

Practical lesson 18: Microbiological diagnosis of mycoses.

Because fungi (yeasts and molds) are eukaryotic organisms, whereas bacteria are prokaryotic, they differ in several fundamental respects. Two fungal cell structures are important medically: (1) The fungal cell wall consists primarily of chitin (not peptidoglycan as in bacteria); thus fungi are insensitive to certain antibiotics, such as penicillins and cephalosporins, that inhibit peptidoglycan synthesis. (2) Chitin is a polysaccharide composed of long chains of N-acetylglucosamine. The fungal cell wall contains other polysaccharides as well, the most important of which is β -glucan, a long polymer of d-glucose.

Pathogenesis:

The response to infection with many fungi is the formation of granulomas. Granulomas are produced in the major systemic fungal diseases (e.g., coccidioidomycosis, histoplasmosis, and blastomycosis, as well as several others). The cell-mediated immune response is involved in granuloma formation. Acute suppuration, characterized by the presence of neutrophils in the exudate, also occurs in certain fungal diseases such as aspergillosis and sporotrichosis. Fungi do not have endotoxin in their cell walls and do not produce bacterial-type exotoxins. Activation of the cell-mediated immune system results in a delayed hypersensitivity skin test response to certain fungal antigens injected intradermally. A positive skin test indicates exposure to the fungal antigen.

TABLE 40-1 Features of Important Fungal Diseases

Type	Anatomic Location	Representative Disease	Genus of Causative Organism(s)	Seriousness of Illness ¹
Cutaneous	Dead layer of skin	Tinea versicolor	<i>Malassezia</i>	1+
	Epidermis, hair, nails	Dermatophytosis (ringworm)	<i>Microsporum</i> , <i>Trichophyton</i> , <i>Epidermophyton</i>	2+
Subcutaneous	Subcutis	Sporotrichosis	<i>Sporothrix</i>	2+
		Mycetoma	Several genera	2+
Systemic	Internal organs	Coccidioidomycosis	<i>Coccidioides</i>	4+
		Histoplasmosis	<i>Histoplasma</i>	4+
		Blastomycosis	<i>Blastomyces</i>	4+
		Paracoccidioidomycosis	<i>Paracoccidioides</i>	4+
Opportunistic	Internal organs	Cryptococcosis	<i>Cryptococcus</i>	4+
		Candidiasis	<i>Candida</i>	2+ to 4+
		Aspergillosis	<i>Aspergillus</i>	4+
		Mucormycosis	<i>Mucor</i> , <i>Rhizopus</i>	4+

¹1+ = not serious, treatment may or may not be given; 2+ = moderately serious, treatment often given; 4+ = serious, treatment given especially in disseminated disease.

CUTANEOUS MYCOSES

Dermatophytoses

Dermatophytoses are caused by fungi (dermatophytes) that infect only superficial keratinized structures (skin, hair, and nails), not deeper tissues. The most important dermatophytes are classified in three genera: *Trichophyton*, *Epidermophyton*, and *Microsporum*. They are spread from infected persons by direct contact. *Microsporum* is also spread from animals such as dogs and cats. This indicates that to prevent reinfection, the animal must be treated also. Dermatophytoses (tinea, ringworm) are chronic infections often located in the warm, humid areas of the body (e.g., athlete's foot and jock itch).¹ Typical ringworm lesions have an inflamed circular border containing papules and vesicles surrounding a clear area of relatively normal skin. The lesions are typically pruritic. Broken hairs and damaged nails are often seen. The disease is typically named for the affected body part (i.e., tinea capitis [head], tinea corporis [body], tinea cruris [groin], and tinea pedis [foot]).

Trichophyton rubrum is also a very common cause of tinea capitis. *Trichophyton schoenleinii* is the cause of favus, a form of tinea capitis in which crusts are seen on the scalp. *Trichophyton* species also cause an inflammatory pustular lesion on the scalp called a kerion. The marked inflammation is caused by an intense T-cell-mediated reaction to the presence of the fungus. In some infected persons, hypersensitivity causes dermatophytid ("id") reactions (e.g., vesicles on the fingers). Id lesions are a response to circulating fungal antigens; the lesions do not contain hyphae. Patients with tinea infections show positive skin tests with fungal extracts (e.g., trichophytin). Scrapings of skin or nail placed in 10% potassium hydroxide (KOH) on a glass slide show septate hyphae under microscopy. Cultures on Sabouraud's agar at room temperature develop typical hyphae and conidia. Tinea capitis lesions caused by *Microsporum* species can be detected by seeing fluorescence when the lesions are exposed to ultraviolet light from a Wood's lamp



Tinea corporis (ringworm). Note oval, ring-shaped inflamed lesion with central clearing. Caused by dermatophytes such as *Epidermophyton*, *Trichophyton*, and *Microsporum*

SUBCUTANEOUS MYCOSES

These are caused by fungi that grow in soil and on vegetation and are introduced into subcutaneous tissue through trauma.

Sporotrichosis

Sporothrix schenckii is a dimorphic fungus. The mold form lives on plants, and the yeast form occurs in human tissue. When spores of the mold are introduced into the skin, typically by a thorn, it causes a local pustule or ulcer with nodules along the draining lymphatics. The lesions are typically painless, and there is little systemic illness. Untreated lesions may wax and wane for years.

Chromomycosis

This is a slowly progressive granulomatous infection that is caused by several soil fungi (*Fonsecaea*, *Phialophora*, *Cladosporium*, etc.) when introduced into the skin through trauma. These fungi are collectively called dematiaceous fungi, so named because their conidia or hyphae are dark-colored, either gray or black. Wartlike lesions with crusting abscesses extend along the lymphatics. The disease occurs mainly in the tropics and is found on bare feet and legs. In the clinical laboratory, dark brown, round fungal cells are seen in leukocytes or giant cells. The disease is treated with oral flucytosine or thiabendazole, plus local surgery.

Mycetoma

Soil fungi (*Petromyces*, *Madurella*) enter through wounds on the feet, hands, or back and cause abscesses, with pus discharged through sinuses. The pus contains compact colored granules. Actinomycetes such as *Nocardia* can cause similar lesions (actinomycotic mycetoma). Sulfonamides may help the actinomycotic form. There is no effective drug against the fungal form; surgical excision is recommended.

Sporotrichosis. Note papular lesions on left hand and forearm. Caused by *Sporothrix schenckii*



SYSTEMIC MYCOSES

These infections result from inhalation of the spores of dimorphic fungi that have their mold forms in the soil. Within the lungs, the spores differentiate into yeasts or other specialized forms, such as spherules. Most lung infections are asymptomatic and self-limited. However, in some persons, disseminated disease develops in which the organisms grow in other organs, cause destructive lesions, and may result in death. Infected persons do not communicate these diseases to others.

COCCIDIOIDES

Disease

Coccidioides immitis causes coccidioidomycosis.

Properties

Coccidioides immitis is a dimorphic fungus that exists as a mold in soil and as a spherule in tissue.

Transmission & Epidemiology

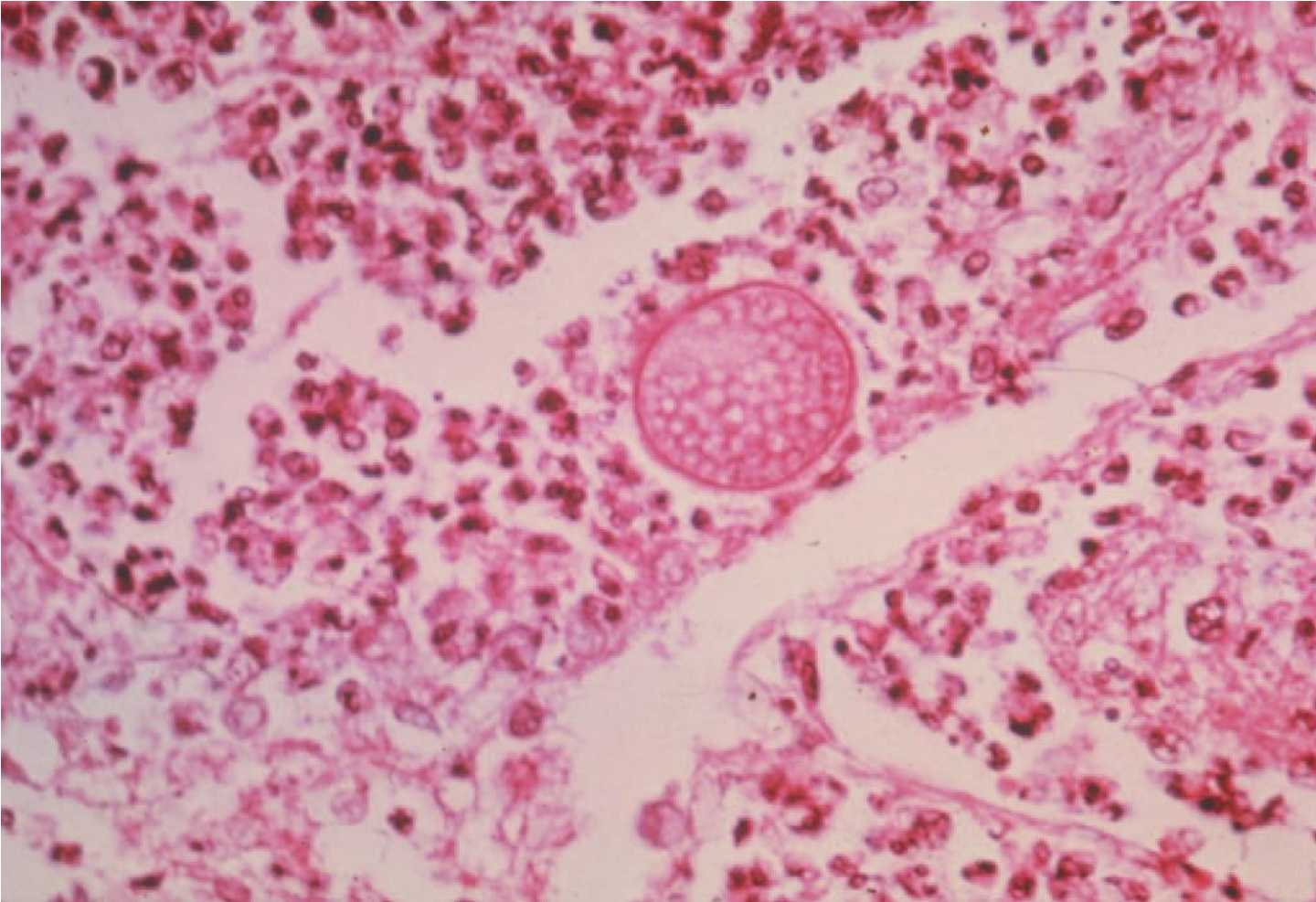
The fungus is endemic in arid regions of the southwestern United States and Latin America. People who live in Central and Southern California, Arizona, New Mexico, Western Texas, and Northern Mexico, a geographic region called the Lower Sonoran Life Zone, are often infected. In soil, it forms hyphae with alternating arthrospores and empty cells. Arthrospores are very light and are carried by the wind. They can be inhaled and infect the lungs.

Laboratory Diagnosis

In tissue specimens, spherules are seen microscopically. Cultures on Sabouraud’s agar incubated at 25°C show septate hyphae with arthrospores . (Caution: Cultures are highly infectious; precautions against inhaling arthrospores must be taken.) In serologic tests, IgM and IgG precipitins appear within 2 to 4 weeks of infection and then decline in subsequent months. Complement-fixing antibodies occur at low titer initially, but the titer rises greatly if dissemination occurs. A PCR assay that detects nucleic acids of Coccidioides is available.

Treatment & Prevention

No treatment is needed in asymptomatic or mild primary infection. Amphotericin B (Fungizone) or itraconazole is used for persisting lung lesions or disseminated disease. Fluconazole is also effective in lung disease. If meningitis occurs, fluconazole is the drug of choice. Intrathecal amphotericin B may be required and may induce remission, but long-term results are often poor. There are no means of prevention except avoiding travel to endemic areas. Patients who have recovered from coccidioidal meningitis should receive long-term suppressive therapy with fluconazole to prevent a recurrence.



Coccidioides immitis—spherule. Long arrow points to a spherule in lung tissue. Spherules are large thick-walled structures containing many endospores. Short arrow points to an endospore

HISTOPLASMA

Histoplasma capsulatum causes histoplasmosis.

Properties

Histoplasma capsulatum is a dimorphic fungus that exists as a mold in soil and as a yeast in tissue. It forms two types of asexual spores (Figure 49–4): (1) tuberculate macroconidia, with typical thick walls and fingerlike projections that are important in laboratory identification; and (2) microconidia, which are smaller, thin, smooth-walled pores that, if inhaled, transmit the infection.

Transmission & Epidemiology

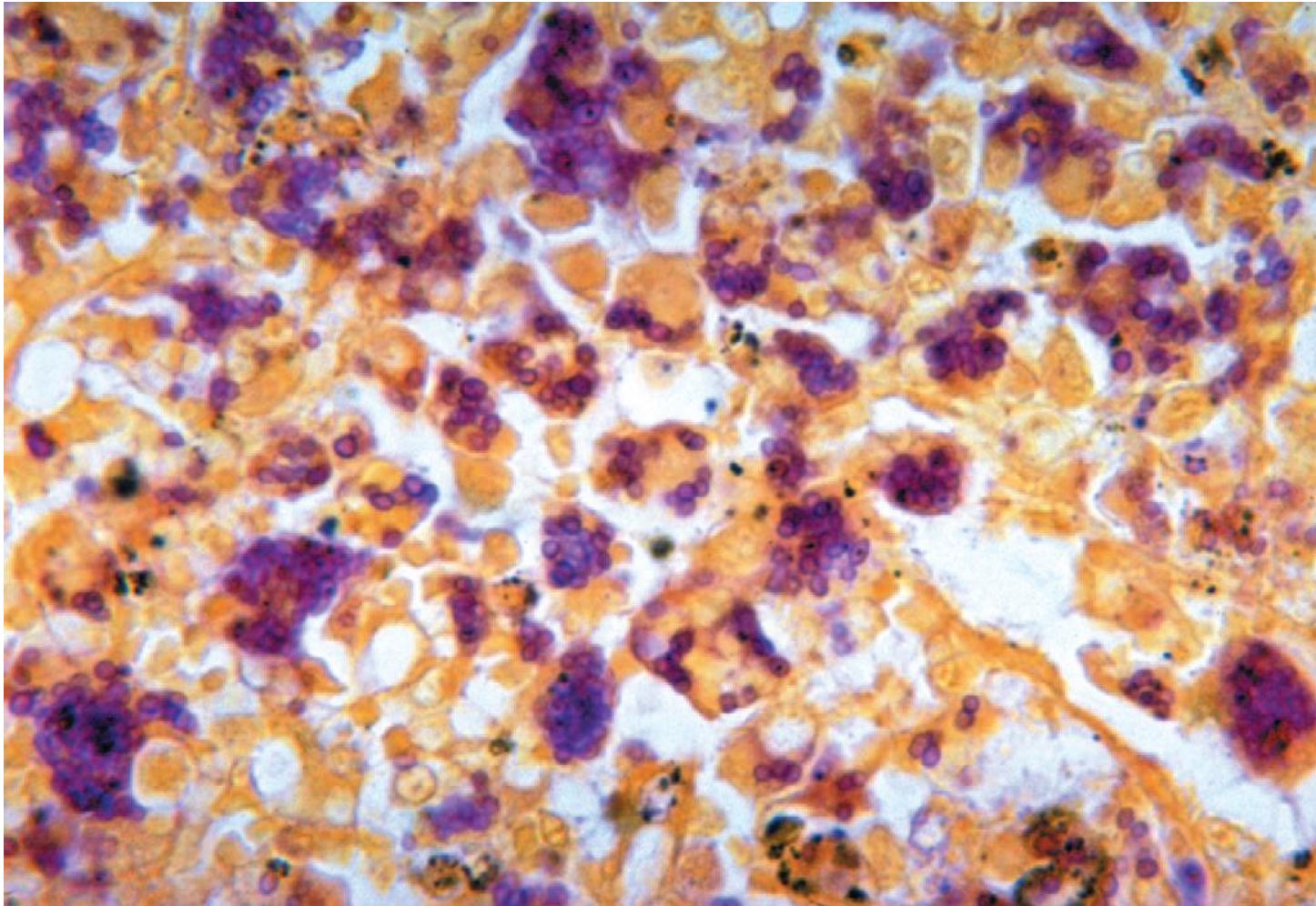
This fungus occurs in many parts of the world. In them United States, it is endemic in central and eastern states, especially in the Ohio and Mississippi River valleys. It grows in soil, particularly if the soil is heavily contaminated with bird droppings, especially from starlings. Although the birds are not infected, bats can be infected and can excrete the organism in their guano. In areas of endemic infection, excavation of the soil during construction or exploration of bat-infested caves has resulted in a significant number of infected individuals.

Laboratory Diagnosis

In tissue biopsy specimens or bone marrow aspirates, oval yeast cells within macrophages are seen microscopically. Cultures on Sabouraud’s agar show hyphae with tuberculate macroconidia when grown at low temperature (e.g., 25°C) and yeasts when grown at 37°C. Tests that detect a Histoplasma polysaccharide antigen by enzyme-linked immunosorbent assay (ELISA) and Histoplasma RNA with DNA probes are also useful. In immunocompromised patients with disseminated disease, tests for Histoplasma antigen in the urine are especially useful because antibody tests may be negative.

Treatment & Prevention

No therapy is needed in asymptomatic or mild primary infections. With progressive lung lesions, oral itraconazole is effective. In disseminated disease, parenteral itraconazole (or amphotericin B) is the treatment of choice. Liposomal amphotericin B should be used in patients with preexisting kidney damage. In meningitis, fluconazole is often used because it penetrates the spinal fluid well. Oral itraconazole is used for chronic suppression in patients with AIDS.



Histoplasma
capsulatum—yeasts
within macrophages.

BLASTOMYCES

Blastomyces dermatitidis causes blastomycosis, also known as North American blastomycosis.

Properties

Blastomyces dermatitidis is a dimorphic fungus that exists as a mold in soil and as a yeast in tissue. The yeast is round with a doubly refractive wall and a single broad-based bud. Note that this organism forms a broad-based bud, whereas Cryptococcus neoformans is a yeast that forms a narrow-based bud.

Transmission & Epidemiology

This fungus is endemic primarily in eastern North America, especially in the region bordering the Ohio, Mississippi, and St. Lawrence rivers, and the Great Lakes region. Less commonly, blastomycosis has also occurred in Central and South America, Africa, and the Middle East. It grows in moist soil rich in organic material, forming hyphae with small pear-shaped conidia. Inhalation of the conidia causes human infection.

Pathogenesis & Clinical Findings

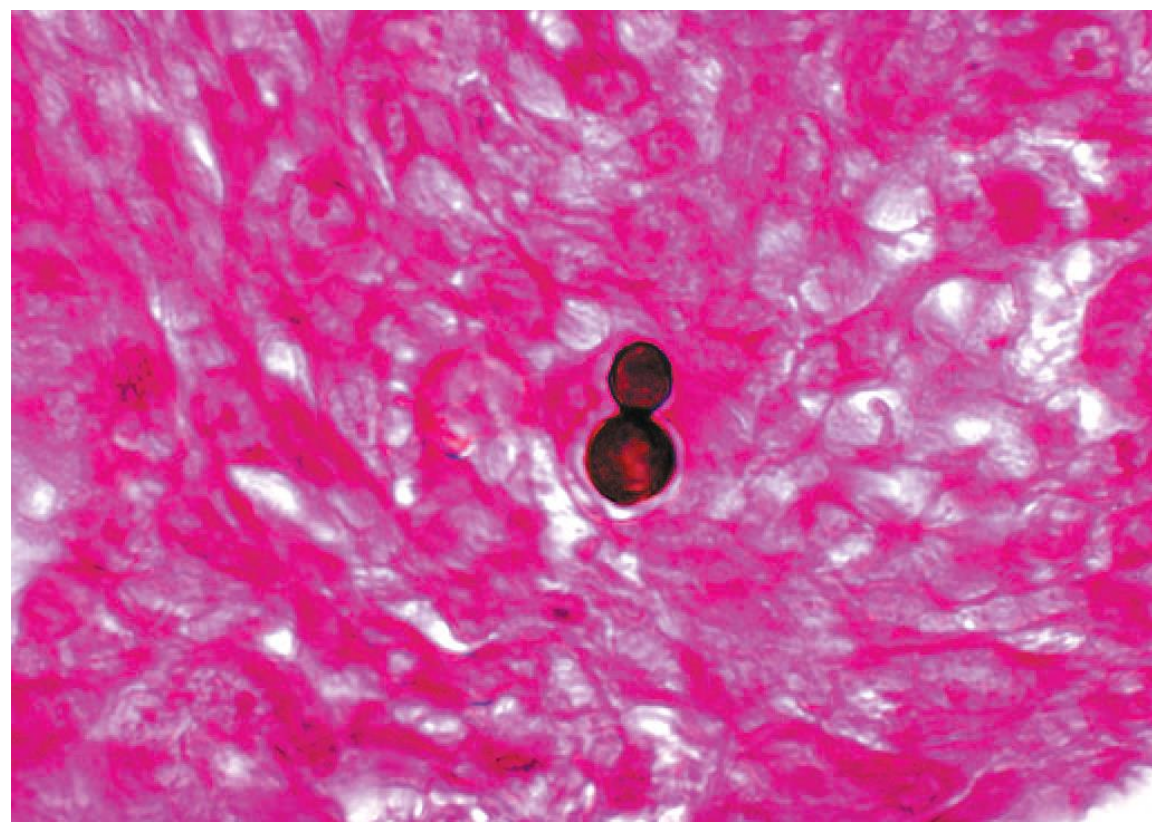
Infection occurs mainly via the respiratory tract. Asymptomatic or mild cases are rarely recognized. Dissemination may result in ulcerated granulomas of skin, bone, or other sites.

Laboratory Diagnosis

In tissue biopsy specimens, thick-walled yeast cells with single broad-based buds are seen microscopically/ Hyphae with small pear-shaped conidia are visible on culture. The skin test lacks specificity and has little value. Serologic tests have little value. A PCR assay that detects nucleic acids of Blastomyces is available.

Treatment & Prevention

Itraconazole is the drug of choice for most patients, but amphotericin B should be



PARACOCCIDIOIDES

Disease

Paracoccidioides brasiliensis causes paracoccidioidomycosis, also known as South American blastomycosis.

Properties

Paracoccidioides brasiliensis is a dimorphic fungus that exists as a mold in soil and as a yeast in tissue. The yeast is thick-walled with multiple buds, in contrast to B. dermatitidis, which has a single bud .

Transmission & Epidemiology

This fungus grows in the soil and is endemic in rural Latin America. Disease occurs only in that region.

Pathogenesis & Clinical Findings

The spores are inhaled, and early lesions occur in the lungs. Asymptomatic infection is common. Alternatively, oral mucous membrane lesions, lymph node enlargement, and sometimes dissemination to many organs develop.

Laboratory Diagnosis

In pus or tissues, yeast cells with multiple buds resembling a “ship captain’s wheel” are seen microscopically. A specimen cultured for 2 to 4 weeks may grow typical organisms. Skin tests are rarely helpful. Serologic testing shows that when significant antibody titers (by ID or CF) are found, active disease is present.

Treatment & Prevention

The drug of choice is itraconazole taken orally for several months. There are no means of prevention



Paracoccidioides—yeasts with multiple buds resembling a “ship captain’s wheel.”
Methenamine silver stain.

OPPORTUNISTIC MYCOSES

Opportunistic fungi fail to induce disease in most immunocompetent persons but can do so in those with impaired host defenses. There are five genera of medically important fungi: *Candida*, *Cryptococcus*, *Aspergillus*, *Mucor*, and *Rhizopus*.

CANDIDA

Diseases

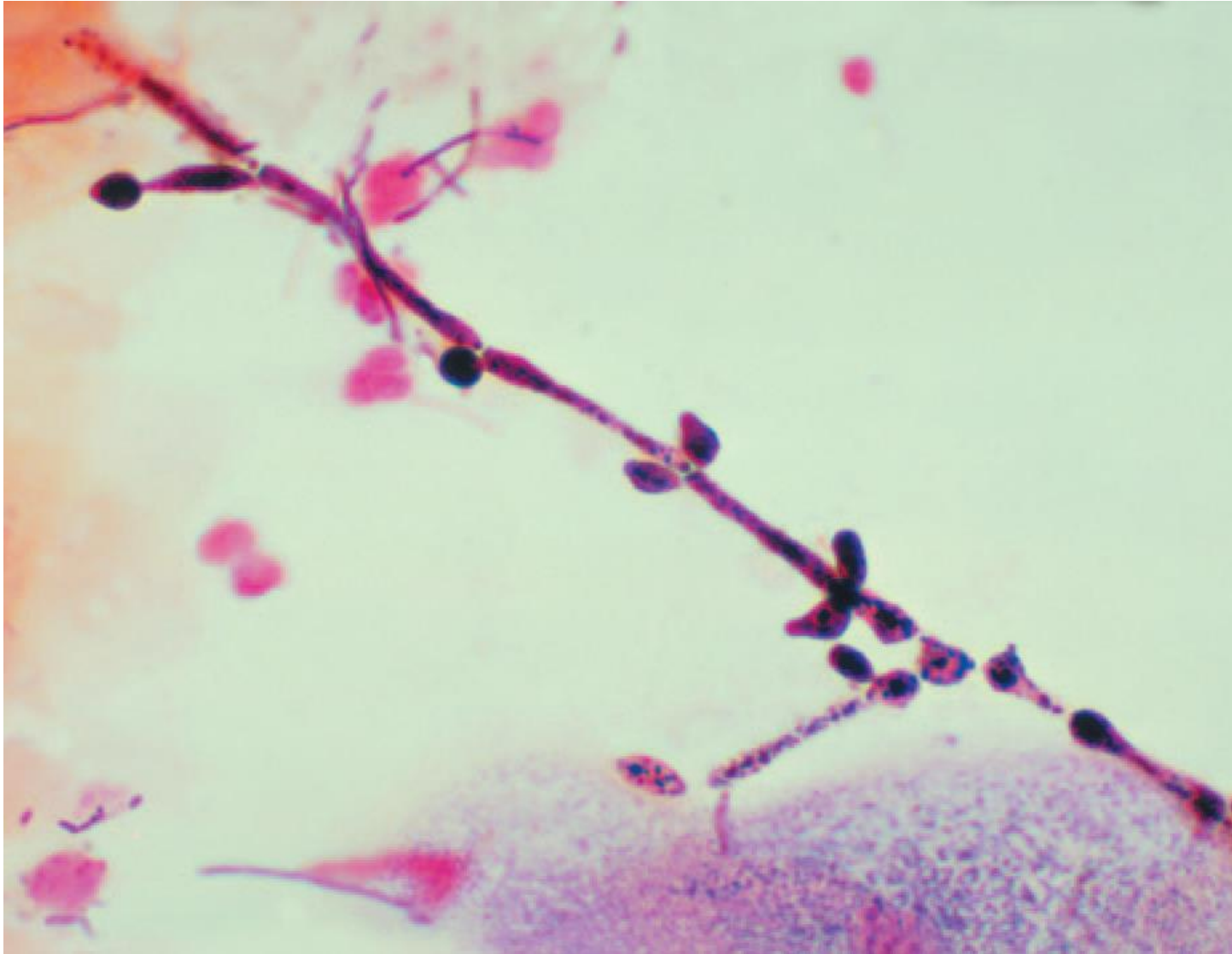
Candida albicans, the most important species of *Candida*, causes thrush, vaginitis, esophagitis, diaper rash, and chronic mucocutaneous candidiasis. It also causes disseminated infections such as right-sided endocarditis (especially in intravenous drug users), bloodstream infections (candidemia), and endophthalmitis. Infections related to indwelling intravenous and urinary catheters are also important. *Candida glabrata* is the second most common cause of disseminated candidal infections and is more drug resistant than *C. albicans*.

Properties

Candida albicans is an oval yeast with a single bud. It is part of the normal flora of mucous membranes of the upper respiratory, gastrointestinal, and female genital tracts. In tissues it appears most often as yeasts or as pseudohyphae. Pseudohyphae are elongated yeasts that visually resemble hyphae but are not true hyphae. True hyphae are also formed when *C. albicans* invades tissues. Carbohydrate fermentation reactions can be used to differentiate it from other species (e.g., *Candida tropicalis*, *Candida parapsilosis*, *Candida krusei*, and *C. glabrata*) that cause human infections. *Candida dubliniensis* is closely related to *C. albicans*. It also causes opportunistic infections in immunocompromised patients, especially AIDS patients. Both species form chlamydospores but *C. albicans* grows at 42°C whereas *C. dubliniensis* does not.

Transmission

As a member of the normal flora, *C. albicans* is already present on the skin and mucous membranes. In addition to the skin, *C. albicans* is found throughout the GI tract (especially the mouth and esophagus) and in the vagina. Thrush in the newborn is the result of passage through a birth canal heavily colonized by the organism. The presence of *C. albicans* on the skin predisposes to infections involving instruments that penetrate the skin, such as needles (intravenous drug use) and indwelling catheters. It is often found in the urine of patients with indwelling urinary (Foley) catheters.



Candida albicans— pseudohyphae

Laboratory Diagnosis

In exudates or tissues, budding yeasts and pseudohyphae appear gram-positive and can be visualized by using calcofluor-white staining. In culture, typical yeast colonies are formed that resemble large staphylococcal colonies. *Candida albicans* forms germ tubes in serum at 37°C, whereas most other species of pathogenic *Candida* species do not. Chlamydospores are typically formed by *C. albicans* but not by most other species of *Candida*. Note that *C. dubliniensis* also forms chlamydospores but will not grow at 42°C whereas *C. albicans* will. Serologic testing is rarely helpful. A laboratory test that can identify *C. albicans* and four other *Candida* species in blood cultures in 3 to 5 hours instead of the usual several days was approved in 2014. The test uses magnetic resonance technology to detect the presence of yeast DNA and then to identify the species. Skin tests with *Candida* antigens are uniformly positive in immunocompetent adults and are used as an indicator that the person can mount a cellular immune response.

CRYPTOCOCCUS

Disease

Cryptococcus neoformans causes cryptococcosis, especially cryptococcal meningitis. Cryptococcosis is the most common, life-threatening invasive fungal disease worldwide. It is especially important in AIDS patients. Another species, *Cryptococcus gattii*, causes human disease less frequently than *C. neoformans*.

Properties

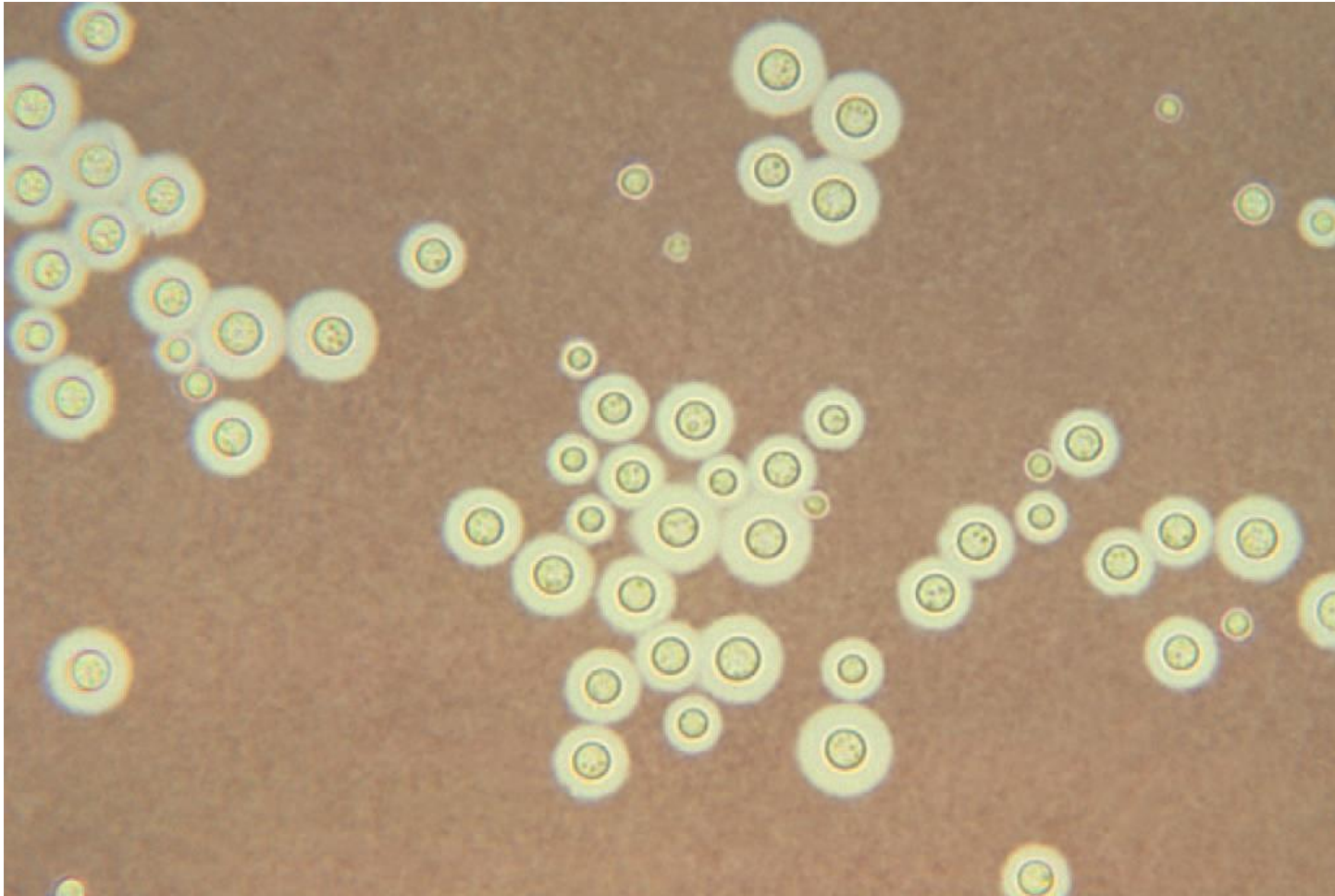
Cryptococcus neoformans is an oval, budding yeast surrounded by a wide polysaccharide capsule . It is not dimorphic. Note that this organism forms a narrow-based bud, whereas the yeast form of *Blastomyces dermatitidis* forms a broad-based bud.

Transmission

Cryptococcus neoformans occurs widely in nature and grows abundantly in soil containing bird (especially pigeon) droppings. The birds are not infected. Human infection results from inhalation of the organism. There is no human-to-human transmission. *Cryptococcus gattii* is associated with eucalyptus trees, most often in the northwestern states of the United States. It is also found in subtropical and tropical areas of many countries.

Pathogenesis & Clinical Findings

Lung infection is often asymptomatic or may produce pneumonia. Disease caused by *C. neoformans* occurs mainly in patients with reduced cell-mediated immunity, especially AIDS patients, in whom the organism disseminates to the central nervous system (meningitis) and other organs. Subcutaneous nodules are often seen in disseminated disease. Note, however, that roughly half the patients with cryptococcal meningitis fail to show evidence of immunosuppression. In some patients with AIDS who are infected with *Cryptococcus*, treating the patient with highly active antiretroviral therapy (HAART) causes an exacerbation of symptoms.



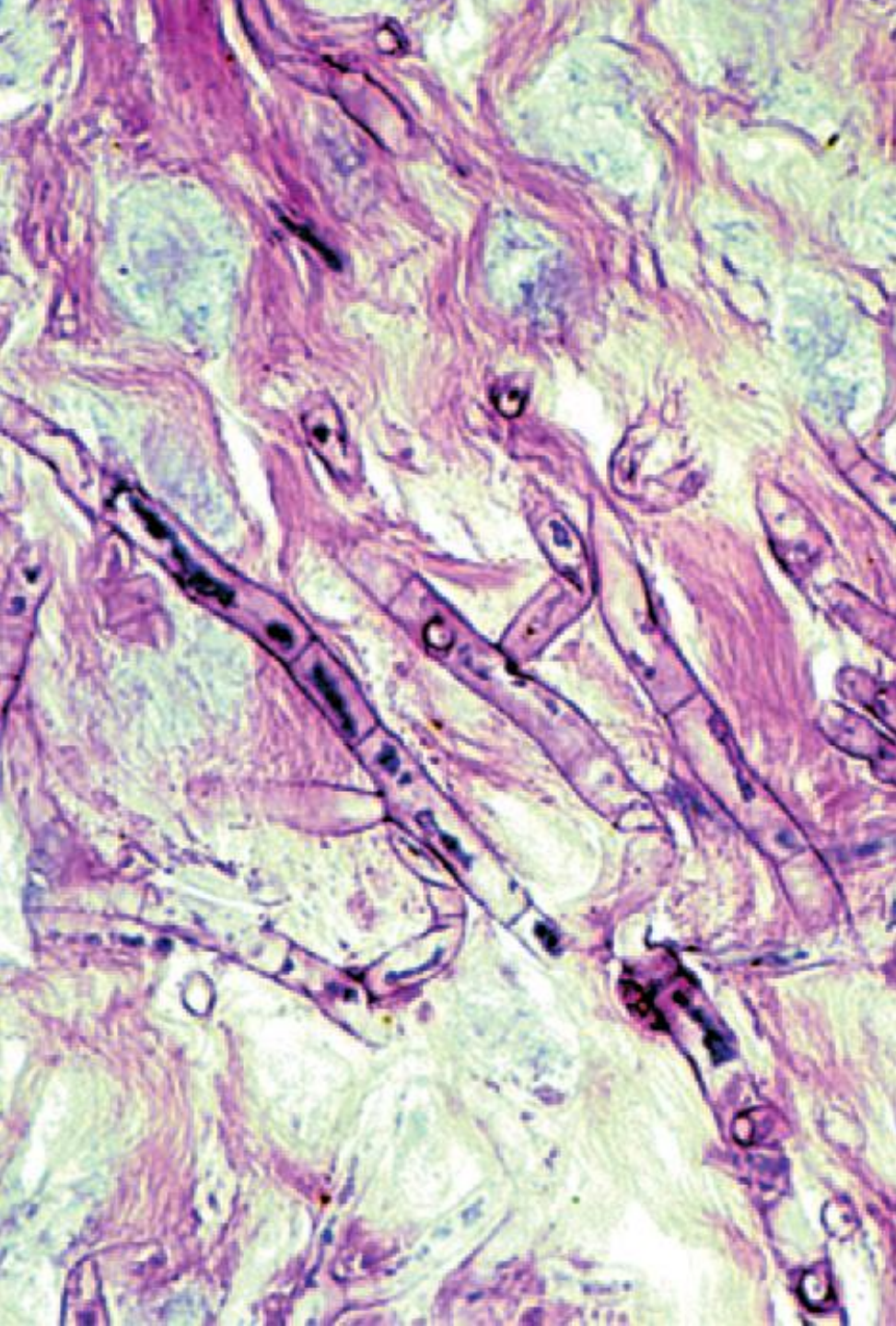
Cryptococcus
neoformans—India
ink preparation.

Laboratory Diagnosis

In spinal fluid mixed with India ink, the yeast cell is seen microscopically surrounded by a wide, unstained capsule. Appearance of the organism in Gram stain is unreliable, but stains such as periodic acid–Schiff (PAS stain), methenamin silver, and mucicarmine will allow the organism to be visualized. The organism can be cultured from spinal fluid and other specimens. The colonies are highly mucoid—a reflection of the large amount of capsular polysaccharide produced by the organism. Serologic tests can be done for both antibody and antigen. In infected spinal fluid, capsular antigen occurs in high titer and can be detected by the latex particle agglutination test. This test is called the cryptococcal antigen test, often abbreviated as “crag.” Distinguishing between *C. neoformans* and *C. gattii* in the laboratory requires specialized media not generally available, so many *C. gattii* infections may go undiagnosed.

Treatment & Prevention

Combined treatment with amphotericin B and flucytosine is used in meningitis and other disseminated disease. Liposomal amphotericin B should be used in patients with preexisting kidney damage. There are no specific means of prevention. Fluconazole is used in AIDS patients for long-term suppression of cryptococcal meningitis. *Cryptococcus gattii* is less responsive to antifungal drugs than is *C. neoformans*.



ASPERGILLUS

Disease

Aspergillus species, especially *Aspergillus fumigatus*, cause infections of the skin, eyes, ears, and other organs; “fungus ball” in the lungs; and allergic bronchopulmonary aspergillosis.

Properties

Aspergillus species exist only as molds; they are not dimorphic. They have septate hyphae that form V-shaped (dichotomous) branches . The walls are more or less parallel, in contrast to *Mucor* and *Rhizopus* walls, which are irregular . The conidia of *Aspergillus* form radiating chains, in contrast to those of *Mucor* and *Rhizopus*, which are enclosed within a sporangium.

Transmission

These molds are widely distributed in nature. They grow on decaying vegetation, producing chains of conidia. Transmission is by airborne conidia.

Pathogenesis & Clinical Findings: *Aspergillus fumigatus* can colonize and later invade abraded skin, wounds, burns, the cornea, the external ear, or paranasal sinuses. It is the most common cause of fungal sinusitis. In immunocompromised persons, especially those with neutropenia, it can invade the lungs producing hemoptysis and the brain causing an abscess. Neutropenic patients are also predisposed to intravenous catheter infections caused by this organism. In 2012, an outbreak of *A. fumigatus* infections, especially meningitis, occurred caused by injectable corticosteroid solutions that were contaminated with the fungus. Aspergilli are well-known for their ability to grow in cavities within the lungs, especially cavities caused by tuberculosis.

Laboratory Diagnosis

Biopsy specimens show septate, branching hyphae invading tissue . Cultures show colonies with characteristic radiating chains of conidia . However, positive cultures do not prove disease because colonization is common. In persons with invasive aspergillosis, there may be high titers of galactomannan antigen in serum. Patients with ABPA have high levels of IgE specific for *Aspergillus* antigens and prominent eosinophilia. IgG precipitins are also present.

Treatment & Prevention

Voriconazole is the drug of choice for invasive aspergillosis. Liposomal amphotericin B, posaconazole, and caspofungin are alternative drugs. A fungus ball growing in a sinus or in a pulmonary cavity can be surgically removed. Patients with ABPA can be treated with corticosteroids and antifungal agents, such as itraconazole. There are no specific means of prevention.



- Mucor species—mucormycosis.
Note necrotic area involving the nose and face

MUCOR & RHIZOPUS

Mucormycosis (zygomycosis, phycomycosis) is a disease caused by saprophytic molds (e.g., Mucor, Rhizopus, and Absidia) found widely in the environment. They are not dimorphic. These organisms are transmitted by airborne asexual spores and invade tissues of patients with reduced host defenses. They proliferate in the walls of blood vessels, particularly of the paranasal sinuses, lungs, or gut, and cause infarction and necrosis of tissue distal to the blocked vessel. Patients with diabetic ketoacidosis, burns, bone marrow transplants, or leukemia are particularly susceptible. Diabetic patients are particularly susceptible to rhinocerebral mucormycosis, in which mold spores in the sinuses germinate to form hyphae that invade blood vessels that supply the brain. One species, *Rhizopus oryzae*, causes about 60% of cases of mucormycosis. In biopsy specimens, organisms are seen microscopically as nonseptate hyphae with broad, irregular walls and branches that form more or less at right angles. Cultures show colonies with spores contained within a sporangium. These organisms are difficult to culture because they are a single, very long cell, and damage to any part of the cell can limit its ability to grow. If diagnosis is made early, treatment of the underlying disorder, plus administration of amphotericin B and surgical removal of necrotic infected tissue, has resulted in some remissions and cures. Liposomal amphotericin B should be used in

PNEUMOCYSTIS

Pneumocystis jiroveci is classified as a yeast on the basis of molecular analysis, but it has many characteristics of a protozoan. Some regard it as an “unclassified” organism. In 2002, taxonomists renamed the human species of *Pneumocystis* as *P. jiroveci* and recommended that *P. carinii* be used only to describe the rat species of *Pneumocystis*. *Pneumocystis* is acquired by inhalation of airborne organisms into the lungs. An inflammatory exudate composed primarily of plasma cells occurs, oxygen exchange is reduced, and dyspnea occurs. A reduced number of CD 4-positive T lymphocytes, such as occurs in AIDS, predisposes to pneumonia. Most immunocompetent people have asymptomatic infections. The clinical findings of *Pneumocystis* pneumonia include fever, nonproductive cough, and dyspnea. Rales are heard bilaterally and the chest X-ray shows a “groundglass” pattern. The mortality rate of untreated *Pneumocystis* pneumonia is approximately 100%.