

COURSE: Medical Microbiology (MBIM 650/720) - Fall 2005
TOPIC: MYCOLOGY
FACULTY: Dr. Arthur Di Salvo

Hour 1

TEACHING OBJECTIVE: To impart sufficient basic science of the medically important fungi to assist you in diagnosing mycotic diseases and to impart sufficient clinical knowledge to raise your index of suspicion for mycotic diseases.

I. INTRODUCTION

What is mycology ? Mycology is the study of fungi and their multiple functions in nature.

A. CLASSIFICATION

Fungi are **eukaryotic** organisms that do **not** contain chlorophyll, but have cell walls, filamentous structures, and produce spores. These organisms grow as saprophytes and decompose dead organic matter. There are between 100,000 to 200,000 species depending on how they are classified. About 300 species are presently known to be pathogenic for man. There are five kingdoms of living things. The fungi are in the Kingdom Fungi.

TAXONOMY

KINGDOM	CHARACTERISTIC	EXAMPLE
Monera	Prokaryocyte	Bacteria Actinomyces
Protista	Eukaryocyte	Protozoa
Fungi	Eukaryocyte *	Fungi
Plants	Eukaryocyte	Plants Moss
Animals	Eukaryocyte *	Arthropods Mammals Man

* This common characteristic is responsible for the therapeutic dilemma in anti-mycotic therapy.

The taxonomy of the Kingdom Fungi is evolving and is controversial. Formerly based on gross and light microscopic morphology, studies of ultra structure, biochemistry and molecular biology provide new evidence on which to base taxonomic positions.

MEDICAL MYCOLOGY

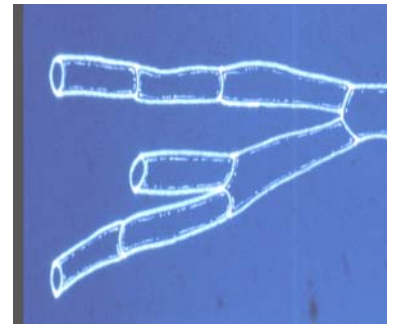
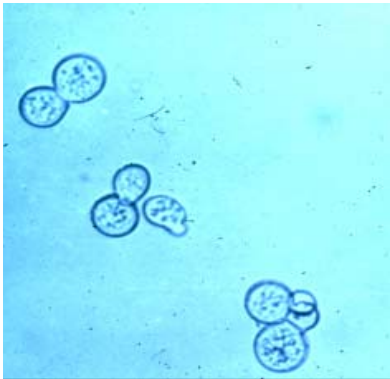
There are four types of mycotic diseases:

1. **Hypersensitivity** - an allergic reaction to molds and spores. Indoor air pollution
2. **Mycotoxicoses** - poisoning of man and animals by feeds and food products contaminated by fungi which produce toxins from the grain substrate.
3. **Mycetismus**- the ingestion of pre-formed toxin (mushroom poisoning).
4. **Infection**

These lectures are concerned only with the last type: pathogenic fungi which cause infections. Most common pathogenic fungi do **not** produce toxins.

B. MORPHOLOGY

Pathogenic fungi can exist as **yeasts** or as **hyphae**. Yeasts are unicellular organisms, which reproduce by budding, and mycelia are multicellular filamentous structures, constituted by tubular cells with cell walls. A mass of hyphae (mycelium) is called **mycelia**. The mycelial forms branch; the pattern of branching and the width of the mycelium are aids to the morphological identification. If the mycelia do not have SEPTA, they are called **coenocytic** (non-septate). The terms "hypha", "mycelium" and "mold" are frequently used interchangeable. Some fungi may occur in both the yeast and mycelial forms. These are called **dimorphic** fungi.



Dimorphic fungi

The dimorphic fungi have two forms:

1. YEAST - (**parasitic or pathogenic** form). This is the form usually seen in tissue, in exudates, or if cultured in an incubator at 37°C.
2. MYCELIUM - (**saprophytic or mold** form). The form observed in nature or when cultured at 25°C. Conversion to the yeast form appears to be essential for pathogenicity in the dimorphic fungi.

Spores

Fungi are identified by several morphological or biochemical characteristics, including the appearance of their fruiting bodies. The asexual spores may be large (macroconidia, chlamydoconidia) or small (microconidia, blastospores, arthroconidia).

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C. EPIDEMIOLOGY

Fungi are ubiquitous in nature and human beings are constantly exposed to them. Most mycotic agents are soil saprophytes and mycotic diseases are generally **not communicable** from person-to-person (occasional exceptions: *Candida* and some dermatophytes). Outbreaks of fungal disease may occur, but these are due to a common environmental exposure, not communicability.

The establishment of a mycotic infection usually depends on the **size of the inoculum** and on the **resistance of the host**. The severity of the disease seems to depend mostly on the immunologic status of the host. Thus, the demonstration of fungi, for example, in blood drawn from an intravenous catheter can correspond to **colonization** of the catheter, to transient **fungemia** (i.e., dissemination of fungi through the blood stream), or to a true **infection**. The physician must decide which is the clinical status of the patient based on clinical parameters, general status of the patient, laboratory results, etc. The decision is not trivial, since treatment of systemic fungal infections requires the aggressive use of drugs with considerable toxicity.

GEOGRAPHICAL DISTRIBUTION

Most of the fungi, which cause systemic infections, have a peculiar, characteristic ecologic niche in nature. This habitat is specific for several fungi which will be discussed later. In this environment, the normally saprophytic organisms proliferate and develop. This habitat is also the source of fungal elements and/or spores, where man and animals, incidental hosts, are exposed to the infectious particles. It is important to be aware of these associations to diagnose mycotic diseases. The physician must be able to **elicit a complete history from the patient including occupation, avocation and travel history**. This information is frequently required to raise, or confirm, your differential diagnosis.

D. DIAGNOSIS

1. **Wet Mount:** Skin scrapings suspected to contain dermatophytes or pus from a lesion can be mounted in KOH on a slide and examined directly under the microscope.
2. **Dermal Hypersensitivity:** Skin Testing used to be popular as a diagnostic tool, but this use is now discouraged because the skin test may interfere with serological studies, by causing false positive results. It may still be used to evaluate the patient's immunity, as well as a population exposure index in epidemiological studies.
3. **Serology:** This tool may be helpful when it is applied to a specific fungal disease; there are no screening antigens for "fungi" in general. Because fungi are poor antigens, the efficacy of serology varies with different fungal agents. The serologic tests will be discussed under each mycosis.

The most common serological tests for fungi are based on latex agglutination, immunodiffusion, complement fixation and enzyme immunoassays. While latex agglutination may favor the detection of IgM antibodies, double immunodiffusion and complement fixation usually detect IgG antibodies. Some EIA tests are being developed to detect both IgG and IgM antibodies. There are some serologic tests which can detect specific fungal **antigens**, e.g. cryptococcosis and histoplasmosis.

4. **Direct fluorescent microscopy:** may be used for identification of fungi, even on non-viable cultures or on fixed tissue sections. The reagents for this test are presently difficult to obtain.

5. **Biopsy and histopathology:** A biopsy may be very useful as a source of the tissue-invading fungi and for the identification of the organism. Usually the Gomori methenamine silver (GMS) stain is used to reveal the organisms which stain black against a green background. The H&E stain does not always tint the organism, but it will demonstrate the inflammatory cells.

6. **Culture:** A definitive diagnosis requires a culture and identification. Pathogenic fungi are usually grown on **Sabouraud dextrose agar (SDA)**. It has a slightly acidic pH (~5.6); cyclohexamide, penicillin, streptomycin or other inhibitory antibiotics are often added to prevent bacterial contamination and overgrowth. Each specimen is inoculated on duplicate sets of media and incubated separately at 25°C and 37°C to reveal dimorphism. The cultures are examined macroscopically and microscopically. The cultures are not considered negative for growth until after 4 weeks of incubation.

7. **DNA probes:** Ribosomal DNA is hybridized to a labeled DNA probe. This test is rapid (1 Hour) and species specific.

E. TREATMENT

Mammalian cells do not contain the enzymes which will degrade the cell wall polysaccharides of fungi. Therefore, these pathogens are difficult to eradicate by the animal host defense mechanisms. Because mammals and fungi are both eukaryotic, the cellular milieu is biochemically similar in both. The cell membranes of all eukaryotic cells contain sterols; ergosterol in the fungal cell membrane and cholesterol in the mammalian cell membrane. Thus, most substances which may impair the invading fungus will usually have serious side effects on the host. Although one of the first chemotherapeutic agents (oral iodides) was an anti-mycotic used in 1903, the further development of such agents has been slower than the development of anti-bacterial agents. The selective toxicity necessary to inhibit the invading organism with minimal damage to the host has been difficult to establish within eukaryotic cells.

The primary antifungal agents follow:

1. **Polyene Derivatives:**

Amphotericin B, a polyene antimycotic, has been the drug of choice for most systemic fungal infections. It has a greater affinity for ergosterol in the cell membranes of fungi than for the cholesterol in the host's cells; once bound to ergosterol it causes disruption of the cell membrane and death of the fungal cell. Amphotericin B is usually administered intravenously (patient usually needs to be hospitalized), often for 2-3 months. The drug is rather toxic; thrombo-phlebitis, nephrotoxicity, fever, chills and anemia frequently occur during administration. Although newer drugs have been shown to be as efficacious and less toxic, amphotericin B is still the gold standard for comparison as well as the therapy of last resort for severe infections.

Lipid based amphotericin: As effective, less toxic, very expensive.

Nystatin. Very limited use, primarily for candida infections. There is now an IV lipid formulation under clinical trials.

2. **Azoles** The azoles (imidazoles and triazoles), including ketoconazole, fluconazole, itraconazole, and voriconazole are being used for muco-cutaneous candidiasis, dermatophytosis, and for some systemic fungal infections. Fluconazole is presently essential for the maintenance of AIDS patients with cryptococcosis because it will penetrate the spinal fluid. The general mechanism of action of the azoles is the inhibition of ergosterol synthesis which affects cell wall synthesis. Oral administration and reduced toxicity are distinct advantages.

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3. Griseofulvin. Griseofulvin is a very slow-acting drug which is used for severe skin and nail infections. Its effect depends on its accumulation in the *stratum corneum* where it is incorporated into the tissue and forms a barrier, which stops further fungal penetration and growth. It is administered orally.

4. 5-fluorocytosine

5-fluorocytosine (5-FC) inhibits RNA synthesis and has found its main application in cryptococcosis (to be discussed later). It is administered p.o.

5. Allylamines

Terbinafine (Lamisil). For dermatophyte infections

6. Echinocandins (caspofungin)

A new antifungal agent recently approved by the FDA

F. CLINICAL CLASSIFICATION OF THE MYCOSES

- a. Superficial mycoses
- b. Subcutaneous mycoses and
- c. Systemic mycoses
- d. Opportunistic mycoses

The **Superficial mycoses** (or cutaneous mycoses) are fungal diseases that are confined to the outer layers of the **skin, nail, or hair**, (keratinized layers) rarely invading the deeper tissue or viscera. The fungi involved are called dermatophytes.

The **Subcutaneous mycoses** are confined to the **subcutaneous tissue** and only rarely spread systemically. They usually form deep, ulcerated skin lesions or fungating masses, most commonly involving the lower extremities. The causative organisms are soil saprophytes, which are introduced through trauma to the feet or legs.

The **Systemic mycoses** may involve deep viscera and become widely disseminated. Each fungus type has its own predilection for various organs which will be described as we discuss the individual diseases.

The **opportunistic mycoses** are caused by ubiquitous saprophytes and occasional pathogens that invade the tissues of those patients who have:

- 1) Predisposing disease (diabetes, cancer, leukemia, etc.) or
- 2) Predisposing conditions (agammaglobulinemia, steroid or antibiotic therapy).

II. ACTINOMYCETES

We will discuss three genera of actinomycetes: Actinomyces, Nocardia, and Streptomyces. These organisms have been shown to be higher bacteria, but they were thought to be fungi for many years because they have filamentous forms, 0.5 to 0.8 microns in diameter, which appear to branch. Some species form aerial mycelia in culture. The clinical manifestations of infection are similar to those of a systemic fungal infection. It is now clear that they are not fungi but are closely related to the mycobacteria. Some facts that you must know about these genera are that **Actinomyces are**

anaerobic, while *Nocardia* and *Streptomyces* are aerobic. **Nocardia stain partially acid-fast**, *Actinomyces* and *Streptomyces* are not acid-fast. **Actinomyces produce granules**. Most actinomycetes in tissue do not stain with the H & E stain commonly used for general histopathology. All genera may produce granules; *Actinomyces* almost always produces granules.

A. ACTINOMYCOSIS

Actinomycosis is a chronic suppurative and granulomatous disease of the cervico-facial, thoracic or abdominal areas

The most common cause of actinomycosis is the organism *Actinomyces israelii* which infects both man and animals. In cattle, the disease is called "lumpy jaw" because of the huge abscess formed in the angle of the jaw. In man, *A. israelii* is an endogenous organism that can be isolated from the mouths of healthy people. Frequently, the infected patient has a tooth abscess or a tooth extraction and the endogenous organism becomes established in the traumatized tissue and causes a suppurative infection. These abscesses are not confined to the jaw and may also be found in the thoracic area and abdomen. The patient usually presents with a pus-draining lesion, so the pus will be the clinical material you send to the laboratory.

This organism, which occurs worldwide, can be seen histologically as "sulfur granules" surrounded by polymorphonuclear cells (PMN) forming the purulent tissue reaction. The organism is a gram-positive rod that frequently branches. The laboratory must specifically be instructed to culture for this **anaerobic** organism.

These lesions must be surgically drained prior to antibiotic therapy and the drug of choice is large doses of penicillin (18-20 million units q day).

B. NOCARDIOSIS

Nocardiosis primarily presents as a pulmonary disease or brain abscess in the U.S. In Latin America, it is more frequently seen as the cause of a subcutaneous infection, with or without draining abscesses. Brain abscesses are frequent secondary lesions. The most common species of *Nocardia* which cause disease in human beings are *N. brasiliensis* and *N. asteroides*. These are soil organisms which can also be found endogenously in the sputum of apparently healthy people. *N. asteroides* is usually the etiologic agent of pulmonary nocardiosis while *N. brasiliensis* is frequently the cause of sub-cutaneous lesions. The geographic distribution of these organisms is worldwide.

The material sent to the laboratory, depending on the presentation of the disease, is sputum, pus, or biopsy material. These organisms rarely form granules. The *Nocardia* are aerobic, gram-positive rods and stain partially acid-fast (i.e., the acid-fast staining is not uniform). There are no serological tests, and the drug of choice is Bactrim (Trimethoprim plus sulfamethoxazole). The *nocardia* grow readily on most bacteriologic and *M. tuberculosis* media.

C. STREPTOMYCOSIS

The streptomyces species usually cause the disease entity known as **mycetoma** (fungus tumor). These infections are usually subcutaneous, but they can penetrate deeper and invade the bone. Some species produce a protease which inhibits macrophages.

Material sent to the laboratory may be pus or skin biopsy. The streptomycetes are aerobic like *Nocardia*, and can grow on both bacterial and fungal (Sabouraud) media. They produce a chalky aerial mycelium with much branching. It is important to let the laboratory know the organism you suspect

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because most bacterial pathogens will grow out overnight, but the actinomycetes take longer to be visible on the culture plates (48-72 hours).

The various species of streptomyces produce granules of different size, texture and color. These granules along with colonial growth and biochemical tests allow the bacteriologist or mycologist to identify each species. The organisms are found world-wide.

There are no serological tests, and the drugs of choice are the combination of sulfamethoxazole/trimethoprim or amphotericin B. In the tropics this disease may go undiagnosed or untreated for so long that surgical amputation may be the only effective treatment.

HOUR 2

III. YEASTS

Yeasts are single-celled budding organisms. They do not produce mycelia. The colonies are usually visible on the plates in 24-48 hours. Their soft, moist colonies resemble bacterial cultures rather than molds. There are many species of yeasts, which can be pathogenic for humans. We will discuss only the two most significant species: *Candida albicans* and *Cryptococcus neoformans*

A. CANDIDIASIS (*Candida albicans*)

There are many species of the genus *Candida* that cause disease. The infections caused by all species of *Candida* are called candidiasis. Although this discussion is limited to *Candida albicans* it is important in clinical practice to speciate *Candida* because drug resistance and treatment varies with species.

Candida albicans is an endogenous organism. It can be found in 40-80% of normal human beings. It is present in the mouth, gut, and vagina. It may be present as a commensal or a pathogenic organism. The establishment of infection with *Candida* species appears to be a property of the host - not the organism. The more debilitated the host, the more invasive the disease. Infections with *Candida* usually occur when a patient has some alteration in cellular immunity, normal flora or normal physiology. Patients with decreased cellular immunity have decreased resistance to fungal infections. Prolonged antibiotic or steroid therapy destroys the balance of normal flora in the intestine allowing the endogenous *Candida* to overcome the host. Invasive procedures, such as cardiac surgery and indwelling catheters, produce alterations in host physiology and some of these patients develop *Candida* infections. Although it most frequently infects the skin and mucosae, *Candida* can cause pneumonia, septicemia or endocarditis in the compromised patient. The organism occurs world-wide.

The clinical material to be sent to the laboratory depends on the presentation of the disease: blood cultures, vaginal discharge, urine, feces, nail clippings or material from cutaneous or mucocutaneous lesions. *Candida* is a polymorphic yeast, i.e., yeast cells and pseudohyphae are produced. It has been shown that *Candida* needs a transcription repressor to maintain the yeast form. This ability to assume various forms may be related to the pathogenicity of this organism. The yeast form is 10-12 microns in diameter, gram positive, and it grows overnight on most bacterial and fungal media. Pseudohyphae may be formed from budding yeast cells that remain attached to each other. Spores (chlamydo spores) may be formed on the pseudomycelium which can be used to identify different species of *Candida*. *Candida albicans* (specifically) also produces germ tubes which serve as a key to speciation. Some mycologists think that the pseudomycelia represent a more invasive form of the organism. The species are identified by biochemical reactions and morphology.

Detection: Beta-glucan assay. This test has been used for only a few years.

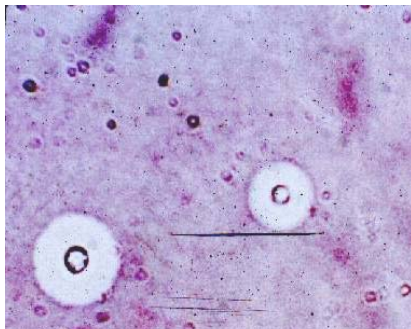
The drug of choice for vaginitis and cutaneous infections is nystatin (topical) and those for systemic infections are itraconazole and fluconazole. If an artificial heart valve or in-dwelling catheter becomes infected, it must be replaced. Drug therapy alone will not suppress the organism if the foreign body remains in the host. This resistance is due to biofilm production which we will discuss in the last hour.

Candida species account for an increasing number of nosocomial infections

B. CRYPTOCOCCOSIS (*Cryptococcus neoformans*)

Cryptococcosis manifests itself most commonly as meningitis but in recent years many pulmonary infections have been recognized.

Infection may be subacute or chronic. The highly fatal meningoencephalitis caused by *C. neoformans* has a prolonged clinical evolution of several months. The patients symptoms may begin with vision problems, lethargy and headache, which then progress to delirium, nuchal rigidity leading to coma and death; unless the physician is thinking about cryptococcus and does a spinal tap for diagnosis and institutes aggressive anti-mycotic therapy. The CSF is examined for its characteristic chemistry (elevated opening pressure, elevated protein and decreased glucose), cells (usually monocytes), and evidence of the organism. The latter is measured by the visual demonstration of the organism (India Ink preparation) or by a serologic assay for the **antigen** of *C. neoformans*. Death usually occurs due to cerebral edema and increased intracranial pressure.



C. neoformans is a very distinctive yeast. The cells, which are spherical, and 5-10 microns in diameter, produce buds that characteristically are narrow-based and a polysaccharide capsule surrounds the organism. There is evidence that the capsule may suppress T-cell function and can be considered a virulence factor. *C. neoformans* also produces an enzyme called phenoloxidase (melanin) which appears to be another virulence factor.

The geographical distribution of this organism is world-wide.

The ecological niche of *C. neoformans* is pigeon and chicken droppings. However, although this organism can be easily recovered from pigeon droppings, a direct epidemiological link has yet to be established between exposure to pigeon droppings and a specific human infection. Infection and disease production is probably a property of the host--not the organism. This organism is ubiquitous, especially in areas like abandoned buildings contaminated with pigeon droppings. The portal of entry is the respiratory system. Evidence is developing which indicates that the initial exposure may be many years prior to the manifestation of disease. The organism can be sequestered for this time.

The India Ink test, which demonstrates the capsule of this yeast, is supplemented by the latex agglutination test for antigen which is more sensitive and more specific. The Latex Agglutination test measures **antigen**, NOT antibody. A decreasing titer indicates a good prognosis, while an increasing titer has a poor prognosis.

When you consider **Cryptococcosis**, think of **Capsules** and **CNS** disease.

In addition to causing meningitis, *C. neoformans* may also infect lungs and skin. The disease in the lungs and skin is characterized by the formation of a granulomatous reaction with giant cells. As with other fungal diseases, there has been an increase in the recognition of pulmonary infection. The yeast may also form a mass in the mediastinum called a cryptococcoma.

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The clinical material sent to the laboratory is CSF, biopsy material, and urine (for some unexplained reason the organism can be isolated from the urine in both the CNS and systemic infections). This organism will grow overnight on bacterial or fungal media at 37 C. but growth is a little slower at room temperature. In culture the organism grows as creamy, white, mucoid (because of the capsule) colonies. Growth in culture is usually visible in 24-48 hours. As the culture ages, it turns brown due to a melanin produced by the phenoloxidase. The organism is a round, single cell, yeast surrounded by a capsule. Identification is based on physiological reactions. Pathologists use a mucicarmine stain, which stains the capsule, to identify the organism in tissue sections. Usually there is little or no inflammatory response. In chronic cryptococcosis the tissue reaction is granulomatous.

The Direct Fluorescent Antibody test identifies the organism in culture or tissue section specifically, by causing the yeast cell wall to fluoresce green. To test the patient's serum there are 3 serologic tests: The Indirect Fluorescent Antibody test, the Tube Agglutination test for antibody, and the Latex Agglutination test for antigen. The latex agglutination test can be used as a prognostic test. As the patient improves, the serum and CSF antigen titer will decrease.

The drugs of choice to treat cryptococcus infection are amphotericin B plus 5-flucytosine (5-FC) or amphotericin plus fluconazole. 5-FC and flucytosine are oral drugs. If either is given as the only treatment, there are relapses so most physicians use two drugs simultaneously. Actually, these two drugs are synergistic with amphotericin B and thus, their association is advantageous.

IV. SUPERFICIAL MYCOSES

The superficial (cutaneous) mycoses are usually confined to the outer layers of skin, hair, and nails, and do not invade living tissues. These fungi are called **dermatophytes**. Dermatophytes, or more properly, keratinophilic fungi, produce extracellular enzymes (keratinases) which are capable of hydrolyzing keratin.

A. CLINICAL MANIFESTATIONS

Tinea means "ringworm" or "moth-like". Dermatologists use the term to refer to a variety of lesions of the skin or scalp.

Tinea corporis – small lesions occurring anywhere on the body.

Tinea pedis – "athlete's foot." Infection of toe webs and soles of feet.

Tinea unguium (onychomycosis) – nails. Clipped and used for culture. Infection usually lifelong.

Tinea capitis – Fungus infection of the hair. Frequently found in children.

Tinea cruris – "jock itch." Infection of the groin, perineum or perianal area.

Tinea barbae – ringworm of the bearded areas of the face and neck.

Tinea versicolor –Characterized by a blotchy discoloration of skin which may itch. Up to 25% of the general population may have this lesion.. Diagnosis is usually possible by direct microscopic examination of KOH-treated skin scrapings which show a typical aspect of mycelia and spores described as "spaghetti and meatballs." Caused by *Malassezia furfur*,

B. ECOLOGY

The dermatophytes (skin plants) causing human infections may have different natural sources and modes of transmission:

Anthropophilic – usually associated with humans only; transmission from man to man by close contact or through contaminated objects.

Zoophilic – usually associated with animals; transmission to man by close contact with animals (cats, dogs, cows) or with contaminated products.

Geophilic – usually found in the soil, transmitted to man by direct exposure.

Knowledge of the species of dermatophyte and source of infection are important for proper treatment of the patient and control of the source. Invasion by zoophilic or geophilic organisms may cause inflammatory disease in man.

Geographic distribution: Dermatophytes occur worldwide, but some species have geographically limited distribution.

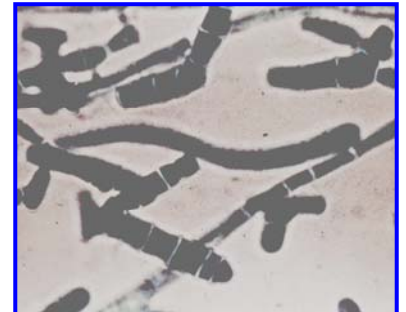
Note: In South Carolina, *Trichophyton tonsurans* is more common in Blacks and *Microsporum canis* is found more commonly in Caucasians.

C. ETIOLOGIC AGENTS

There are three genera of dermatophytes:

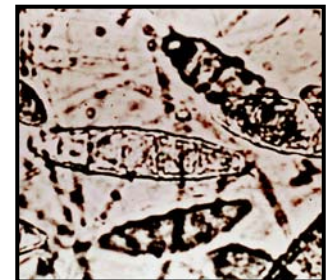
1. **Trichophyton** sp. (19 species)

Infect **skin, hair and nails**. Take 2-3 weeks to grow in culture. The conidia are large (**macroconidia**), smooth, thin-wall, septate (0-10 septa), and pencil-shaped; colonies have loose aerial mycelia, which produce a variety of pigments. Identification requires special biochemical and morphological techniques. *Trichophyton rubrum* is presently the most common cause of tinea in Blacks in South Carolina. Rarely can cause subcutaneous infections (kerion) in immunocompromised individuals, particularly patients with chronic myelogenous leukemia.



2. **Microsporum** sp. (13 species)

May infect **skin and hair**, rarely nails. Its prevalence has decreased significantly. One species (*M. audouini*) when prevalent (15-20 years ago) could easily be identified on the scalp because infected hairs fluoresce a bright green color when illuminated with a UV-emitting Wood's light. The loose, cottony mycelia produce macroconidia which are thick-walled, spindle-shaped, multicellular, and **echinulate** (spiny). *Microsporum canis* is one of the most common dermatophyte species infecting humans and the most commonly found in whites in South Carolina.



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3. ***Epidermophyton floccosum*** (Only one species in this genus)
Infect **skin and nails** and rarely hair. Yellow-colored, cottony cultures; usually readily identified by the thick, bifurcated hyphae with multiple, smooth, club-shaped macroconidia of 2-4 cells.



D. THERAPY

Skin infections can be treated (more or less successfully) with a variety of drugs, such as:

Tolnaftate (Tinactin)	available over the counter - Topical
Clotrimazole Topical	
Miconazole Topical	"Step up to the mike."
Ketoconazole Oral	seems to be most effective for tinea versicolor and other dermatophytes.
Itraconazole Oral	
Terbinafine (Lamisil)	Oral, topical. For skin and nail infections. (Digger Dermatophyte).

For infections involving the scalp and particularly the nails, **griseofulvin** is commonly used. This antimycotic must be incorporated into the newly produced keratin layer to form a barrier against further invasion by the fungus. This is a very slow process requiring oral administration of the drug for long periods - up to 6-9 months for fingernail infections and 12-18 months for toenail infections, however it is of low cost and an oral medication.

Itraconazole and terbinafine are now the drugs of choice for onychomycoses – but there is still about 20% treatment failures. Itraconazole best for tinea versicolor.

E. The Id reaction

Patients infected with a dermatophyte may show a lesion, often on the hands, from which no fungi can be recovered or demonstrated. It is believed that these lesions, which often occur on the dominant hand (i.e. right-handed or left-handed) are secondary to immunological sensitization to a primary (and often unnoticed) infection located somewhere else (e.g. feet). These secondary lesions will not respond to topical treatment but will resolve if the primary infection is successfully treated either with topical or systemic drugs.

F. CLINICAL MATERIAL FOR THE LABORATORY

Hair, skin or Nails.

HOOR 3

V. FILAMENTOUS FUNGI

A. CHROMOBLASTOMYCOSIS - A chronic, localized infection of subcutaneous tissues caused by several species of dematiaceous (black pigmented) fungi. The 3 most common agents are:

1. *Fonsecaea pedrosoi*
2. *Cladosporium carrionii*
3. *Phialophora verrucosa*

These fungi, recognized by a variety of names, are saprobes located in soil and decaying vegetation. The route of entry is usually by trauma. The lesions are sub-cutaneous and the surface can be flat or verrucous and they take several years to develop. These organisms are called dematiaceous fungi, because they have a black pigment in the mycelium cell wall (in culture and in tissue). In tissue these fungi form **sclerotic bodies** which are the reproductive forms dividing by fission. These organisms induce a granulomatous reaction.

The etiologic agents of chromoblastomycosis are septate, mold-like, branching, darkly pigmented mycelia which produce asexual fruits called conidia. We identify these fungi in culture by the shape and formation of the conidia and biochemical tests. The melanin in the pigment may be a virulence factor.

The fungi have a world-wide distribution especially in warmer climates like the tropics or southern U.S.

The specimens to send to the laboratory are: pus or tissue.

There is still no really successful therapy. It is chronic, fibrotic, and usually with superimposed bacterial infection. The sclerotic bodies protect the fungus from host defenses. Excision and local heat have been used with some success. Terbinafine and itraconazole are now being used to treat (or control) this disease.

There are no serological tests to aid in the diagnosis.

B. MYCETOMA

Mycetoma (fungous tumors) are also chronic, subcutaneous infections. These are called eumycotic mycetoma (tumors caused by the TRUE fungi as opposed to those caused by actinomycetes). These tumors frequently invade contiguous tissue, particularly the bone. A diagnosis of the etiologic agent is essential for patient management because the prognosis and therapy differs.

Mycetoma characteristics:

1. tumefaction - swelling
2. granules - a variety of colors (white, brown, yellow, black, etc.).
3. draining sinus tracts

The three most common etiologic agents are:

1. *Madurella mycetomatis*
 2. **Exophiala jeanselmei*
 3. **Pseudallescheria boydii*
- *The most common in the US.

Geographic distribution: World-wide and the organisms are associated with the soil, thus infections of the feet and legs are most common.

Clinical specimens for diagnosis:

1. pus – with granules
2. tissue – for histological examination

The color, size and texture of the granules are an aid in the diagnosis of mycetomas. The agents of mycetoma are all filamentous fungi, which require 7-10 days for visible growth on the culture media, and then another several days for specific identification. These fungi are identified by the colonial

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morphology, conidia formation and biochemical reactions. The species of fungi cannot be distinguished in histopathological tissue sections.

There are no serologic tests except for *Pseudallescheria boydii*.

Treatment is very difficult, but amphotericin B, ketoconazole and itraconazole have been used with some success.

C. MUCORMYCOSIS

Mucormycosis is an acute inflammation of soft tissue, usually with fungal invasion of the blood vessels. This rapidly fatal disease is caused by several different species in this class. The mucormycetes, like the candida species, are ubiquitous and rarely cause disease in an immunocompetent host. Some characteristic underlying conditions which cause susceptibility are: diabetes, severe burns, immunosuppression or intravenous drug use. The portal of entry is inhalation, ingestion, surface contamination (burns).

The three most common genera causing this clinical entity are:

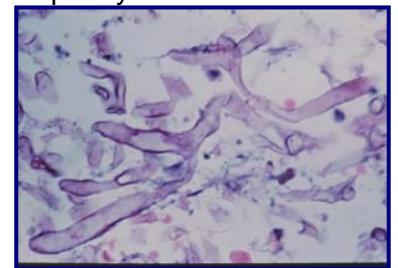
1. *Rhizopus* species *
2. *Mucor* species
3. *Absidia* species

Characteristics: world-wide distribution, commonly in soil, food, organic debris, seen on decaying vegetables in the refrigerator and on moldy bread. Rhinocerebral and pulmonary infections are common. This disease is frequently seen in neutropenic patients and the **uncontrolled diabetic**.

Typical case: An uncontrolled diabetic patient comes to ER (may be comatose depending on the state of diabetes) and a cotton-like growth is observed on the roof of the mouth or in the nose. These are the hyphae of the organism. If untreated, the patient will die within a few hours or days. What do you do to help this patient first? Controlling the diabetic state is most important before administering amphotericin B.

These fungi have a tendency to invade blood vessels, particularly arteries, (angioinvasive), adhere to endothelial cells, invade and cause necrosis. They then enter the brain via the blood vessels and by direct extension through the cribiform plate. This is why they cause death so quickly.

Culture: A rapid growing, loose, white mold which is visible in 24-48 hours. With age, and the formation of sporangia, the colony becomes dark gray. The sporangia contain the dark spores. The mycelium is, wide (15-20 microns), ribbon-like and non-septate (coenocytic). This same appearance is clear in tissue sections. The species are identified by the morphology in culture.



Treatment consists of debridement and amphotericin B.

There is an immunodiffusion test available, but the physician cannot wait for these results before instituting rapid, vigorous intervention. The diagnosis and treatment must be immediate and based primarily on clinical observations.

D. ASPERGILLOSIS

Aspergilli produce a wide variety of diseases. Like the mucormycetes, they are ubiquitous in nature and play a significant role in the degradation of plant material as in composting. Similar to candida and the mucormycetes, they rarely infect a normal host.

There are three clinical types of pulmonary aspergillosis:

1. Allergic - hypersensitivity to the organism. Symptoms may vary from mild respiratory distress to alveolar fibrosis.
2. Fungus ball – which is characteristically seen in the old cavities of TUBERCULOSIS patients. This is easily recognized by x-ray, because the lesion (actually a colony of mold growing in the cavity) is shaped like a half-moon (crescent). The patients may cough up the fungus elements because the organism frequently invades the bronchus. Chains of conidia can sometimes be seen in the sputum. lesions.
3. Aggressive tissue invasion. Primarily a pulmonary disease, but the aspergilli may disseminate to any organ. They may cause endocarditis, osteomyelitis, otomycosis and cutaneous

Aspergillosis is difficult to diagnose:

1. Clinical symptoms are not specific
2. Radiography is non-specific (except for fungus ball).
3. Blood cultures seldom positive
4. Serology seldom positive (early enough for intervention)
5. Invasive procedures required for early detection

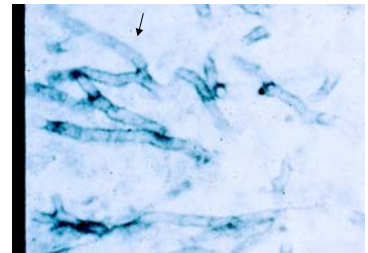
There are more than one hundred species of aspergilli. The most common etiologic agents of aspergillosis in the United States:

1. *Aspergillus fumigatus*
2. *A. niger*
3. *A. flavus*

The organism is distributed world-wide and is commonly found in soil, food, paint, air vents. They can even grow in disinfectant.

Culture: Aspergilli require 1-3 weeks for growth. the colony begins as a dense white mycelium which later assumes a variety of colors, according to species, based on the color of the conidia. The hyphae are branching and septate. Species differentiation is based on the formation of spores as well as their color, shape and texture.

Histopathology: The septate hyphae are wide and form dichotomous branching, i.e., a single hypha divides into two even branches of hyphae, and then the mycelium continues branching in this fashion.



Serology: There are two serological tests for aspergillosis; the first is an Immunodiffusion test. There may be 1 to 5 precipitin bands. Three or more bands usually indicate increasingly severity of the disease. i.e., tissue invasion. The second is an EIA measure of galactomannan – specificity –99.6 % but only 50 % sensitive.

Treatment: Voriconazole and Amphotericin B.

VI. DIMORPHIC FUNGI

A. BLASTOMYCOSIS (*Blastomyces dermatitidis*)

Blastomycosis is a chronic granulomatous disease which progresses slowly. Although the **pulmonary** and **skin** involvement is the most common, *B. dermatitidis* frequently affects **bone**, **prostate** and other organs.

Blastomycosis frequently presents as a cutaneous or a respiratory disease. The cutaneous lesions may be primary (usually self-limiting) or secondary (a manifestation of systemic disease). The patient who presents with a complaint of respiratory symptoms will frequently remark about loss of appetite, loss of weight, fever, productive cough, hemoptysis and night sweats. These symptoms resemble those of tuberculosis. The X-ray shows obvious pulmonary disease. To make the specific diagnosis the physician must be aware of blastomycosis. Sputum sent to the laboratory for "culture" will not yield the organism. The laboratory must be alerted to look for fungal organisms or to look specifically for blastomyces. Some patients have a sub-clinical or "flu-like" response to infection.

Laboratory specimens: depend on the manifestation of the disease: If there are skin lesions, send skin scrapings or **pus**. If there is pulmonary involvement, send **sputum**. Other specimens include **biopsy material** and **urine**. Occasionally, the organism can be isolated from urine as it often infects the prostate.

As was noted earlier, most of the systemic fungi have a specific niche in nature where they are commonly found. This organism survives in soil that contains organic debris (rotting wood, animal droppings, plant material) and infects people collecting firewood, tearing down old buildings or engaged in other outdoor activities, which disrupt the soil.

In addition to an ecological niche, most dimorphic fungi, which cause systemic infections, have a limited geographic distribution where they occur most frequently. Blastomycosis occurs in eastern North America and Africa. The vast majority of patients with blastomycosis in S.C. are infected in the northern part of the state, above the Fall Line (Augusta, GA, Aiken, Columbia, Cheraw, Raleigh, NC).

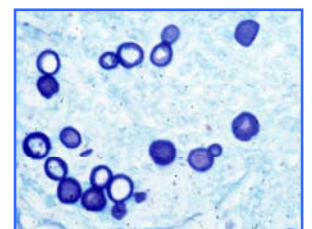
A specific gene (BAD-1) which appears to be a virulence factor for *B. dermatitidis* has recently been described. Once the conversion to the yeast form begins BAD1 rapidly accumulates on the surface of the yeast cell.

Mycology

If you request a fungus culture from the microbiology laboratory, the cultures will be incubated at 25°C and at 37°C because most of the significant systemic pathogenic fungi are dimorphic.

A culture of *B. dermatitidis* takes 2 to 3 weeks to grow at 25°C. It appears as a white, cottony mold (mycelium) on **Sabouraud dextrose agar**. Most specimens for fungus culture are plated on Sabouraud's dextrose agar for the 25 C incubation.

Microscopically, the mycelia and the fruiting bodies (conidia) are evident. However, the mold cannot be identified by its fruiting bodies. The fruiting bodies are called microconidia, but they are not distinctive. Other fungal saprophytes and pathogens have similar conidia. At 37°C the yeast form grows in about 7-10 days. It appears as a buttery-like, soft colony with a tan color. Microscopically, we see the typical



yeast form of a THICK WALL and a SINGLE BUD with a WIDE BASE. This wide base is characteristic of *B. dermatitidis*, and it is important to be able to recognize this. The cells are 12-15 microns in diameter.

The yeast will convert to the mycelial form when incubated at 25°C, taking from 3 to 4 days up to a few weeks. Similarly, the mycelial growth can be converted to yeast form when incubated at 37°C. In the past, the only way to identify the dimorphic fungi was to convert from one form to the other, but now it is possible to take the mycelial growth (which is the easiest to grow), and confirm the isolate with a DNA probe in a matter of hours.

Histopathology

B. dermatitidis produces both a granulomatous and suppurative tissue reaction. A typical cutaneous lesion shows central healing with microabscesses at the periphery. The yeast forms can frequently be demonstrated in a KOH preparation of pus from a skin lesion. A pus specimen may be obtained by nicking the top of a microabscess with a scalpel, obtaining the purulent material and making the diagnosis in 5 minutes by microscopic examination with KOH. This organism has a characteristic appearance of a double contoured wall with a single bud on a wide base.

Serology

There are three serological tests used for blastomycosis:

1. Immunodiffusion test (precipitin) It requires 2 to 3 weeks after onset of illness to become positive. This test is positive in about 80% of the patients with blastomycosis. When it is positive, there is close to 100% specificity.
2. Complement fixation (CF) test. This test requires 2 to 3 months after the onset of disease to develop detectable antibody. Besides the long delay before there is measurable antibody, another disadvantage of the C-F is that it **cross reacts** with other fungal infections (coccidioidomycosis and histoplasmosis). The advantage is that it is a **quantitative** test. The physician can follow the patient's response to the disease by monitoring the antibody titer.
3. Enzyme Immunoassay (EIA). The test is easy to perform and antibody is detected early in the disease process.

TREATMENT:

Itraconazole is the drug of choice for mild cases of blastomycosis while amphotericin B is used for patients with life threatening disease. The new antimycotic, voriconazole is now being used.

HOUR 4

B. HISTOPLASMOSIS (*Histoplasma capsulatum*)

Histoplasmosis is a systemic disease, primarily of the **reticuloendothelial** system, manifesting itself in the **bone marrow, lungs, liver**, and the **spleen**. In fact, hepatosplenomegaly is the primary sign of infection in children, while in adults, histoplasmosis more commonly appears as a pulmonary disease.

The lungs are the portal of entry. There is generally complete recovery from the acute pulmonary form (another "flu-like" illness). In the endemic area the majority of patients who develop histoplasmosis (95%) are asymptomatic. The diagnosis is made from their history, serologic testing or skin testing. In the patients who are clinically ill, histoplasmosis generally occurs in one of three forms: acute

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pulmonary, chronic pulmonary or disseminated. However, if untreated, the disseminated form of disease is usually fatal.

Patients will first notice shortness of breath and a cough which becomes productive. The sputum may be purulent or bloody. Patients will become anorexic and lose weight. They have night sweats. These symptoms resemble tuberculosis, and the lung x- ray also looks like tuberculosis with calcifications, but radiologists can distinguish between these diseases on the chest film.

This is one of the most common fungal infections, occurring frequently in S.C., particularly the northwestern portion of the state. The ecological niche of *H. capsulatum* is in **blackbird roosts, chicken houses, and bat guano**. Typically, a patient will have spread chicken manure around his garden, and 3 weeks later will develop pulmonary infection. There have been several outbreaks in S.C. where workers have cleared canebrakes which served as blackbird roosts with bulldozers. All who were exposed, workers and bystanders, contracted histoplasmosis. Histoplasmosis is a significant occupational disease in bat caves in Mexico when workers harvest the guano for fertilizer.

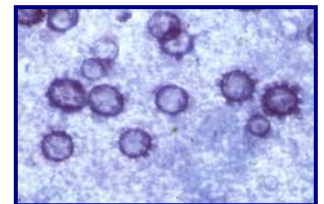
Histoplasmosis is prevalent primarily in the eastern U.S. and parts of Central and South America. In S.C., a histoplasmin skin test survey of lifetime, one county residents, white males, 17 to 21 years old, was performed on Navy recruits. The greatest number of positive skin tests appeared in the northwestern part of the state. A similar study of medical students conducted at MUSC about 30 years ago bore the same distribution (Goodman and Ever, J.S.C.M.A. 67:53-55, 1971).

The skin test is NOT used for diagnostic purposes, because it interferes with serological tests. Skin tests are reserved for epidemiological surveys.

Clinical specimens sent to the laboratory depend on the presentation of the disease; **Sputum** or **Bronchial alveolar lavage** if its pulmonary disease, or **Biopsy** material from the diseased organ. **Bone marrow** is an excellent source of the fungus, which tends to grow in the reticulo-endothelial system. **Peripheral blood** is also a source of visualizing the organism histologically. The yeast is usually found in **monocytes** or in **PMN's**. Many times an astute medical technologist performing a white blood cell differential count will be the first one to make the diagnosis of histoplasmosis. In peripheral blood, *H. capsulatum* appears as a small yeast about 5-6 microns in diameter. (*Blastomyces* is 12 to 15 microns). **Gastric washings** are also a source of *H. capsulatum* as people with pulmonary disease produce sputum and frequently swallow their sputum.

Mycology

When it is cultured on Sabouraud dextrose agar and incubated at 25°C, *H. capsulatum* appears as a white, cottony, aerial mycelium after 2 to 3 weeks. As the colony ages, it becomes tan. In the mold form, Histoplasma has a very distinct spore called a tuberculate macroconidium (10-15 microns diameter). The tubercles are diagnostic, however there are some nonpathogens which appear similar. A medical mycologist will be able to distinguish between them.



Grown at 37°C the yeast form appears. It is a soft, white to tan colony. The yeast cell is 5-6 microns in diameter and slightly oval in shape. This is not diagnostic. To confirm the diagnosis, one must convert the organism from yeast to mycelium or vice-versa or use the DNA probe.

Serology for histoplasmosis is a little more complicated than for other mycoses, but it provides more information than blastomycosis serology.

There are 3 serologic tests available:

1. Complement Fixation
2. Immunodiffusion
3. EIA (antibody)
4. EIA (antigen)

Each of these serological tests has different characteristics, which make them useful. The complement fixation test is like the one for blastomycosis, except there are 2 antigens, one to the yeast form of the organism and the other to the mycelial form. Some patients react to one form and not the other, while some individuals react to both. The reason for the different responses is not clear. One disadvantage is that complement-fixing antibody develops late in the disease, about 2 to 3 months after onset. A second disadvantage is that it cross reacts with other mycotic infections. An advantage of the C-F test is that it is quantitative, so the physician can follow the course of the disease by observing the titer of several samples.

The interpretation of the immunodiffusion test is a little more complicated than with blastomycosis because there are two bands which may appear. An H band indicates active disease and will appear in 2 to 3 weeks. An M band can indicate past or present disease, or result from a skin test. This is one reason why skin tests are not used for diagnosis because they can interfere with other tests. Skin tests will also affect the complement fixation test.

The EIA test for the detection of **antigen** is only available in the author's laboratory but it appears to be sensitive in detecting systemic histoplasmosis.

The drugs of choice (DOC) are Itraconazole (static) for mild disease and amphotericin B for severe disease (cidal).

C. COCCIDIOIDOMYCOSIS (*Coccidioides immitis*)

Coccidioidomycosis is primarily a pulmonary disease. About 60 % of the infections in the endemic area are asymptomatic. About 25 % suffer a "flu-like" illness and recover without therapy. This disease exhibits the typical symptoms of a pulmonary fungal disease: arthralgia, anorexia, weight loss, cough, hemoptysis, and resembles tuberculosis. CNS infection with *C. immitis* is more common while it is less frequent with the other fungal diseases. There is a much greater mortality rate in dark-skinned people (Mexicans, Filipinos, and Blacks). They are 25 times more likely to develop progressive disease and death. The reason for this is obscure.

Geographic Distribution

The ecological niche of *C. immitis* is the Sonoran desert, which includes the deserts of the Southwest, USA (California, Arizona, New Mexico, Nevada, Utah and Texas) and northern Mexico. It is also found in small foci in Central and South America. **Desert soil, pottery, archaeological middens, cotton, and rodent burrows** all harbor *C. immitis*.

C. immitis is a dimorphic fungus with 2 life cycles. The organism follows the SAPROPHYTIC cycle in the soil and the PARASITIC cycle in man or animals. The saprophytic cycle starts in the soil with spores (arthroconidia) that develop into mycelium. The mycelium then matures and forms alternating spores within itself. The arthroconidia are then released, and germinate back into mycelia. The parasitic cycle involves the inhalation of the arthroconidia by animals which then form spherules which become filled with endospores. The ambient temperature and availability of oxygen appear to govern the pathway. The spores of the organism are readily airborne and can be carried by the wind

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and therefore spread hundreds of miles in storms so the distribution is quite wide. In 1978 cases were seen in Sacramento 500 miles north of the endemic area, from a dust storm in Southern California. .

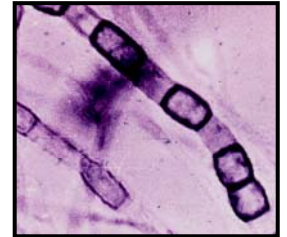
The cases that occur in South Carolina are usually in patients who have visited an endemic area and brought back pottery, or blankets purchase from a dusty roadside stand, or in Navy and Air Force personnel who were exposed when they were stationed in the endemic area. The disease manifests itself after they are transferred to a base in South Carolina. A few interesting cases occurred in cotton mills in Burlington and Charlotte, N.C. The cotton, grown in the desert of the Southwest, was contaminated with the fungus and the mill workers inhaled the spores while handling the raw cotton and developed coccidioidomycosis.

Clinical Specimens

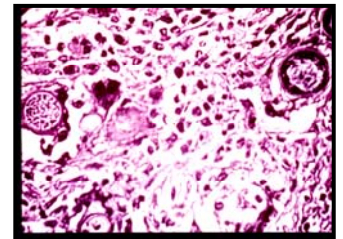
Clinical specimens include **sputum**, **pus** from skin lesions, **gastric washings**, **CSF**, and **biopsy material** from skin lesions.

Mycology

C. immitis is a dimorphic fungus. Cultured on Sabouraud's agar at 25°C it grows as a mold in 2 to 3 weeks. Characteristically, the mycelia develop arthroconidia. ("By their fruits ye shall know them"). It is a barrel-shaped (smaller at the edges, wider at the middle) asexual spore. Typically, the arthroconidia alternate with non spore-forming cells in the mycelium.



When grown *in vitro* at 37°C there is no yeast form!! *C. immitis* is a dimorphic fungus; *in vivo* (pus or tissue) one sees the **pathogenic** or **invasive** form – which is a **spherule**. The organism develops into spherules (30-60 microns) that are filled with endospores which are 3 to 5 microns in diameter. A spherule will develop endospores within, then break apart, releasing the endospores. This is the tissue form seen in pus or histological sections: spherules and loose endospores. They can also be seen in a KOH preparation of sputum. It is pathognomonic for coccidioidomycosis.



Serology

There are four tests for diagnosis:

1. Complement-Fixation
2. Slide agglutination
3. Immunodiffusion
4. EIA

C-F antibody is slow to rise and develops in about 1 month after exposure. This test is excellent for coccidioidomycosis because it is quantitative. However, these antibodies cross-react with some other fungi (*Blastomyces* and *Histoplasma*) but this is not a problem in the endemic area. The C-F test is also a PROGNOSTIC test. If the titer keeps rising, then the patient is responding poorly and the course may be fatal. If the C-F titer is dropping then the prognosis for that patient is favorable. A titer of greater than 1:128 usually indicates extensive dissemination.

Life-long immunity usually follows infection with *C. immitis*.

Fluconazole and itraconazole are the drugs of choice with amphotericin B reserved for more severe infections

D. PARACOCCIDIOIDOMYCOSIS (*Paracoccidioides brasiliensis*)

This is a chronic granulomatous disease of mucous membranes, skin, and pulmonary system. This disease occurs from the middle of Mexico (North America) to Central and South America. Most cases are reported from Brazil. The ecological niche of this organism is **probably** the soil.

A common triad of symptoms that are seen in Latin America is **pulmonary lesions, edentulous mouth, and cervical lymphadenopathy**. Prior to the recognition of this disease, patients in Latin America with paracoccidioidomycosis were often sent to tuberculosis sanatoria, just as patients with histoplasmosis were in the U.S. The organisms invade the mucous membranes of the mouth causing the teeth to fall out. White plaques are also found in the buccal mucosa, and this along with the triad is now used to clinically differentiate clinically between tuberculosis and paracoccidioidomycosis.

This disease has a long latency period. 10-20 years may pass between infection and manifestation of the infection in the non-endemic areas of the world. Typically, a case of paracoccidioidomycosis seen in the U.S. occurs in someone who worked in South America for some period of time and then returned to the U.S. and years later, develop this disease. The patient does not realize the importance of this past history. Almost all diagnoses of fungal diseases depend upon careful questioning and a probing history.

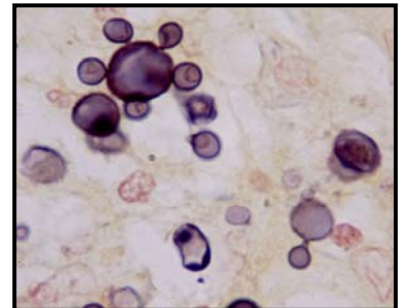
The clinical material which should be sent to the laboratory for examination is sputum, biopsy material, pus, and crust from the lesions. Examination of sputum or crust from one of the lesions with KOH reveals a yeast because this is a dimorphic fungus. In contrast to the other yeasts, particularly blastomyces, paracoccidioides has **multiple** buds, a **thin** cell wall, and a **narrow** base.

Mycology

At 25° C, the colony is a dense, white mycelium, not loose and cottony like the others. On Sabouraud's agar it takes 2-3 weeks to grow. When cultured at 37° C, it is slow growing with a white-tan, thick colony. Microscopically, these yeasts appear as described above ranging in size from 5 to 15 microns.

Histopathology

Histologically, one sees multiple buds forming a "Captain's wheel." This is diagnostic of paracoccidioidomycosis. In this case, the mother cell is 40-50 microns in diameter and the buds are 2-5 microns in size.



Serology

The best serological test for paracoccidioidomycosis is the immunodiffusion test. It is better than 99% specific and almost 85% sensitive.

Therapy

The D.O.C. is amphotericin B. Sulphonamide-trimethoprim has also been used. Presently Itraconazole appear to provide the best recovery.

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E. SPOROTRICHOSIS (*Sporothrix schenckii*)

Sporotrichosis is usually a chronic infection of the cutaneous or subcutaneous tissue, which tends to suppurate, ulcerate and drain. In recent years, a pulmonary disease has been seen more frequently. Occasionally, infection with *S. schenckii* may result in a mycetoma.

Sporotrichosis is caused by another dimorphic fungus. The infection is also known as "rose growers disease." The ecologic niche for this organism is rose thorns, sphagnum moss, timbers and soil. A study on the occupational distribution of sporotrichosis showed that forest employees accounted for 17% of the cases, gardeners and florists, 10%; and other soil-related occupations another 16%. Sporotrichosis occurs worldwide.

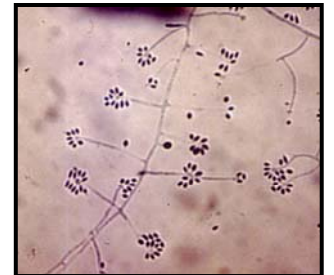
Every aspect of this disease (clinical, pathology, mycology, ecology) was investigated during an epidemic of 3,000 patients in a gold mine in South Africa during the 1940's. The mold was growing on mine timbers and every time a worker bruised himself on the timber, the spores were inoculated and a lesion developed. Patient history is very important in this disease also. It is often seen in gardeners and begins with a thorn prick on the thumb. A pustule develops and ulcerates. It infects the lymphatic system and then the disease progresses up the arm with ulceration, abscess formation, break down of the abscess with large amounts of pus followed by healing. Progression usually stops at the axilla.

Geographical distribution: Worldwide.

Clinical material to be sent to the laboratory may be pus, biopsy material, or sputum from pulmonary patients. The yeast form of this fungus in tissue or in culture, can be round (6-8 μm) or fusiform. The fusiform shape is not the usual form but if a cigar-shaped yeast is observed in tissue, it is usually diagnostic of sporotrichosis.

Mycology

At 37° C *Sporothrix* grows as a white pasty yeast with small round to oval forms (3-10 μm). At 25° C this colony is white-cream and very membranous, but as it ages (2-3 weeks) it becomes black and leathery. Microscopically, the mycelium is branching, septate and very delicate, 2-3 μm in diameter. The pyriform conidia, 2-4 μm , form a typical arrangement in radial groups at the end of a conidiophore called "daisies."



Histopathology

S. schenckii stains poorly with the usual histopathological stains. If sporotrichosis is suspected, the pathologist must be informed so he can use special stains. Histologically asteroid bodies, a tissue reaction (also known as **Splendori** reaction) may be seen around the yeast cell.

Serologic tests are not commercially available.

The drug of choice for the cutaneous form and the systemic form is itraconazole.

HOUR 5

VII. OPPORTUNISTIC MYCOSES

Opportunistic mycoses are infections due to fungi with low inherent virulence. These pathogens are an almost limitless number of fungi. The etiologic agents are organisms which are common in all environments. The host/pathogen equilibrium is as follows:

$$\frac{\text{Number of organisms} \times \text{Virulence}}{\text{Host resistance}} = \text{Disease}$$

is tilted in favor of "disease" because the host resistance is lowered. **For the immunocompromised host, there is no such thing as a non-pathogenic fungus.** The fungi most frequently isolated from immunocompromised patients are saprophytic (i.e. from the environment) or endogenous (a commensal). The most common species are *Candida* sp., *Aspergillus*, and mucorales.

The **upward trend in the diagnoses of opportunistic mycoses** reflects increasing clinical awareness by physicians, improved clinical diagnostic procedures and better laboratory identification techniques. Another important factor contributing to the increasing incidence of infections with fungi that have not been previously known to be pathogenic has been the rise in the number of **immunocompromised** patients who are susceptible hosts for the most uncommon agents. Patients with primary immunodeficiencies are susceptible to mycotic infections particularly when cell-mediated immunity is compromised. In addition, several types of secondary immunodeficiencies may be associated with an increased frequency of fungal infections.

When a fungus is isolated from an immunocompromised patient, the attending physician has to distinguish between: **1) colonization** (which is of no major concern), **2) transient fungemia** (often involving *C. albicans*) and **3) systemic infection**. A great deal of clinical judgment is required to reach these conclusions, which imply important therapeutic decisions, such as the institution of therapy.

The diagnosis of opportunistic infections requires a **high index of suspicion**. Without this curiosity the clinician may not consider mycotic infections in the compromised patient because:

1. Patients present with atypical signs and symptoms.
2. The etiological agent may be considered a saprophyte or contaminant.
3. The systemic mycoses may occur outside the known endemic area.

Causes of immunodeficiency commonly encountered:

Malignancies. (Leukemias, lymphomas, Hodgkin's disease). In one study of cancer patients, fungal septicemia and pneumonias accounted for almost a third of deaths.

Drug therapies. Anti-neoplastic substances, steroids, immunosuppressive drugs.

Antibiotics. Over-use or inappropriate use of antibiotics can also contribute to the development of fungal infections by altering the normal flora of the host and facilitating fungal overgrowth or by selecting for resistant organisms.

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Therapeutic procedures can predispose for fungal infections:

1. Solid Organ and Bone Marrow transplantation
2. Open heart surgery
3. Indwelling catheters (urinary, I.V. drugs or parenteral hyperalimentation). In cases of fungemia, the contaminated catheter must be removed before starting anti-fungal therapy.
4. Artificial heart valves can be colonized by a variety of infectious agents, including *Candida* species. In a case of *Candida* infection of an artificial heart valve, antifungal treatment is only efficient if the infected valve is replaced.
5. Radiation therapy.

Other factors associated with increased frequency of mycotic infections:

1. Severe burns
2. Diabetes
3. Tuberculosis
4. I.V. drug use

AIDS. Virtually all AIDS patients will have a fungal infection sometime during the course of disease. Certain fungi may be frequently associated with some of the predisposing factors listed above. However, any one of the ubiquitous saprophytes (most of which do not cause disease in immunocompetent hosts) as well as occasional pathogens may cause disease in these patients. In the last few years, the use of HAART therapy has reduced the number of fungal infections.

Biofilm Formation: It has long been recognized that in patients with a microbial infection, any artificial device, such as an indwelling catheter or prosthetic valve, must be removed prior to initiating antibiotic therapy. The foreign body will act as a nidus, seeding the infection if it remains present. The exact mechanism was not clear. A biofilm is a microcolony of organisms which adheres to a surface (catheter, implant, or dead tissue) and which resist removal by fluid movement and have a decreased susceptibility to antimicrobials. This biofilm phenomenon, which occurs on the rocks in a stream, was first recognized as a public health problem in drinking water distribution pipes and was regarded as a source of coliform contamination of drinking water. Recent work in clinical microbiology has shown that these organisms develop a resistance to therapy because they are contained in a matrix, which acts like a tissue and becomes a barrier to antibodies and antimicrobial agents.

CLINICAL PRESENTATION

Common fungal infections may have an unusual presentation in immunosuppressed patients because:

1. Atypical signs and lesions.

Malassezia furfur usually causes a rather benign and self-limited disease in normal hosts (Tinea versicolor), but in immunocompromised patients may show a rash with disseminated disease and sepsis. This organism requires long-chain fatty acids for growth. Patients receiving parenteral fat emulsions for nutrition become a walking petri plate.

2. Unusual Organ affinity.

Candida may invade liver, heart valves; Oral thrush occurs in people who are relatively immunocompetent while esophageal candidiasis occurs in those patients who are immunologically compromised. .

Cryptococcus may cause pulmonary and cutaneous infections

Studies show that from 10 % to 30 % of AIDS patients have cryptococcal meningitis and they will require maintenance therapy with fluconazole for the remainder of their life. Fluconazole penetrates the CSF

Mortality: Without treatment 100%
With treatment 20%

Relapse:
Non-AIDS 15-20%
AIDS patients 50%

With relapse there is 60% mortality.

Sporotrichosis: Co-infection with other fungi is frequent

Blastomycosis: More frequently disseminated and all patients have done very poorly.

There has been one report on 15 cases of blastomycosis in AIDS patients. Six patients (40%) had CNS involvement. Usually CNS disease only occurs in 3-10% of the patients.

Aspergillosis

Mortality: With amphotericin B 72%
Without amphotericin B 90%

3. Infections with systemic dimorphic fungi occurring outside endemic areas.

These factors complicate the diagnosis and management of these diseases.

Coccidioidomycosis

Mycelial forms seen in tissue. Occurs in patients outside the endemic area. Patients require fluconazole or itraconazole maintenance therapy.

Histoplasmosis

Occurs in patients outside the endemic area and they require fluconazole or itraconazole maintenance therapy

- All cases are disseminated.
- Relapse rate is > 50%
- Rapidly fatal in 10%.

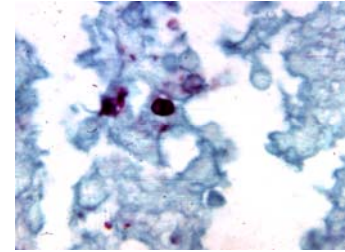
4. Unusual Histopathology.

Even the inflammatory reaction may be different in biopsy specimens. The normal host reaction to fungal invasion is usually pyogenic or granulomatous. In the immunodeficient host the reaction is necrotic.

5. Unusual Pathogens

Penicillium marneffeii

Dimorphic, Produces a red pigment and reproduces by fission. Requires amphotericin B therapy and oral itraconazole maintenance.



Pneumocystis carinii

Formerly thought to be a protozoan. Presently believed to be a fungus.

Table. **Some common associations between fungal organisms and disease conditions.**

<i>Cryptococcus neoformans</i>	<i>Candida albicans</i>	<i>Candida (Torulopsis) glabrata</i>	<i>Mucormycetes</i>	<i>Aspergillus species</i>
Diabetes mellitus	Prolonged antibiotic therapy	Cytotoxic drugs	Diabetes mellitus	Leukemias
Tuberculosis		Immunosuppression	Leukemias	Corticosteroid therapy
Lymphoma	Prolonged intravenous catheters	Diabetes mellitus	Corticosteroid therapy	Tuberculosis
Hodgkin's disease		Hyperalimentation	Intravenous therapy	Immunosuppression
Corticosteroid therapy	Prolonged urinary catheters	Intravenous catheters	Severe burns	I.V. drug abuse
Immunosuppression	Corticosteroid therapy			
	Diabetes mellitus			
	Hyperalimentation			
	Immunosuppression			

IMPROVING TREATMENT:

1. New drugs
2. New therapeutic regimen
3. Aggressive therapy
 - Prophylactic
 - Empirical
 - Pre-emptive
4. Conjunctive therapy

SUMMARY:

"Only the prepared mind can help the impaired host." Dr. Libero Ajello, Opportunistic Fungal Infections. Proceedings of the Second International Conference. Charles C. Thomas, 1975. P. 31-35.

MEDICAL MYCOLOGY GLOSSARY

ACTIDIONE - Trademark name for cycloheximide, a selective antifungal agent.

AERIAL - mycelium: Hyphal units above the colony agar interface.

ANAMORPH - A somatic or reproductive structure that originates without nuclear recombination (asexual reproduction). Cf. Teleomorph.

ANTHROPOPHILIC - A fungus (dermatophyte) that preferentially grows on man rather than other animals or the soil.

ARTHROCONIDIUM - (pl. arthroconidia) A thallic conidium released by the fragmentation or lysis of hypha. It is not notably larger than the hypha from which it was produced, and separation occurs at a septum.

ARTHROSPORE - See arthroconidium.

ASTEROID BODY -(Splendore-Hoeppli phenomenon) An eosinophilic substance which forms a covering of approximately 10 microns thick around a basophilic yeast especially in sporotrichosis.

BASE - The junction of a bud and the mother cell of a yeast.

BIOFILM – Microcolonies of organisms which adhere to a surface (catheter, implant, water pipe, blood vessel) and which resist removal by fluid movement and have a decreased susceptibility to anti-microbials.

BUD - A type of asexual reproduction commonly found in yeasts.

CAPSULE - A hyaline mucopolysaccharide covering around the cell body of certain yeasts (Cryptococcus, Rhodotorula) and some spores and conidia.

CHLAMYDOSPORE - Thick-walled resistant resting spore, especially in *Histoplasma capsulatum*. See macroconidium.

COENOCYTIC - Without septa.

COLONIZATION - growth of an organism in a host without tissue invasion.

COLUMELLA - A sterile invagination of a sporangium, as in the Mucormycetes.

COMMENSALISM - A symbiotic relationship in which there is no damage to either participant.

COMPLEMENT FIXATION - A serologic procedure to determine antibody to fungus infections. Cross reacts with other systemic fungi but is a quantitative test.

CONIDIOGENOUS CELL - The cell that gives rise to a conidium.

CONIDIUM (pl. conidia) - A reproductive propagule produced in the absence of nuclear recombination, thus representing anamorphic or asexual reproduction.

CONIDIOPHORE - A specialized hypha that gives rise to, or bears a conidium.

CYCLOHEXIMIDE - See Actidione.

DERMATOMYCOSIS - An infection of hair, skin and nails caused by the keratinophilic fungi of the genera *Trichophyton*, *Microsporum* and *Epidermophyton* which infect hair, skin and nails.

DERMATOPHYTE - Infection of hair, skin and nails caused by fungi other than dermatophytes.

DEMATIACEOUS - A fungus having brown or black melanotic pigment in the cell wall.

DICHOTOMOUS - A type of branching of hyphae that is frequent and repetitious; the two branches are approximately equal in size.

DIMORPHIC - Having two forms.

ECHINULATE - Covered with delicate spines.

ECOLOGY - The science of organisms as affected by the factors of their environment.

ECTOENDOTHRIX - Arthroconidia formed on the outside and inside of a hair shaft.

ECTOTHRIX - Forming a sheath of arthroconidia on the outside of a hair shaft. The cuticle of the hair is destroyed.

EDENTULOUS - The absence of teeth.

ENDOGENOUS - From within.

ENDEMIC - A disease which occurs in a limited geographic area.

ENDOSPORE - A spore formed within some other unit, such as in a spherule. (Typical of *Coccidioidomycosis*).

ENDOTHRIX - Arthroconidia formed inside a hair shaft. The cuticle of the hair remains intact.

EXOGENOUS - From without. The source of most mycotic infections is exogenous, i.e. outside the body (the environment).

FLOCCOSE - Cottony or wooly.

FOMITE - A substance other than food that may harbor and transmit infectious organisms.

FRUITING BODY - Reproductive structures of fungi. (Spores).

FUNGEMIA - Presence of fungi in the blood.

GMS - Gomori methenamine-silver. An excellent stain for visualizing fungi. The cell wall stains black and the background is green. Advantage: stains all fungi. Disadvantage: the tissue reaction is not visible.

GEOPHILIC - Soil-seeking, having a soil reservoir.

GERM TUBE - Initial hypha from a sprouting conidia, spore or yeast.

GLABROUS - Smooth.

H & E - Hematoxylin and Eosin. A stain used routinely for general pathology. Most fungi are visible, but not distinctive. Fungal walls usually stain blue or purple. Other cells stain pink. Advantage: the tissue reaction is visible.

HYALO - Colorless; also hyaline.

HYPHA (pl. hyphae) - A vegetative filament of a fungus.

HYPHOMYCETE - An fungus that produces mycelium with or without discernible dark pigment in the cell walls. If the hypha is pigmented, it is called dematiaceous; if colorless, hyaline.

IMMUNODIFFUSION - A serologic test to determine the presence of antibody by double diffusion precipitation in auger.

INCIDENCE - The number of new cases of a disease occurring during a specific period.

INCUBATION PERIOD - The time between an infectious agent entering the body and the onset of clinical symptoms.

INDURATED - Hard.

INTERCALARY - Formed within a hyphal unit.

INVASIVE - The entrance and growth of an organism in tissue.

LATEX AGGLUTINATION - A simple serologic procedure to detect antibody by the clumping of antigen coated particles.

MACROCONIDIUM - The larger of two types of conidia produced in the same manner by the same fungus.

MICROCONIDIUM (pl. microconidia) - The smaller of two types of conidia produced in the same manner by the same fungus.

MOLD - See Mycelium.

MURIFORM - Like a wall; multicellular, with transverse and longitudinal septations.

MYCELIUM - The mass of hyphae making up a fungus colony.

MYCOLOGY - The study of fungi.

ORGANOTROPISM - The predilection of a fungus to invade a particular organ.

PHAEO - Darkly pigmented.

PREVALENCE - The total number of cases of a disease in existence at a certain time in a designated area.

Medical Mycology

PROBE – A specific nucleic acid sequence (known) used to detect a complimentary sequence in an unknown fungus.

PSEUDOHYPHA (pl. pseudohyphae) - A fragile string of cells that result from the budding of blastoconidia that have remained attached to each other. The septa separating the cells are complete and there is no cytoplasmic connection, as is found in most true septate hypha.

RHIZOID - A root like structure. Used in the identification of some Mucormycetes.

RESERVOIR - A permanent host or carrier from which infection is spread.

SAPROBE - An organism which requires organic material as a source of energy.

SAPROPHYTE - See Saprobe.

SCLEROTIC BODY - (sclerotic cell). The tissue form (yeast-like) of most agents of chromomycosis. Dark brown, single or in short chains, occasionally septate, 5 - 15 microns in diameter.

SENSITIVITY - The ability to detect all patients with a specific disease.

SEPTUM (pl. septa) - A cross wall.

SEROLOGY - The study of antigens or antibodies in peripheral blood to support, confirm or rule out certain diseases.

SOURCE - The clinical specimen most likely to yield the etiologic agent. ALSO The ecologic niche or natural nidus of the etiologic agent.

SPECIFICITY - The capacity to identify a disease correctly.

SPINOSE - Covered with small spines.

SPORANGIOPHORE - A specialized hypha that gives rise to a sporangium.

SPORANGIOSPORE - A reproductive unit formed in a sporangium.

SPORANGIUM - A cell within which spores are borne by progressive cleavage.

SPORE - A reproductive propagule produced internally by "free cell" formation, as in the ascomycete, i.e., complete spores formed all at once around the nuclei available or by "progressive cleavage," as in a sporangium.

STOLON - Hypha from which rhizoids and sporangiophores are produced, as in the genus *Rhizopus*.

SYNONYM - Another (especially a later or illegitimate) name for a species or taxonomic group.

TELEOMORPH - The sexual state of a fungus.

TERMINAL - Formed at the end of a structure.

TINEA - Literally "moth". A clinical term meaning "ringworm".

THERMOTOLERANT - Ability to grow at high temperatures (usually above 42 C).

TUBERCULATE - Spines or finger-like projections on macroconidia, characteristic of *Histoplasma capsulatum*.

VESICLE - A swollen or bladder-like cell.

VIRULENCE - Degree of pathogenicity; the disease producing capacity of an organism.

YEAST - A unicellular fungus, usually round or ovoid, that reproduces by budding.

ZOOPHILIC - Infecting lower animals rather than man.

The glossary was derived from several sources including: Rippon, Medical Mycology, Third Edition; Emmons, Binford, Utz, and Kwon-Chung, Medical Mycology, third Edition; Ainsworth & Bisby's Dictionary of the Fungi, Seventh Edition; and other keen authorities.