encapsulated yeast. The individual cell has a diameter of 3–5 µm and is surrounded by a polysaccharide capsule several micrometers wide (Fig. 6.**5a**).

C. neoformans can be cultured on Sabouraud agar at 30–35 °C with an incubation period of three to four days (See Fig. 6.**5b**).

Pathogenesis and clinical picture. The normal habitat of this pathogen is soil rich in organic substances. The fungus is very frequently found in bird droppings. The portal of entry in humans is the respiratory tract. The organisms

Cryptococcus neoformans

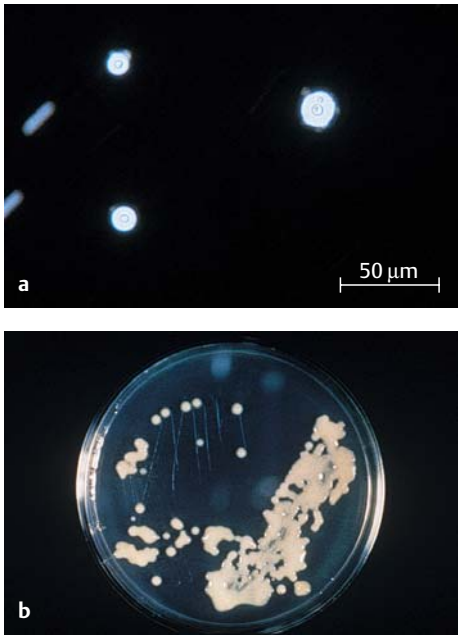


Fig. 6.**5** **a** Ink preparation from cerebrospinal fluid; negative image of thick, mucoid capsule surrounding the yeast cells. Clinical diagnosis: cryptococcal meningitis.
b Culture on Sabouraud agar: whitish, creamy colonies.

are inhaled and enter the lungs, resulting in a pulmonary cryptococcosis that usually runs an inapparent clinical course. From the primary pulmonary foci, the pathogens spread hematogenously to other organs, above all into the central nervous system (CNS), for which compartment *C. neoformans* shows a pronounced affinity. A dangerous meningoencephalitis is the result. Good preconditions for dissemination from the lung foci are provided especially by primary diseases that weaken the immune defenses. Malignancies and steroid therapy are other frequent predisposing factors. AIDS patients also frequently develop cryptococcoses.

Diagnosis. This is particularly important in meningitis. The pathogens can be detected in cerebrospinal fluid sediment using phase contrast microscopy. An ink preparation results in a negative image of the capsule (see Fig. 6.**5a**). Culturing is most successful on Sabouraud agar. *C. neoformans* can be differentiated from other yeasts and identified based on special metabolic properties (e.g., breakdown of urea). A latex agglutination test is available for detection of capsule polysaccharide in cerebrospinal fluid and serum (anticapsular antibodies coupled to latex particles). Identification of antibodies to the capsular polysaccharide is achieved by means of an agglutination test or an enzymatic immunosorbence test.

Therapy. Amphotericin B is the agent of choice in CNS cryptococcosis, often used in combination with 5-fluorocytosine.

Epidemiology and prevention. No precise figures are available on the frequency of pulmonary cryptococcosis. The incidence of the attendant meningoencephalitis is one case per million inhabitants per year. There are no specific prophylactic measures.

Mucor, Absidia, Rhizopus (Mucormycoses)

Mucormycoses are caused mainly by various species in the genera *Mucor*, *Absidia*, and *Rhizopus*. More rarely, this type of opportunistic mycosis is caused by species in the genera *Cunninghamella, Rhizomucor,* and others. All of these fungal genera are in the order *Mucorales* and occur ubiquitously. They are found especially often on disintegrating organic plant materials.

Morphology and culture. Mucorales are molds that produce broad, nonseptate hyphae with thick walls that branch off nearly at right angles (Fig. 6.**6**). Mucorales are readily cultured. They grow on all standard mediums, forming high, whitish-gray to brown, "fuzzy" aerial mycelium.

Culturing is best done on Sabouraud agar.

Pathogenesis and clinical pictures. *Mucorales* are typical opportunists that only cause infections in patients with immune deficiencies or metabolic dis-

***Mucorales* (Zygomycetes)**

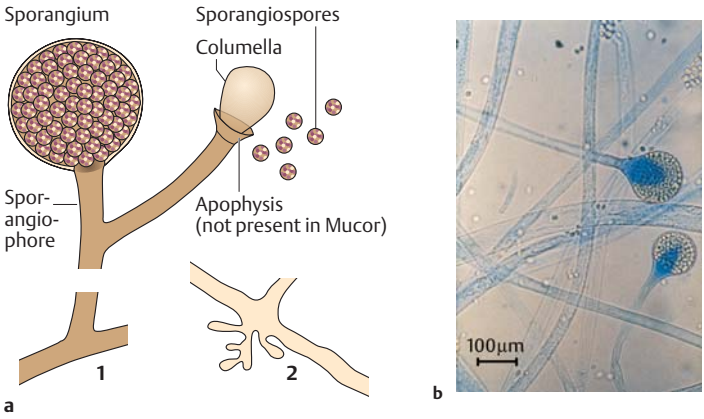


Fig. 6.**6** **a** Morphological elements:
1 = sporangium (60–350 µm) with sporangiospores (5–9 µm),
2 = nonseptate hyphae (diameter 6–15 µm) with rhizoid (→ rootlike structure).
b *Absidia corymbifera:* lactophenol blue preparation. Material from culture.

orders (diabetes). The pathogens penetrate into the target organic system with dust. They show a high affinity to vascular structures, in which they reproduce, potentially resulting in thrombosis and infarction. The infections are classified as follows according to their manifestations:

- **Rhinocerebral mucormycosis**, spreads from the nose or sinuses and may affect the brain. Most often observed as a sequel to diabetic acidosis.
- **Pulmonary mucormycosis**, with septic pulmonary infarctions. Occurs most frequently in neutropenic malignancy patients under remission therapy.
- **Gastrointestinal mucormycosis** (vary rare), seen in undernourished children and accompanied by infarctions of the gastrointestinal tract.
- **Cutaneous mucormycosis**, manifests as a sequel to skin injuries, especially burns.
- **Disseminated mucormycosis**, as a sequel to any of these forms, especially pulmonary mucormycosis.

Diagnosis. Confirmation of diagnosis is based on detection of tissue infiltration by morphologically typical fungal hyphae. Culturing can be attempted on

Sabouraud agar. Identification concerns solely the morphological characteristics of the fructification organs. There is no method of antibody-based diagnosis.

Therapy. Amphotericin B is the antimycotic agent of choice. Surgical measures as required. Control of the primary disease.

Phaeohyphomycetes, Hyalohyphomycetes, Opportunistic Yeasts, Penicillium marneffei

The list of clinically relevant fungi previously not categorized as classic opportunists has lengthened appreciably in recent years. These organisms are now being found in pathogenic roles in patients with malignancies, in AIDS patients, in patients undergoing cytostatic and immunosuppressive therapies, massive corticosteroid therapy, or long-term treatment with broad-spectrum antibiotics. The terms phaeohyphomycetes, hyalohyphomycetes, and opportunistic yeasts have been created with the aim of simplifying the nomenclature.

Phaeohyphomycoses. These are subcutaneous and paranasal sinus infections caused by "dematious" molds or "black fungi." To date, numerous genera and species have been described as pathogenic agents. Common to all is the formation of hyphae, which appear as a brownish black color due to integration of melanin in the hyphal walls. Examples of the genera include *Curvularia*, *Bipolaris*, *Exserohilum*, *Wangiella*, *Dactylaria*, *Ramichloridium*, *Chaetomium*, and *Alternaria*. The natural habitat of these fungi is the soil. They occur worldwide. Phaeohyphomycetes invade the body through injuries in the skin or inhalation of spores. Starting from primary foci (see above), the pathogens can disseminate hematogenously to affect other organs including the CNS. The clinical pictures of such infections most closely resemble the mucormycoses and aspergillosis. If feasible, surgical removal of infected tissues and administration of antimycotic agents is indicated. The prognosis is poor.

Hyalohyphomycoses. This collective term is used for mycoses caused by hyaline (melanin-free) molds. Examples of some of the genera are *Fusarium*, *Scopulariopsis*, *Paecilomyces*, *Trichoderma*, *Acremonium*, and *Scedosporium*. These fungi are also found all over the world. Pathogenesis, clinical pictures, therapy, and prognosis are the same as for the phaeohyphomycoses.

Opportunistic yeast mycoses. Other yeasts besides the most frequent genus by far, *Candida*, are also capable of causing mycoses in immunosuppressed patients. They include *Torulopsis glabrata*, *Trichosporon beigelii*, and species of the genera *Rhodotorula*, *Malassezia*, *Saccharomyces*, *Hansenula*, and others. These "new" mycoses are not endogenous, but rather exogenous infections. In

clinical and therapeutic terms, they are the same as candidiasis. *Malassezia furfur* occasionally causes catheter sepsis in premature neonates and persons who have to be fed lipids parenterally. Lipids encourage growth of this yeast.

Penicilliosis. This fungal infection is caused by the dimorphic fungus *Penicillium marneffei*, which probably inhabits the soil. *P. marneffei* infections are one of the most opportunistic infections most frequently seen in AIDS patients who either live in Southeast Asia or have stayed in that area for a while. The infection foci are located primarily in the lungs, from where dissemination to other organs can take place. The therapeutic of choice in the acute phase is amphotericin B, this treatment must be followed by long-term prophylactic azoles (itraconazole) to prevent remission.

Pneumocystis carinii (Pneumocystosis)

■ *Pneumocystis carinii* is a single-celled, eukaryotic microorganism that was originally classified as a protozoan, but is now considered a fungus. This pathogen can cause pneumonia in persons with defective cellular immune systems, in particular those showing AIDS. Extrapulmonary manifestations are also recorded in a small number of cases. Laboratory diagnostic methods include direct detection of the microbes under the microscope, by means of direct immunofluorescence or PCR. Appropriate anti-infective agents for therapy include cotrimoxazole, pentamidine, or a combination of the two. ■

Pneumocystis carinii is a single-celled, eukaryotic microorganism that was, until recently, classified with the protozoans. Molecular DNA analysis has revealed that it resembles fungi more than it does protozoans, although some of the characteristic properties of fungi, such as membrane ergosterol, are missing in *Pneumocystis carinii*. This microbe occurs in the lungs of many mammalian species including humans without causing disease in the carriers. Clinically manifest infections emerge in the presence of severe underlying defects in cellular immunity, as in AIDS.

Morphology and developmental cycle. Three developmental stages are known for *P. carinii*. The **trophozoites** are elliptical cells with a diameter of 1.5–5 μm. Presumably, the trophic form reproduces by means of binary transverse fission, i.e., asexually. Sexual reproduction does not begin until two haploid trophozoites fuse to make one diploid **sporozoite** (or precyst), which are considered to be an intermediate stage in sexual reproduction. After further nuclear divisions, the sporozoites possess eight nuclei at the end of their development. The nuclei then compartmentalize to form eight spores with a diameter of 1–2 μm each, resulting in the third stage of devel-

opment, the **cyst**. The cysts then release the spores, which in turn develop into trophozoites.

Culture. *P. carinii* cannot be grown in nutrient mediums. It can go through a maximum of 10 developmental cycles in cell cultures. Sufficient propagation is only possible in experimental animals, e.g., rats. This makes it difficult to study the pathogen's biology and the pathogenic process and explains why all aspects of these infections have not yet been clarified.

Pathogenesis and clinical pictures. Humans show considerable resistance to *P. carinii* infections, which explains why about two-thirds of the populace are either carriers or have a history of contact with the organism. Disease only becomes manifest in the presence of defects in the cellular immune system. Of primary concern among the clinical manifestations is the **interstitial pneumonia**. Profuse proliferation of the pathogen in the alveoli damages the alveolar epithelium. The pathogens then penetrate into the interstitium, where they cause the pneumonia. Starting from the primary infection foci, the fungi spread to other organs in 1–2% of cases, causing extrapulmonary *P. carinii* infections (of the middle ear, eye, CNS, liver, pancreas, etc.).

Diagnosis. Suitable types of diagnostic material include pulmonary biopsies or bronchoalveolar lavage (BAL) specimens from the affected lung segments. Grocott silver staining can be used to reveal cysts and Giemsa staining shows up trophozoites and sporozoites. Direct immunofluorescence, with labeled monoclonal antibodies to a surface antigen of the cysts, facilitates detection

Cryptococcus neoformans

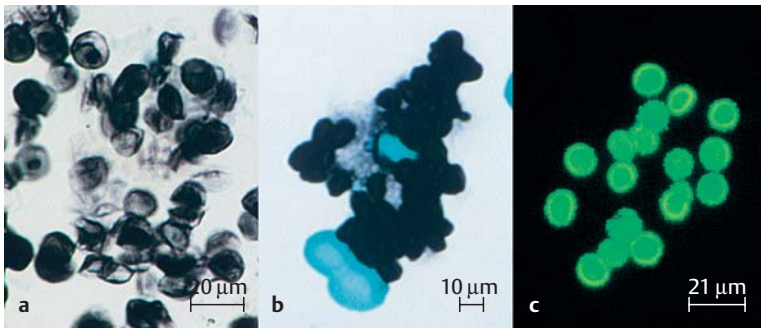


Fig. 6.7 **a** Cysts of *P. carinii*, Grocott staining.
b Yeast fungi, Grocott staining (for differential diagnosis).
c Cysts of *P. carinii*, detection with direct immunofluorescence and monoclonal antibodies.

(see Fig. 6.**7c**). Amplification of specific DNA sequences using the PCR has recently come to the fore as a useful molecular detection method.

Therapy. Acute pneumocystosis is treated with cotrimoxazole (oral or parenteral) or pentamidine (parenteral) or a combination of both of these anti-infective agents. Pentamidine can also be applied in aerosol form to reduce the side effects.

Subcutaneous Mycoses

Fungi that cause classic subcutaneous mycoses grow in the soil and on dying plants. They penetrate through skin injuries into the subcutaneous connective tissue, where they cause local, chronic, granulomatous infections. These infections are seen mainly in the tropics and subtropics.

Sporotrichosis is caused by *Sporothrix schenckii*, a dimorphic fungus that grows as yeast cells in host tissues. Sporotrichosis is characterized by an ulcerous primary lesion, usually on an extremity, and multiple nodules and abscesses along the lymphatic vessels.

Chromomycosis (also chromoblastomycosis) can be caused by a number of species of black molds. The nomenclature of these pathogens is not firmly established. Weeks or months after the spores penetrate into a host, wartlike, ulcerating, granulomatous lesions develop, usually on the lower extremities.

Madura foot or **mycetoma** can be caused by a wide variety of fungi as well as by filamentous bacteria (*Nocardia* sp., *Actinomadura madurae*, *Streptomyces somaliensis*). Potential fungal contributors include *Madurella* sp., *Pseudoallescheria boydii*, and *Aspergillus* sp. The clinical picture is characterized by subcutaneous abscesses, usually on the feet or hands. The abscesses can spread into the musculature and even into the bones. Fistulae are often formed.

Cutaneous Mycoses

Dermatophytes (Dermatomycoses or Dermatophytoses)

Dermatophytes are fungi that infect tissues containing plenty of keratin (skin, hair, nails).

Classification. Dermatophytes are classified in three genera: ***Trichophyton*** (with the important species *T. mentagrophytes*, *T. rubrum*, *T. schoenleinii*, *T. tonsurans*); ***Microsporum*** (*M. audouinii*, *M. canis*, *M. gypseum*); and ***Epidermophyton*** (*E. floccosum*). Some dermatophyte species are anthropophilic, others zoophilic. The natural habitat of the geophilic species *M. gypseum* is the soil.

Morphology and culture. The dermatophytes are filamentous fungi. They grow readily on fungal nutrient mediums at 25–30 °C. After 5–14 days, cultures with a woolly appearance, in different colors, usually develop (Fig. 6.**8**).

Pathogenesis and clinical pictures. Dermatomycoses are infections that are transmitted directly by human contact, animal-human contact or indirectly on inanimate objects (clothes, carpets, moisture, and dust in showers, swimming pools, wardrobes, gyms). The localization of the primary foci corresponds to the contact site. Thus feet, uncovered skin (hair, head, facial skin) are affected most frequently. Different species can cause the same clinical picture. Frequent dermatomycoses include:

Dermatophytes

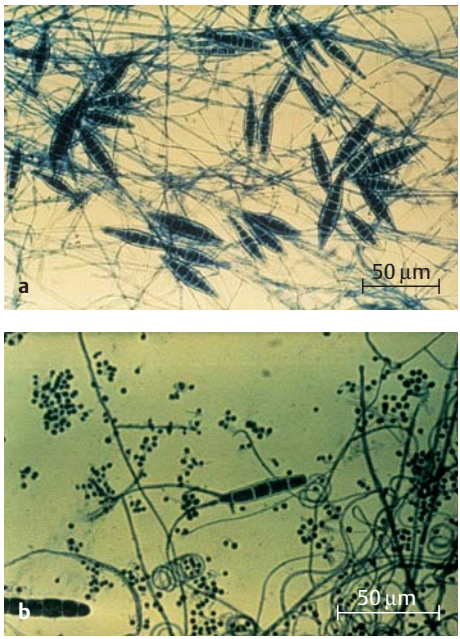


Fig. 6.**8** **a** *Microsporum canis*. Lactophenol blue preparation: large, fusiform macroconidia.
b *Trichophyton mentagrophytes*. Lactophenol blue preparation: thin-walled, cylindrical macroconidia; numerous microconidia, often in clumps; spiral hyphae.

- **Tinea corporis (ringworm):** *Microsporum canis* and *Trichophyton mentagrophytes*. Affects hairless skin.
- **Tinea pedis (athlete's foot):** *T. rubrum*, *T. mentagrophytes*, and *Epidermophyton floccosum*. Affects mainly the lower legs.
- **Tinea capitis:** *T. tonsurans* and *M. canis*. Affects scalp hair.
- **Tinea barbae:** *T. rubrum* and *T. mentagrophytes*. Beard ringworm.
- **Tinea unguium:** *T. rubrum*, *T. mentagrophytes*, and *E. floccosum*.
- **Onychomycosis (nail mycosis):** Various dermatophytes and Candida spp.

Diagnosis. Material suitable for diagnostic analysis include skin and nail scrapings and infected hair. The fungi are observed under the microscope in a KOH preparation. Identification is based on the morphology of the hyphae as well as on the macroconidia and microconidia in the fungal cultures.

Therapy. Dermatomycoses can be treated with locally applied antimycotic agents. In cases of massive infections of the hair, and above all of the nails, the oral allylamine terbinafine or azoles can be used. Griseofulvin is rarely used today.

Epidemiology and prevention. Dermatophytes occur naturally all over the world. The geophilic dermatophyte, *M. gypseum*, can cause infections in persons in constant, intensive contact with the soil (e.g., gardeners). Prophylactic measures for all dermatomycoses consist in avoiding direct contact with the pathogen. Regular disinfection of showers and wardrobes can contribute to prevention of athlete's foot, a very frequent infection.

Other Cutaneous Mycoses

Pityriasis (or **tinea**) **versicolor** is a surface infection of the skin caused by *Malassezia furfur*. This infection is observed mainly in the tropics but is known all over the world. It causes hypopigmentations. *M. furfur* is dependent for its metabolic needs on a source of long-chain fatty acids. This fungus is actually a component of the skin's normal flora. The pathogenesis of the infections has not yet been clarified.

Tinea nigra, which occurs mainly in the tropics, is caused by *Exophiala werneckii*. Infection results in brown to black, maculous efflorescences on the skin.

White and black piedras is an infection of the hair caused by *Trichosporon beigelii* or *Piedraia hortae*.