



Advanced medical Mycology

Systemic mycoses

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General characters

1) Fungi that cause systemic mycoses are:

- *Histoplasma capsulatum* causing histoplasmosis.
- *Blastomyces dermatitidis* causing blastomycosis.
- *Paracoccidioides brasiliensis* causing paracoccidioidomycosis.
- *Coccidioides immitis* causing coccidioidomycosis.

2) All these fungi are **DIMORPHIC**:

- In the soil or culture at 25 degree: *hyphae with spores*.
- In the tissues or culture at 37 degree: *yeast cells*.

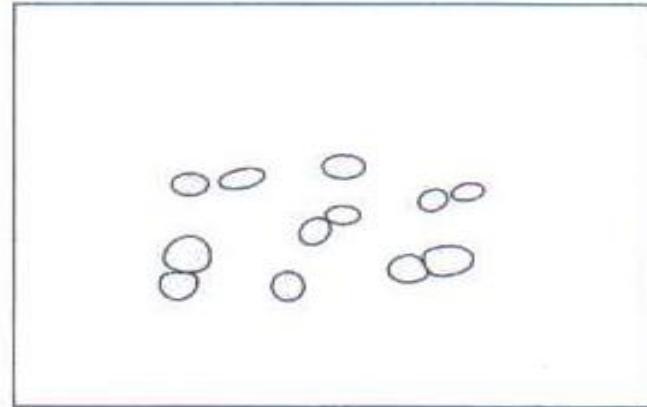
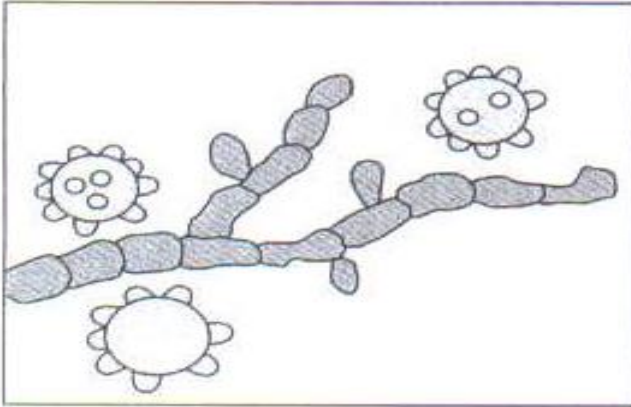
3) Infection by these fungi occurs as follows:

- These fungi grow as hyphae in the soil.
- They release spores into air.
- These spores are inhaled by man.
- Inside the human body: spores will grow as yeast cells.

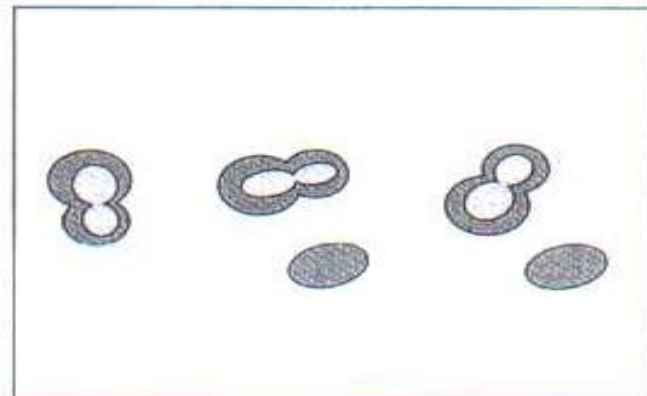
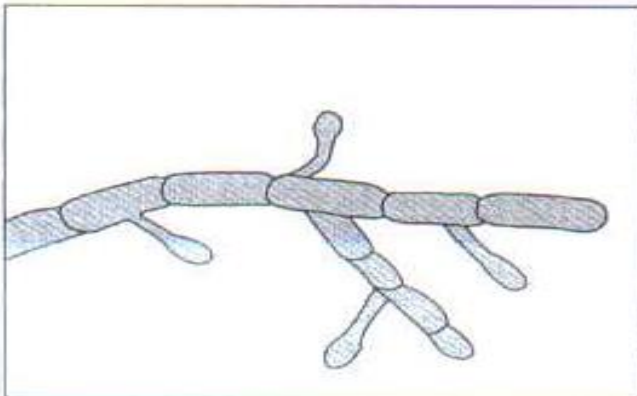
Morphology

	Histoplasma capsulatum	Blastomyces dermatidis
At 25 degree (saprobiic phase)	Septate hyphae + <u>tuberculate</u> macroconidia + microconidia	Septate hyphae + <u>pyriform</u> microconidia
At 37 degree (parasitic phase)	Budding yeast cells found <u>inside macrophages</u>	Yeast cell with <u>a single bud</u> attached by a <u>broad base</u>

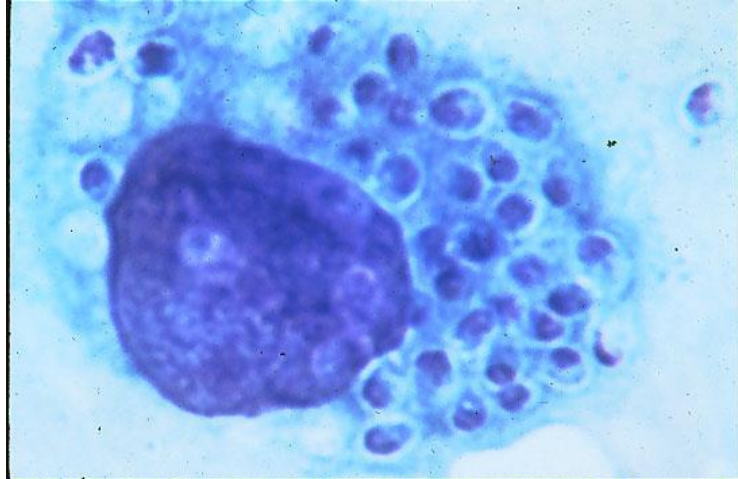
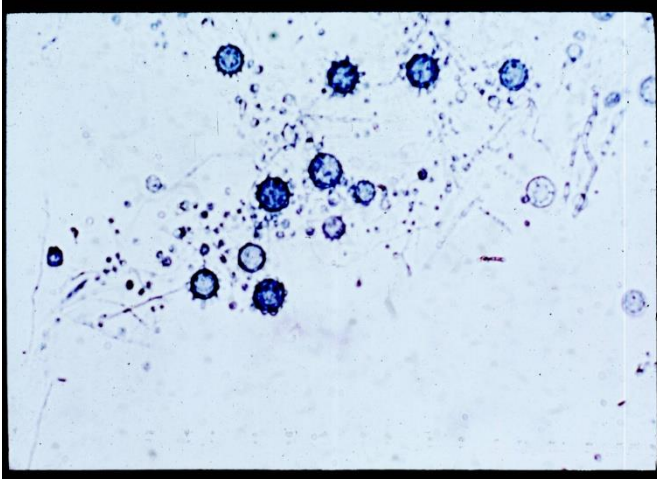
Histoplasma capsulatum



Blastomyces dermatidis



Histoplasma capsulatum

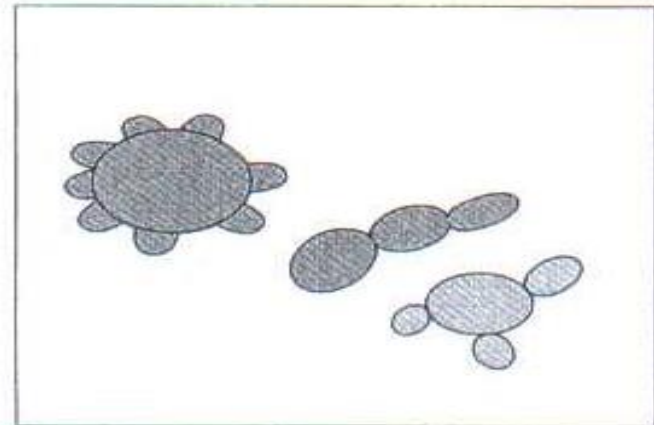
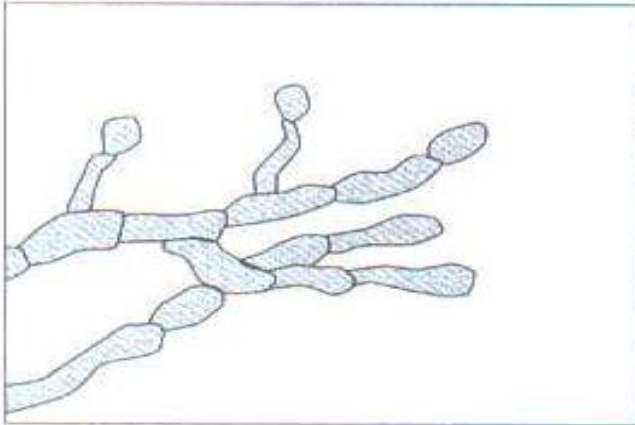


Blastomyces dermatidis

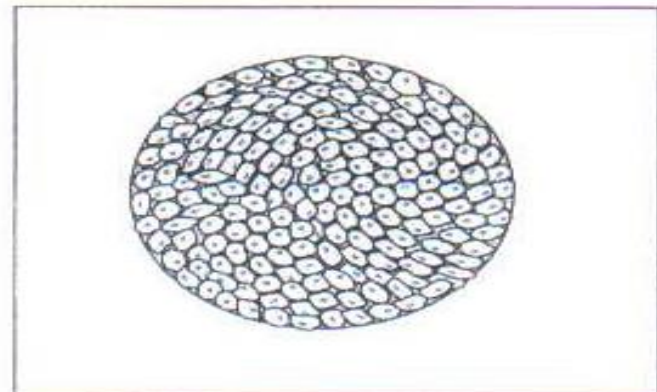
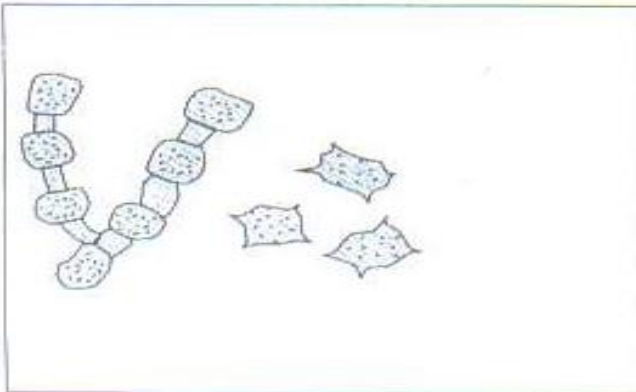


	Paracoccidioides brasiliensis	Coccidioides immitis
At 25 degree (saprobiic phase)	No <u>specific</u> pattern of conidiation	Septate hyphae with alternating <u>arthroconidia</u> which are <u>barrel</u> shaped
At 37 degree (parasitic phase)	Yeast cell with several budding cells (<u>ship wheel arrangement</u>)	Large spherical structure called <u>spherule</u> filled with spores

Paracoccidioides brasiliensis



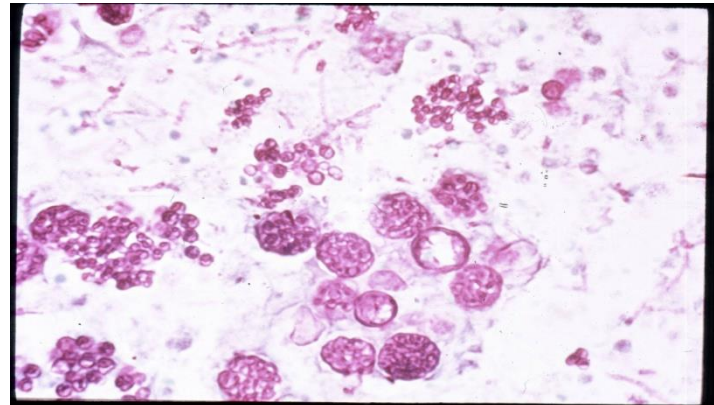
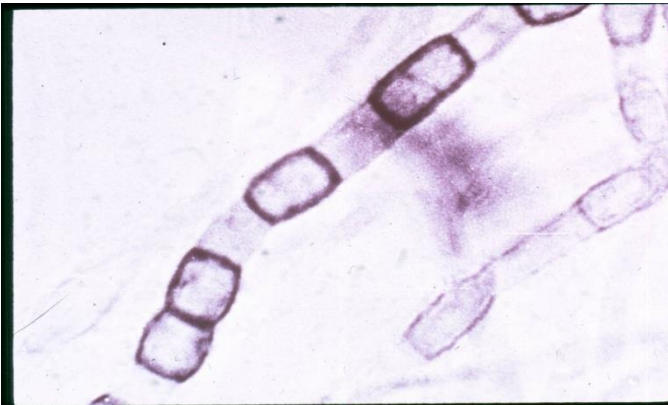
Coccidioides immitis



Paracoccidioides brasiliensis



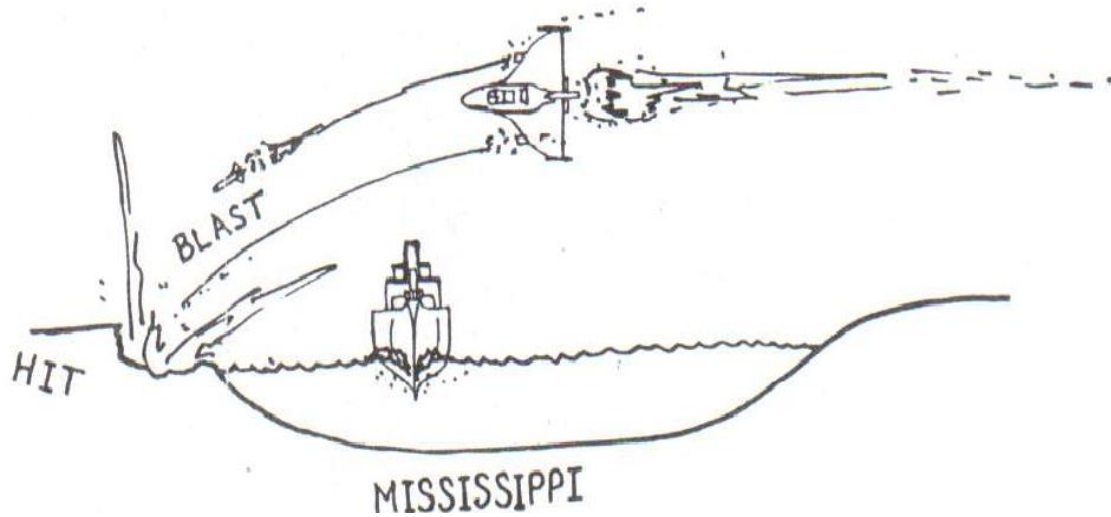
Coccidioides immitis



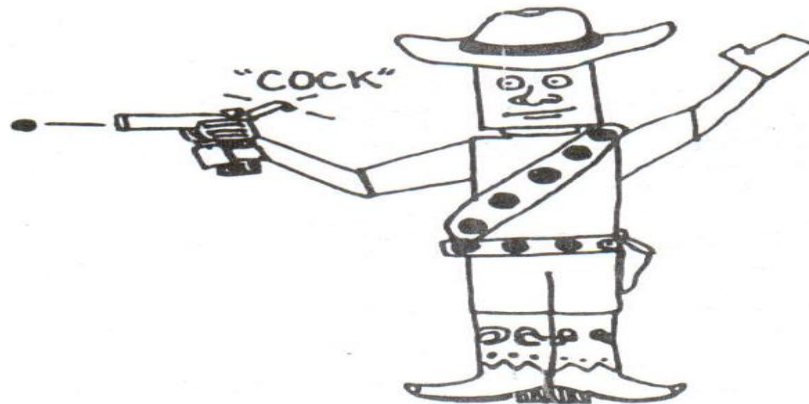
Epidemiology

- ❑ Infection by these fungi is restricted to certain areas in the world.
- ❑ So, SYSTEMIC mycoses is called ENDEMIC mycoses.
 - ❖ Histoplasmosis and blastomycosis are endemic in vast areas that drain into Mississippi river.
 - ❖ Paracoccidioides brasiliensis is endemic in South America especially Brazil.
 - ❖ Coccidioides immitis is endemic in Southwestern USA (Arizona, New Mexico, South California) and North Mexico.

Histoplasma & blastomyces



Coccidioides



Pathogenesis

- ❑ These fungi are acquired by inhalation of spores residing in the soil. (NO PERSON TO PERSON)
- ❑ Once inhaled, spores will grow as yeast cells that infect the lungs.
- ❑ Dissemination may occur.

Histoplasma capsulatum (non capsulated) grows in areas contaminated with birds & bats excreta. So, outbreaks of histoplasmosis occur during clearing chicken coops or spelunking.

Blastomyces dermatidis is isolated from the soil and rotten wood.

Clinical picture

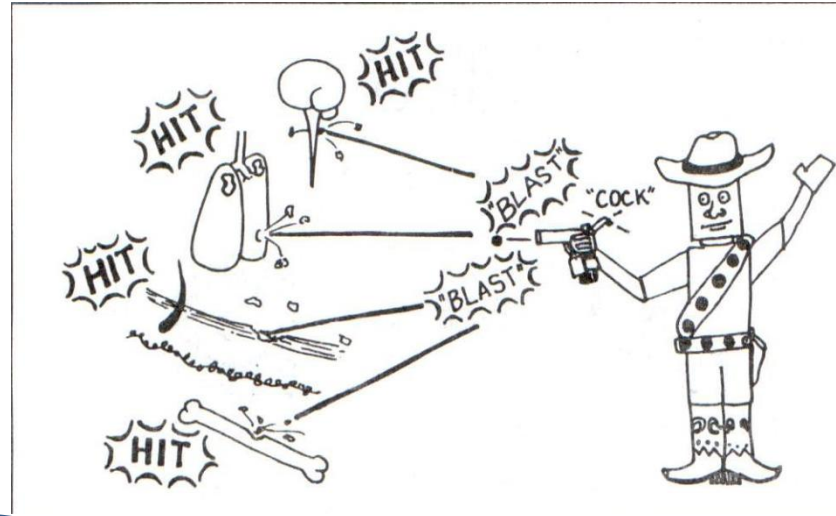
- Asymptomatic: the majority of cases.
- Pneumonia:
 - **Mild** pneumonia with fever & coughing.

Histoplasma lesions may heal with calcification of granuloma

- A small percent will develop **severe** pneumonia.
- A smaller percent will progress to **chronic cavitory** pneumonia.

❑ Disseminated lesion: occurs in immunodeficient patients. Spread occurs to:

- Lung.
- CNS causing meningitis.
- Bone causing lytic lesions.
- Skin causing ulcers.
- Other organs.

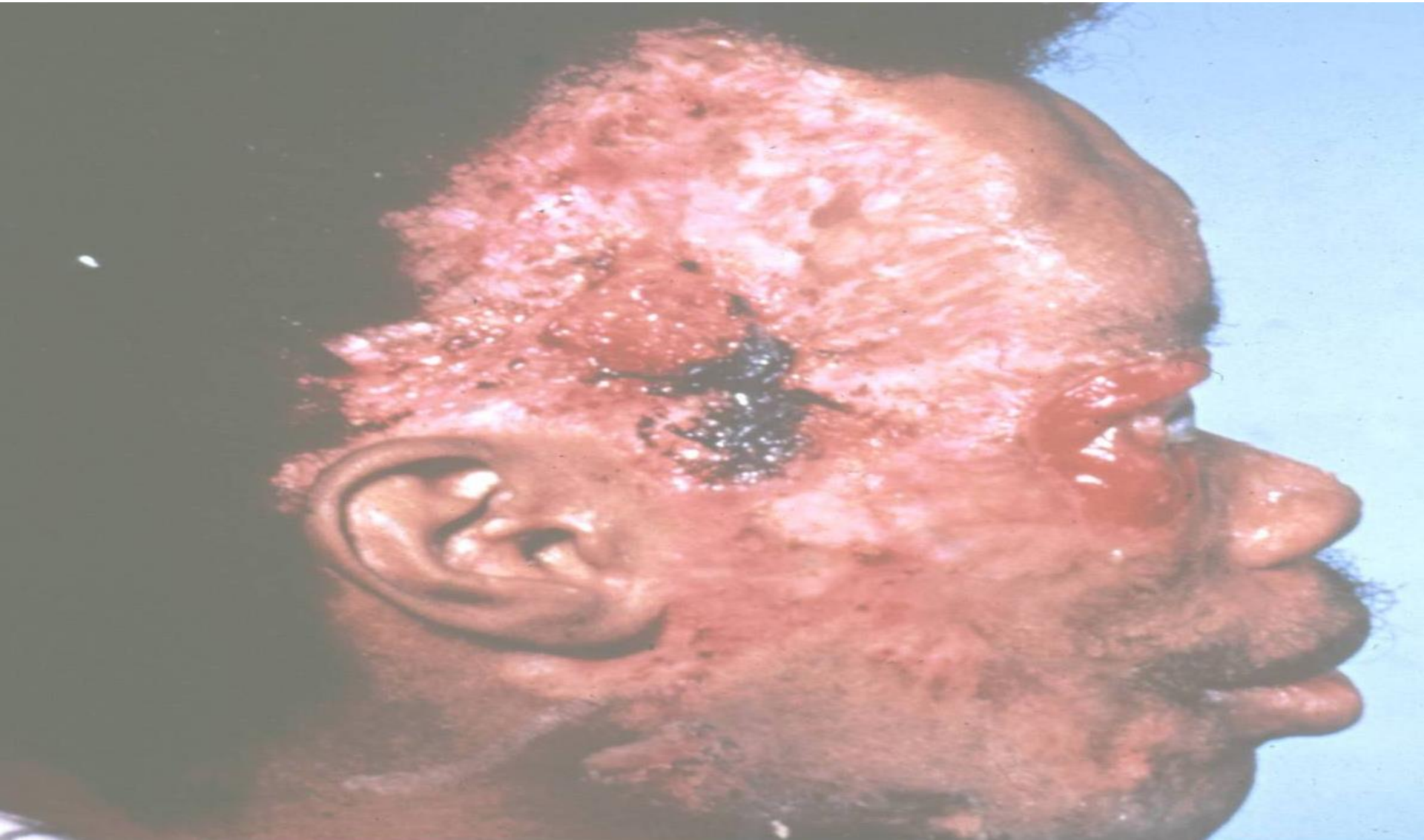


NOTE THAT

Blastomycosis is the hardest to get & the hardest to have

Blastomycosis is the rarest systemic infection. Mostly present as chronic disseminated disease.

Blastomycosis



Laboratory diagnosis

Tissue is the

- ❑: BIOPSY from the affected tissue.
- ❑ Direct microscopic examination of the tissue after staining with PAS, silver to see the yeast phase.
- ❑ Culture on SDA aSpeciment:
 - ❖ At 25 degree: hyphae with characteristic spores.
 - ❖ It converts to yeast after incubation at 37 degree.

□ Serological diagnosis: detect the antibodies against fungi. Detection of **RISING TITER** is more indicative.

❖ For diagnosis of histoplasmosis, we detect antibodies against 2 antigens:

- Cell free culture filtrate of mycelia (histoplasmin antigen).
- Inactivated whole yeast phase cells.

✚ The tests used are:

- Complement fixation test.
- Immunodiffusion test.

❖ For diagnosis of blastomycosis, we detect antibodies against 2 antigens:

- Cell free culture filtrate of mycelia (blastomycin antigen).
- Inactivated whole yeast phase cells.

✚ *Serological diagnosis of blastomycosis is not reliable because these antigens are:*

- Poorly defined.
- Cross reactive with other fungi.

❖ For diagnosis of **paracoccidioidomycosis**, we detect antibodies against 2 antigens:

- Cell free culture filtrate of mycelia (paracoccidioidin antigen).
- Inactivated whole yeast phase cells.

✚ Tests used are:

- Complement fixation test.
- Immunodiffusion test.

❖ For diagnosis of coccidiomycosis, we detect antibodies against 2 antigens:

- Cell free culture filtrate of mycelia (coccidioidin antigen).
- Cell free culture filtrate of yeast phase cells (spherulin antigen).

+ Tests used are:

- Tube precipitin test.
- Complement fixation test.

□ Skin tests:

- ❖ They are histoplasmin, blastomycin, paracoccidiodin and coccidiodin skin tests.
- ❖ Positive result means induration and erythema about 48-72 hours after intradermal injection with the antigen.
- ❖ Demonstrate only previous exposure in epidemiological studies.
- ❖ Should not be used for diagnosis because they may result in false positive serological tests.

Skin tests are not useful in blastomycosis because blastomyces antigens are poorly defined.

Treatment

- Acute lesions: no treatment.
- For chronic & disseminated lesions:
itraconazole or amphotericin B for months.
- But, all infections with blastomyces require aggressive treatment with itraconazole or amphotericin B.

Opportunistic mycoses

Candidiasis



Figure 1. Skin Smear *Candida albicans*
www.meddean.luc.edu

The Situation

Frequency

- most common fungal pathogen worldwide
 - 4th leading causes of nosocomial infections, 40% mortality
 - significant mortality and morbidity in low birth-weight infants
 - affects 75% women, 45% experience recurrence
- > 10 million visits/year
- classified as a STD by CDC

Immunocompromised

- cancer and HIV-AIDs patients
- most commonly manifested in patients with leukemia or HIV-AIDs infections. Oral candidiasis is often a clue to acute primary infection

Public Concerns

- increasing resistance to drug therapies due to antibiotics and antifungals

Biology of *Candida albicans*

- Commensal \longleftrightarrow Pathogen
- A thin-walled dimorphic fungus
- Morphogenesis
 - Unicellular yeast (harmless)
 - Filamentous (pathogenic)
- Principal Cell Wall Polymers
 - Glucan
 - Mannan
- Strict aerobe, favors moist surfaces
- Commensally found in gut, genitals, and lungs
- Body Temp 37° C, neutral pH
- Rapid Multiplication & Spread

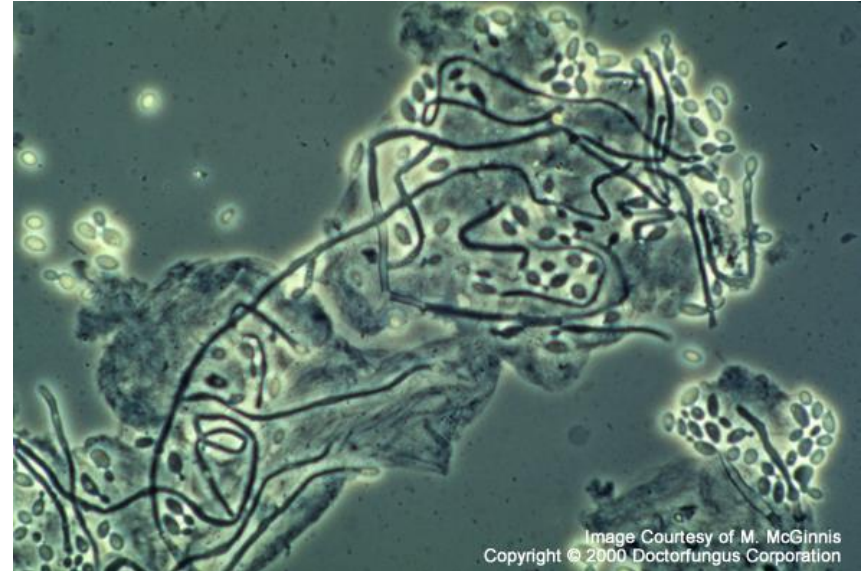
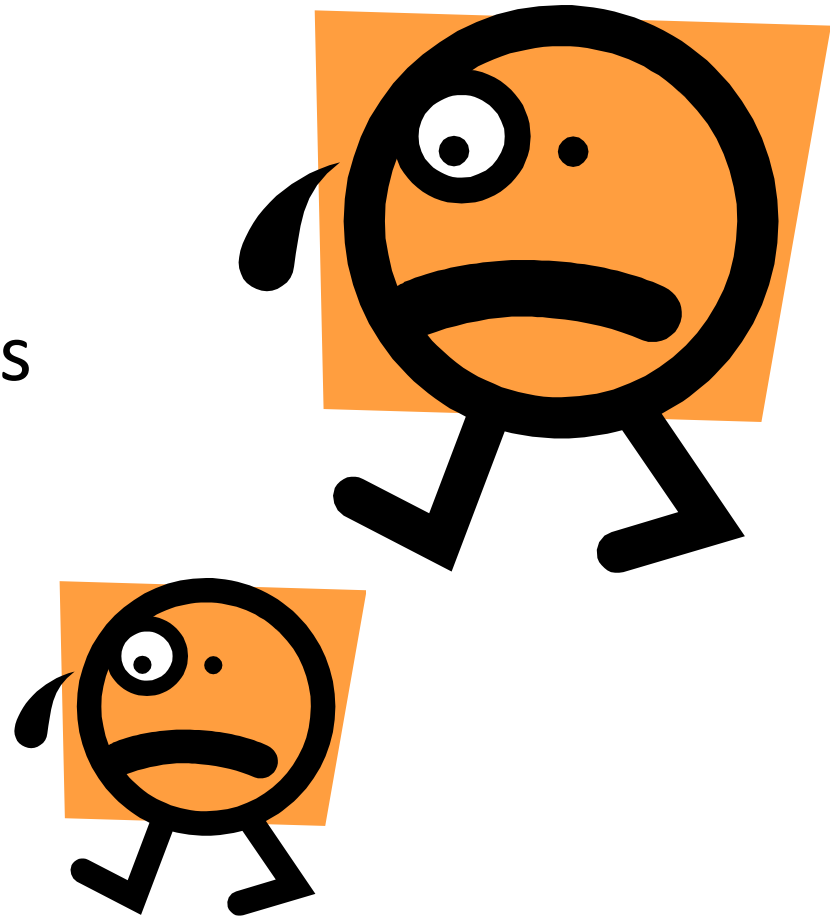


Figure 1. Yeast in Oral Scraping
A sample of an oral scraping contains yeast cells and
pseudohyphae
(www.doctorfungus.org)

Diseases by *C. albicans*

- Thrush
- Esophagitis
- Cutaneous Candidiasis
- Genital Yeast Infections
- Deep Candidiasis



Oropharyngeal Thrush

- * Pseudomembranous
- * Atrophic
- * Angular cheilitis

Symptoms

Risk Factors

HIV

Treatment: topical
antifungals



Figure 1. Angular cheilitis
(www.emed.com)



Figure 2. Oral Thrush, atrophic
(www.mycolog.com)



Figure 3. Oral Thrush, pseudomembranous
(www.emed.com)

Genital Yeast Candidiasis

Symptoms

Risk Factors

- disruption of normal microbiota

Treatment

- direct genital administration
- tablets, suppositories, creams

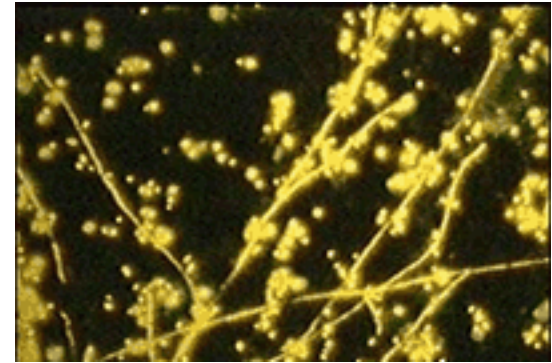


Figure 1. Vaginal Yeast Culture
(www.euromeds.co.uk)

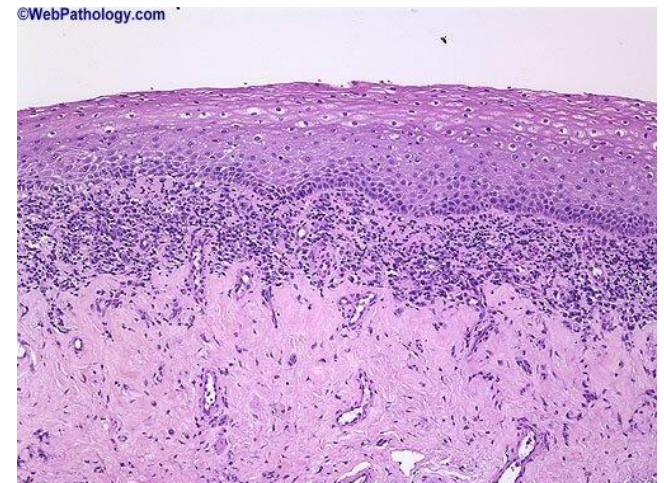


Figure 2. Plasma cell balanitis. A band-like infiltrate of plasma cells is in the dermis of the male penis.
(www.webpathology.com)

Deep Candidiasis

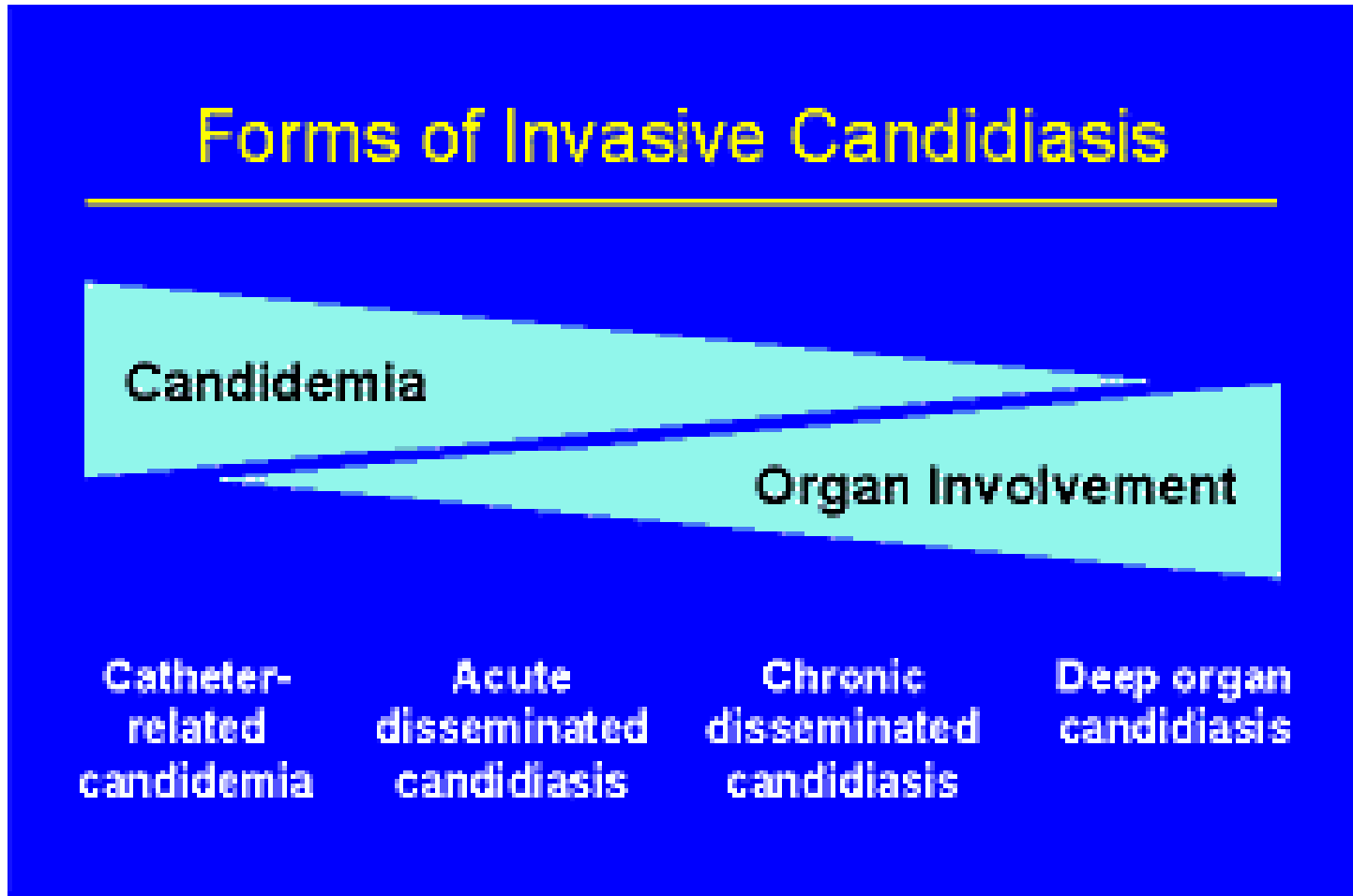
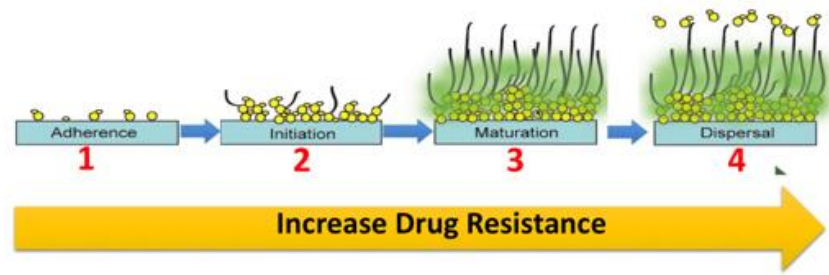


Figure 1. Four forms of invasive candidiasis
(www.doctorfungus.org)

Pathogenesis

- Host Recognition
Adhesions
- Enzymes
Hydrolases: Phospholipases, Lipases, Proteinases
- Morphogenesis
Yeast form to Filamentous hyphae/pseudohyphae
- Phenotypic Switching
- Biofilm formation



Virulence assay of different *C. albicans* strains using the skin equivalent (AST 2000)

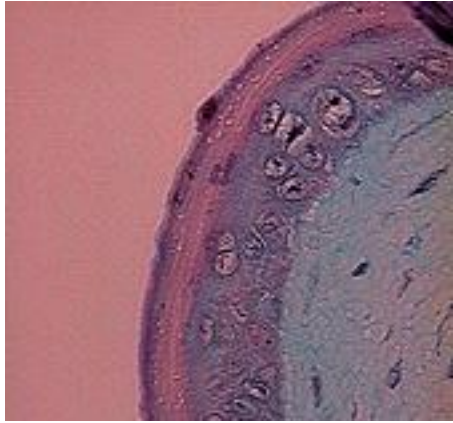


Figure 1. skin equivalent before infection

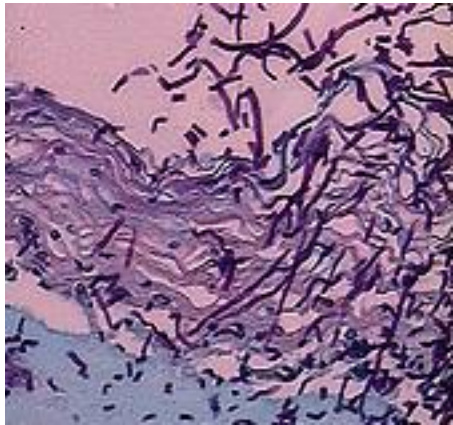


Figure 2. Infection with pathogenic clinical isolate of *C. albicans*. After 48 h the yeast penetrates the skin equivalent and destroys the tissue

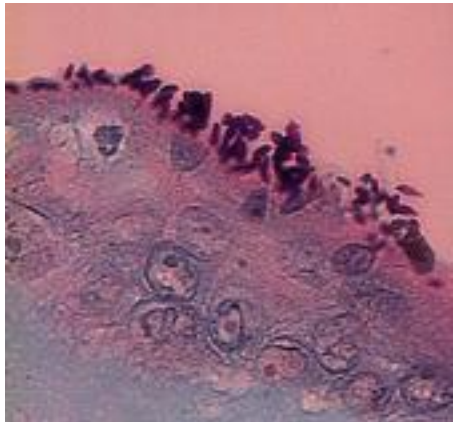


Figure 3. Infection with non-pathogenic *C. albicans*. This strain is not able to penetrate into the tissue and thus behaves as avirulent as shown in the mouse model of systemic infection.

MORPHOGENESIS



Figure 1. Morphogenesis.
Morphogenesis in *C. albicans* is a pivotal virulence factor that allows rapid multiplication and subsequent dissemination in host tissue.
(www.kent.ac.uk)

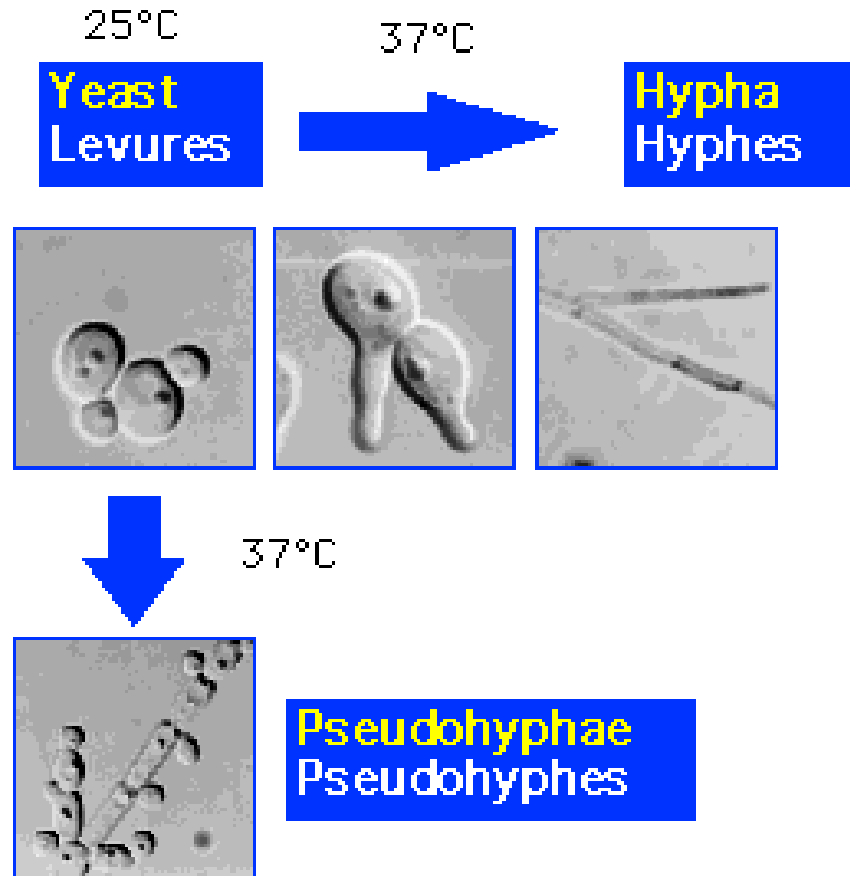


Figure 2. Morphogenic forms of *Candida albicans*
<http://cbr-rbc.nrc-cnrc.gc.ca/thomaslab/candida/caindex.html>

Diagnosis

- Complete medical evaluation and history, with physical exam that focuses on the area of the body with symptoms
- Diagnosis is made based on signs and symptoms, and clusters of budding yeast
- Generally, a doctor takes a sample of the vaginal discharge or swabs an area of oral or skin lesions, urine, feces, and nail clippings
- Fungal blood and stool cultures for detection of *Candida* should be taken for patients suspected of having deep organ candidiasis
- Tissue biopsy may be needed for invasive systemic disease.

Route of Transmission/Portal of Entry

- *C. albicans* is normally present on the skin and in mucous membranes such as the vagina, mouth, rectum, and digestive tract
- The fungus also can travel through the blood stream in immunocompromised individuals
- *Candida* can enter newborn infants during or shortly after birth
- This organism is OPPORTUNISTIC- for example, antibiotics kill the good bacteria leaving *Candida* free to grow

How Candida causes an infection:

- ✘ The infections caused by all species of Candida are called Candidiasis-most commonly Candida albicans
- ✘ Candida is known to impair immune functioning by directly and negatively impacting the helper-suppressor ratio of T lymphocytes
- ✘ Organism causes disease by invading human tissue by means of pseudohyphae (long filaments) which can penetrate intracellular cracks
- ✘ The overgrowth of Candida is complex and a difficult condition to overcome once it enters the body. It is one of the most highly adaptive organisms, and has the ability to mutate and develop stronger forms of itself

How it affects the immune system

- ✘ T-cell efficiency can be influenced to a useful extent by nutrition.
- ✘ It is largely the suppressors which are involved in fighting Candida
- ✘ It's adaptability allows it to produce disguising antigens which deter the immune system from recognizing it as foreign and harmful.
- ✘ In this way the immune system may eventually become non-responsive to the presence of *Candida albicans*.
- ✘ Impaired production and function of the T-cell lymphocytes cannot effectively regulate B-cell immunoglobulin production. As a result the body cannot discriminate between harmless and potentially toxic agents.

Toxins

- ✘ Produces toxins
 - Gliotoxin: inactivates important enzymes, and is cytotoxic
 - Acetaldehyde: normal by-product of metabolism, however excess production of this by Candida can cause impaired neuro-transmission in the brain
- ✘ Candida albicans and its toxins can infiltrate and effect any organ or system in the body and can be responsible for a large variety of physical and mental health conditions that we see so commonly in people today
- ✘ Toxic waste can also be absorbed into the blood stream

Demographics:

- ✘ Candidiasis is an extremely common infection. Thrush occurs in approximately 2–5 percent of healthy newborns and occurs in a slightly higher percentage of infants during their first year of life.
- ✘ Over 1 million adult women and adolescent girls in the United States develop vaginal yeast infections each year. It is not life-threatening, but the condition can be uncomfortable and frustrating.

Common factors that can cause infection:

- ✘ A common cause of Candida albicans fungus is the overuse of antibiotics or oral contraceptives
- ✘ Steroid hormone medication, ex. Cortisone, or corticosteroids often prescribed for skin conditions such as rashes, eczema
- ✘ Suppressed immune systems, ex. Patients with diabetes, HIV/AIDS, and that receive radiation or chemotherapy
- ✘ High sugar and starch diet (yeasts live on sugar, and starches are converted into sugar very quickly during our digestion process)

Areas affected:

- ✘ Candida species can infect tissues in essentially every body system, producing a wide range of clinical manifestations
- ✘ is called thrush when it grows in the mouth, especially in infants
- ✘ shows up on skin as a red, inflamed, and sometimes scaly rash, ex: diaper rash
- ✘ causes vaginitis, commonly known as a yeast infection
- ✘ causes candidal onychomycosis in the nails
- ✘ can affect the esophagus and the digestive tract
- ✘ Candidal infection of the penis may result from sexual intercourse with an infected partner

C. albicans in AIDS patients

- ✘ In immunocompromised patients, when body resistance is low as in leukemia or HIV/AIDS, *Candida albicans* can enter the bloodstream and causes systemic infection
- ✘ The more debilitated the host the more invasive the disease
- ✘ *Candida albicans* is one of several mycoses (fungal disease) that account for most deaths associated with AIDS
- ✘ So common in HIV+ individuals that their mycoses are part of what defines end-stage AIDS

Systemic Candida

Paranasal Sinuses

Blood vessels

Lungs



Esophagus

Skin

Stomach

Intestine

Deep Candida Mycoses

Brain

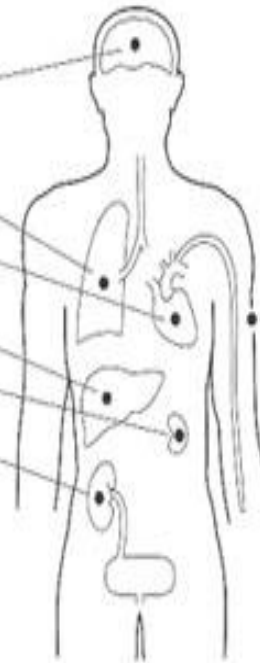
Lungs

Heart

Liver

Spleen

Kidney



Superficial Mycoses
(hair, nail, skin)

Cutaneous Mycoses
(hair, nail, skin)

Subcutaneous Mycoses

Symptoms of Infection:

- Oxygen-carrying red blood cells are rigid and stiff when compared to patients without candida
- Fatigue
- Carbohydrate craving
- Topical infections of fingernails, vaginal infections, underarm infections
- White coated tongue
- Increased allergic symptoms
- Reduced thyroid function
- Lowered immune system and increased susceptibility to viral infections
- T-cell helper/suppressor ratios radically lowered
- Up to 3/4 of the lymphocytes become paralyzed
- Candida seems to upset the function of organs as opposed to damaging them

Treatment:

- ✘ Antifungal medications -- these include oral rinses and tablets, vaginal tablets and suppositories, and creams. For vaginal yeast infections, medications that are available over the counter include creams and suppositories such as Monistat, Vagistat, and Gyne-Lotrimin. Your doctor may prescribe a pill, Diflucan
- ✘ Creams combined with low-strength corticosteroids -- reduce inflammation and itching.
- ✘ The “Candida diet”- detox, probiotics, improve what you eat
- ✘ Most treatments last from 2 - 3 days to 2 weeks. Be sure to take all medicine exactly as prescribed. If you do not, the same infection could come back, or you could become infected with a new strain of candida.
- ✘ For severe candidiasis that could be life threatening to someone with a compromised immune system, your doctor may prescribe an IV medication, amphotericin B.

Vaccine/Cure?

- Recently researchers used recombinant DNA to create a live vaccine, and have conjugated fungal antigens with diphtheria toxoid to create a vaccine against *Candida*

Prevention:

- Good personal hygiene
- Keeping skin clean, dry, and free from abrasions or cuts
- Avoid high sugar, starch, and carbohydrates in your diet
- Taking sufficient amounts of probiotics, to repopulate normal flora

Current Drug Therapies

Major Drug Categories

Polyenes

Problems: ↓ Catalase activity, ergosterol production

Azoles

Problems: Enhanced drug efflux

FDA approved antifungal drugs

Amphotericin B (Fungizone)

Clotrimazole (Mycelex)

Fluconazole (Diflucan)

Itraconazole (Sporanox)

Ketoconazole (Nizoral)

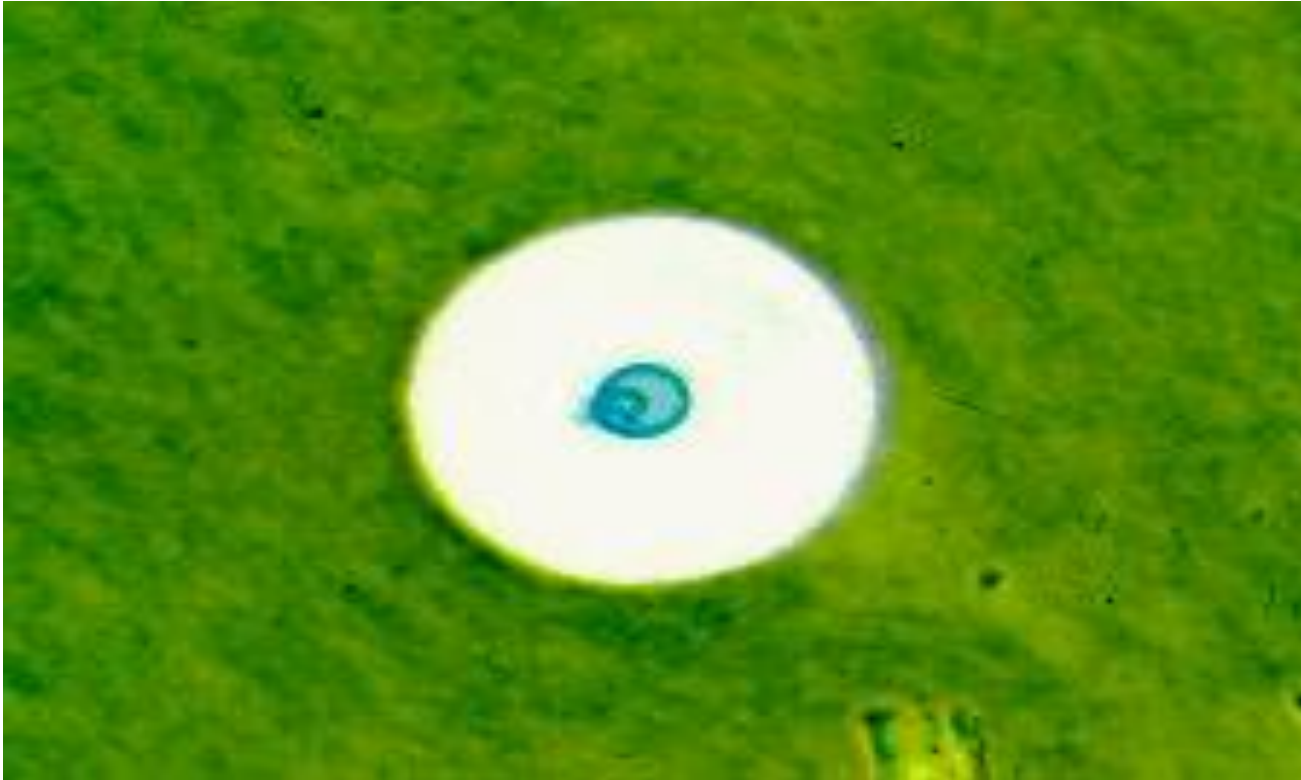
Nystatin (Mycostatin)

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Fig. 1. Fungizone
(www.bms.se)

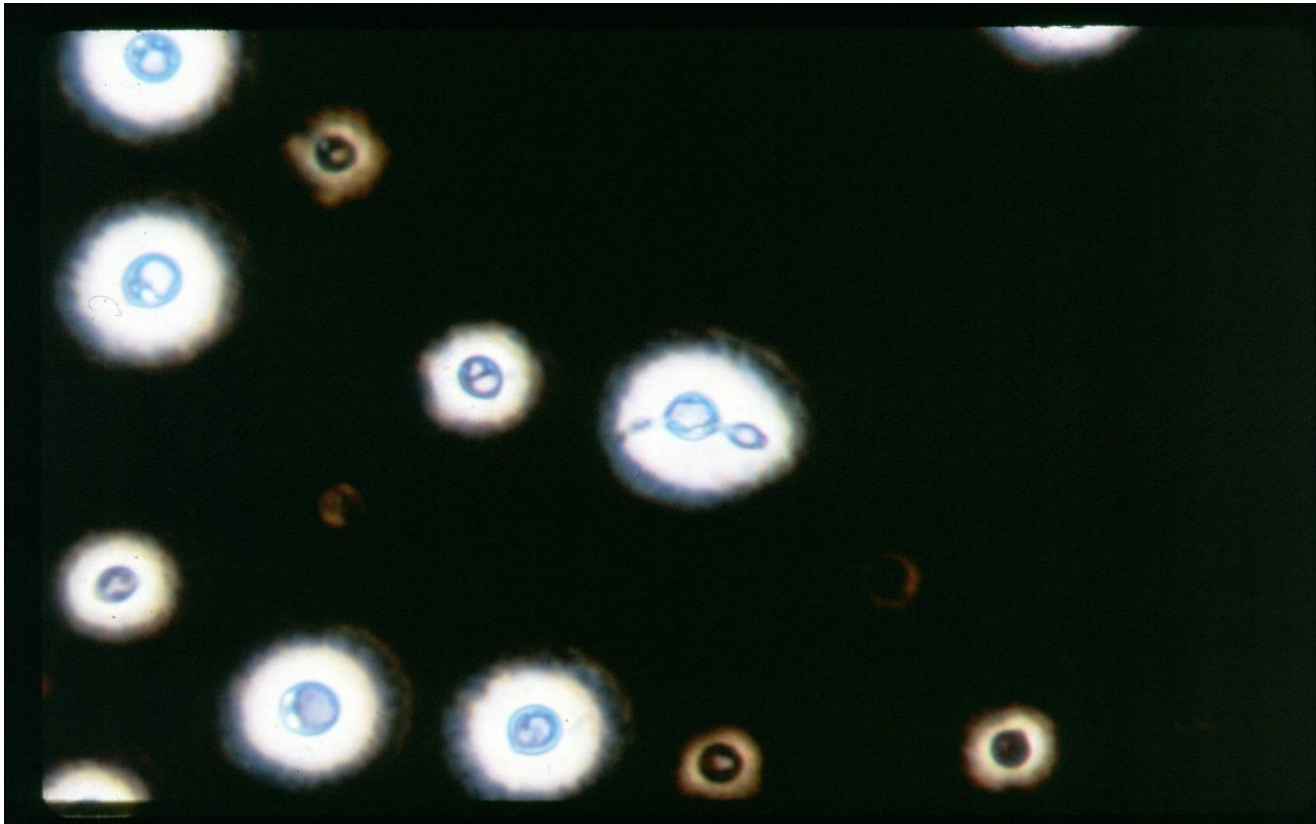
Opportunistic mycoses

Cryptococcus neoformans •



Morphology

- Yeast cells surrounded by gelatinous capsule.



Virulence factors

- ❑ Capsule interferes with phagocytosis.
- ❑ Phenol oxidase enzyme
 - It acts on diphenolic substrate producing melanin in the fungal cell wall.

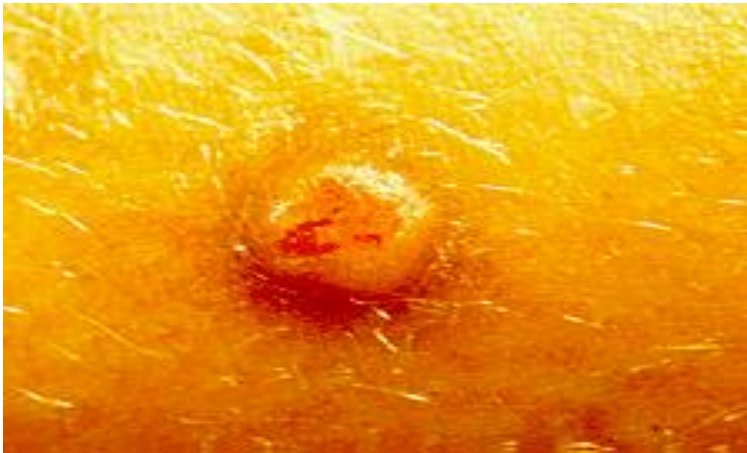
Pathogenesis

- ❑ It lives in soil contaminated with excreta of birds especially pigeons.
- ❑ Infection occurs by inhalation.
- ❑ It affects immunocompromised patients especially those with AIDS.

Diseases

- ❑ Pneumonia
- ❑ It may spread systemically to meninges causing **meningitis.**
- ❑ Skin lesions

Cryptococcal Skin lesions



Laboratory diagnosis

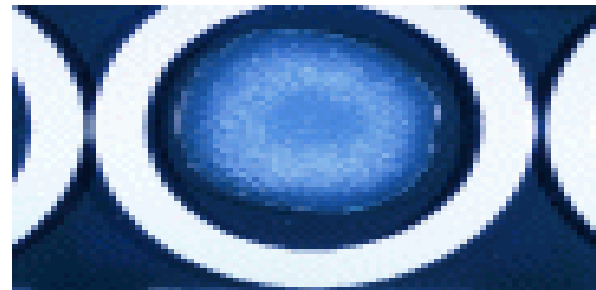
- ❑ **Specimen:** sputum, CSF
- ❑ **Direct examination of the specimen after staining using india ink:** large gelatinous capsule around budding yeast cells.
- ❑ **Culture:** on SDA or bird seed agar at 25 – 37 degree.

Cryptococcus on bird seed agar



Identification of cryptococcus on the plate is done by:

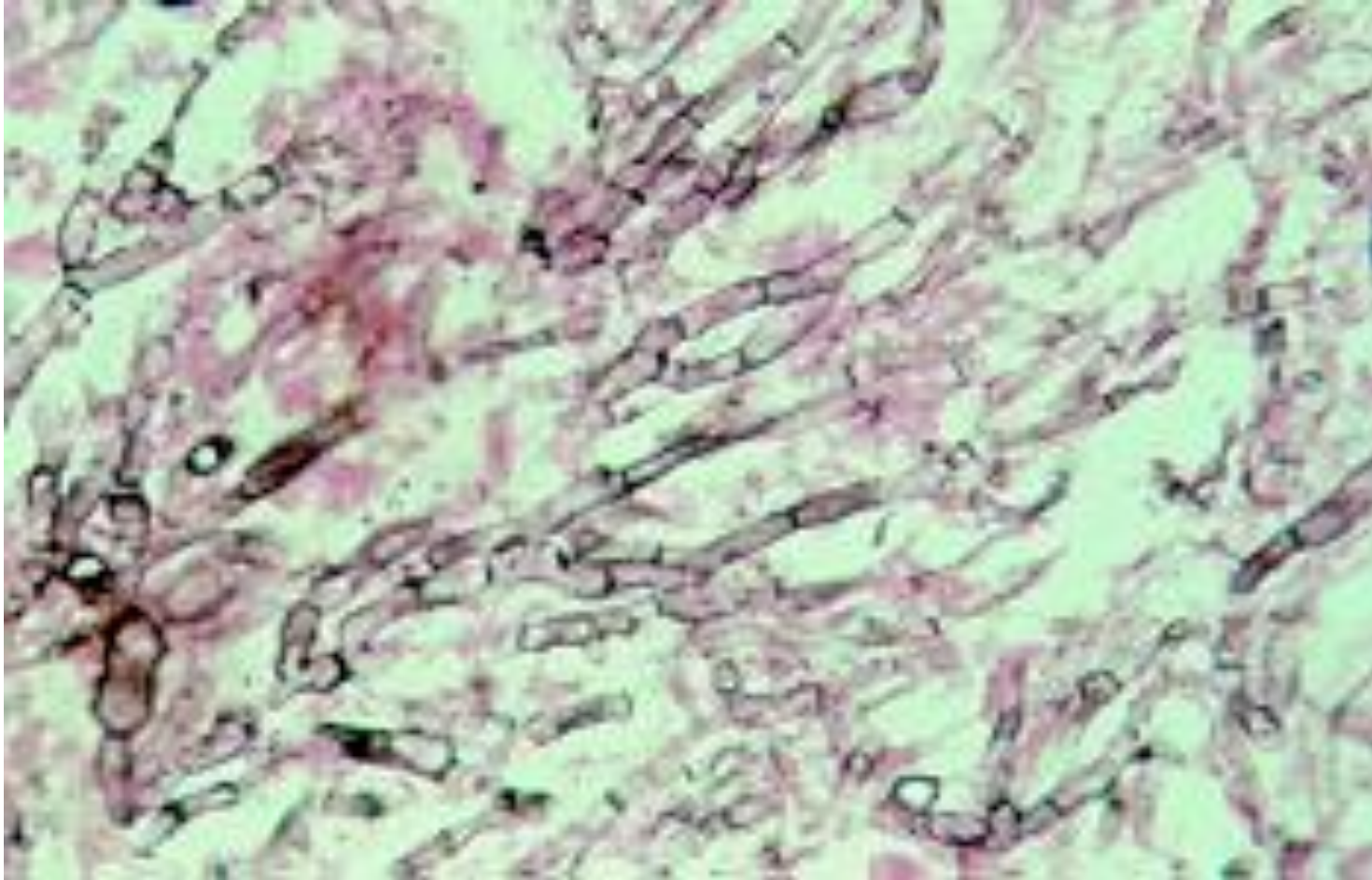
1. **Morphology**: muroid colonies. It appears brown to black on bird seed agar.
 2. **India ink staining**
 3. **Biochemical reactions**: urease positive + phenol oxidase positive.
 4. **DNA probes**
- Direct detection of capsular antigen in CSF by latex agglutination test.**



Treatment

- Amphotericin B and flucytosine are given for 6 months. Given for life with AIDS patients.

Aspergillus

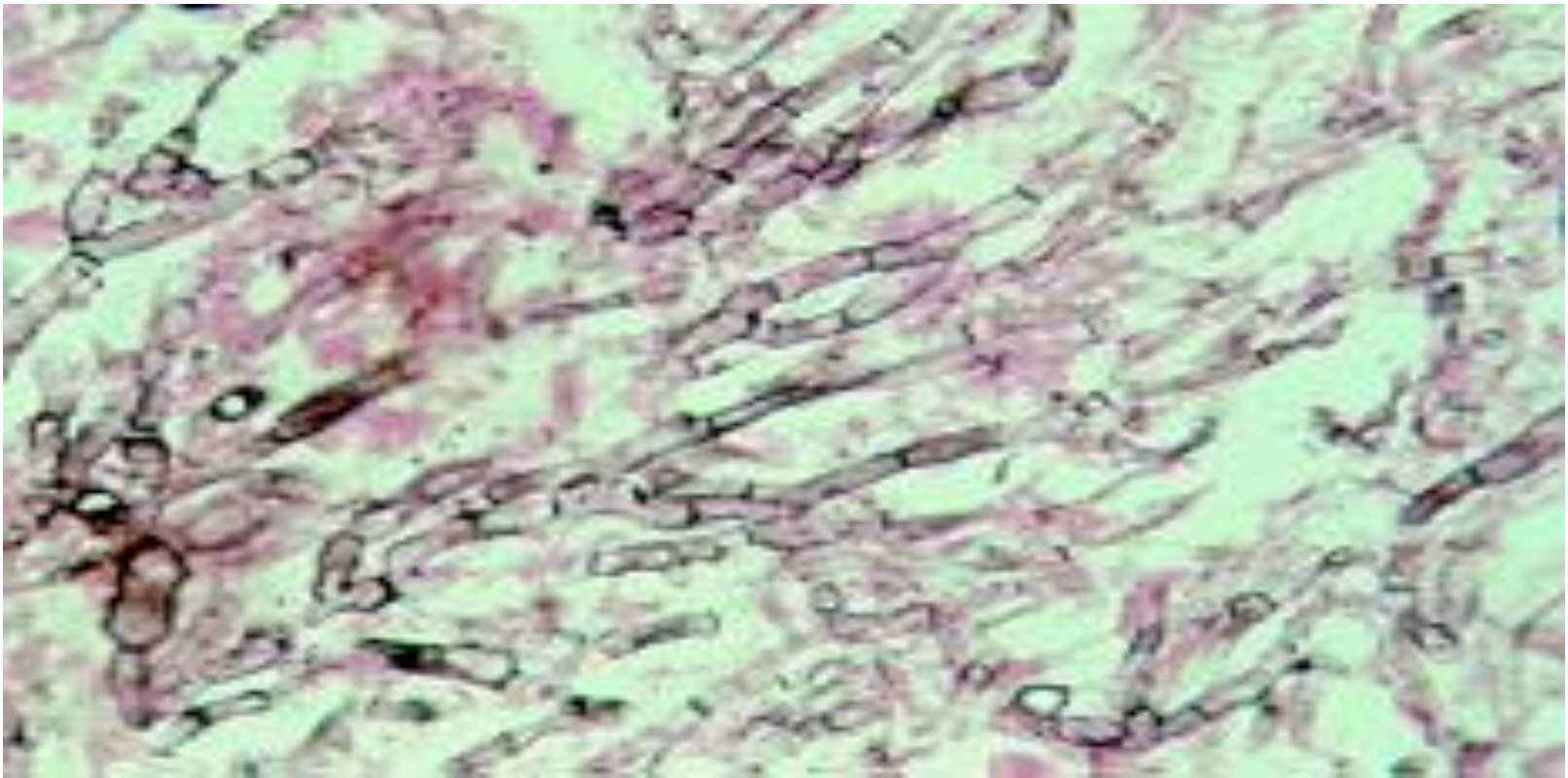


Species

- ❑ Aspergillus is present every where.
- ❑ It is a frequent laboratory contaminant.
- ❑ Species include:
 - *Aspergillus fumigatus* (*the most common human pathogen*)
 - *Aspergillus flavus* (*produces aflatoxin*)
 - *Aspergillus niger*
- ❑ These fungi produce sexual spores called ascospores. So, they are ascomycetes.

Morphology

- ❑ It is a mold.
- ❑ It is formed of hyphae which are: hyaline, septate, uniform in width and branch dichotomously.

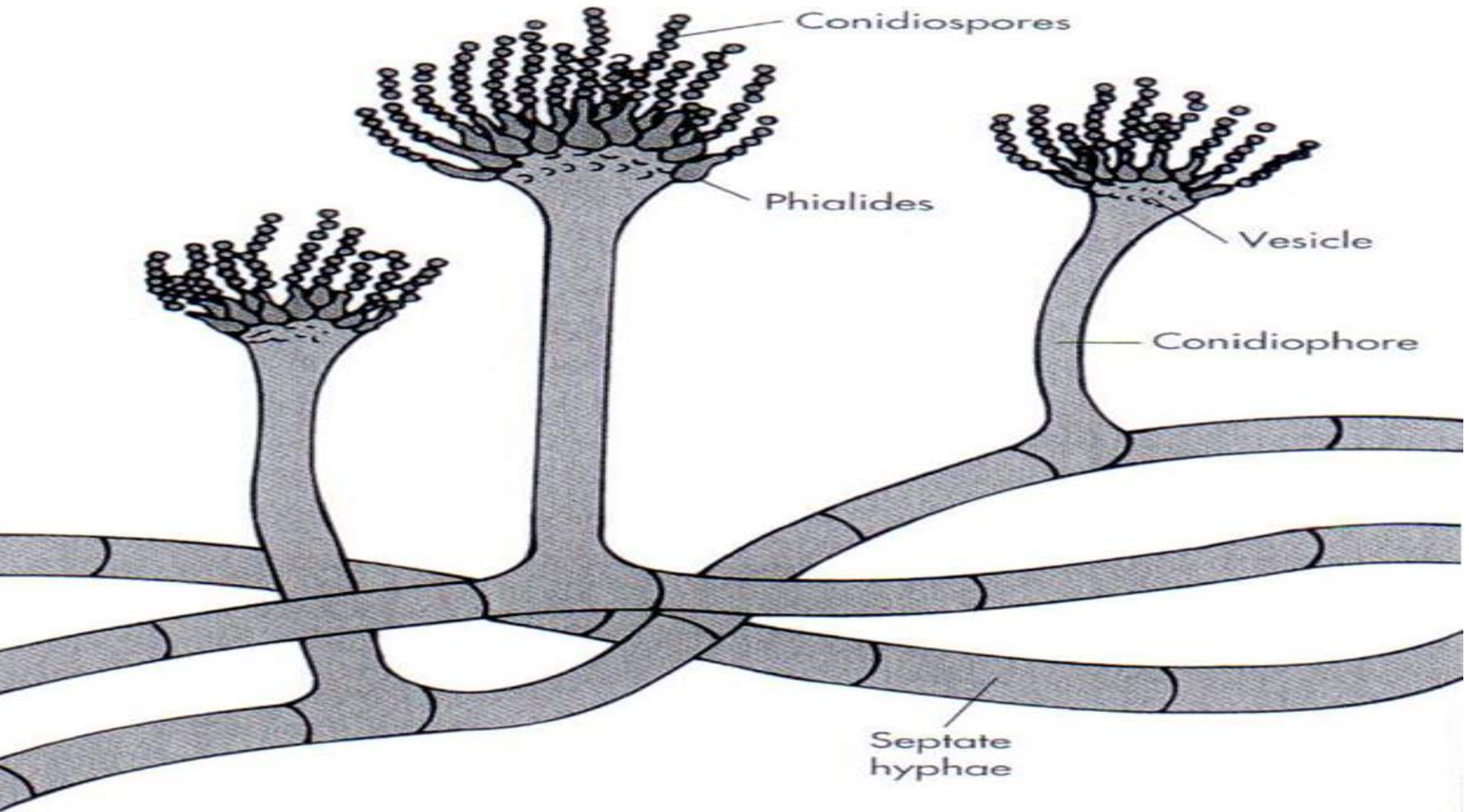


□ Hyphae produce:

❖ Long conidiophore with terminal vesicle covered with either:

- Layer of phialides that produce chains of microconidia (uniseriate).
- Layer of metulae which bear the phialides that produce chains of microconidia (biseriate).

Uniseriate structure



Diseases

- Mode of infection: inhalation
- ❑ Allergic bronchopulmonary aspergillosis (asthma mediated by IgE).
- ❑ Aspergilloma (fungus ball): the fungus enters preexisting lung cavity as in patients with tuberculosis.
- ❑ Invasive aspergillosis:
 - Invasion of blood vessels by hyphae producing bloody sputum.
 - May disseminate to other organs.
- ❑ Aflatoxin consumption in contaminated peanuts may cause liver damage and liver cancer.

Laboratory diagnosis

- Allergic bronchopulmonary aspergillosis:
 - High level of IgE and IgG against aspergillus.
 - Increased level of eosinophils.
 - Skin test: intradermal injection of the patient with aspergillus fungus produces hotness, redness and edema in the injected area within 20 minutes.
 - Sputum examination and culture.

Aspergilloma:

- Chest X-ray.

□ Invasive aspergillosis:

- Sputum examination and culture.
- Positive circulating cell wall galactomannan.

Sputum examination for aspergillus

❑ Direct microscopic examination:

- Reveal hyaline, septate hyphae with uniform width and branch dichotomously.

❑ Culture:

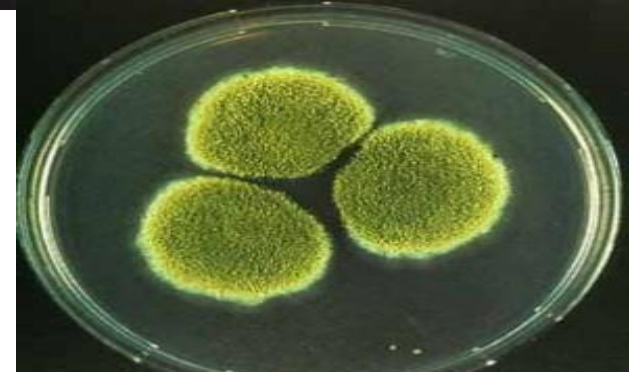
- On SDA with no cycloheximide and incubated at the room temperature.
- **Identification of the species is done by:**
 - ✓ Colonial morphology.
 - ✓ Slide culture to study the morphology of conidia.

Colonial morphology

A. fumigatus



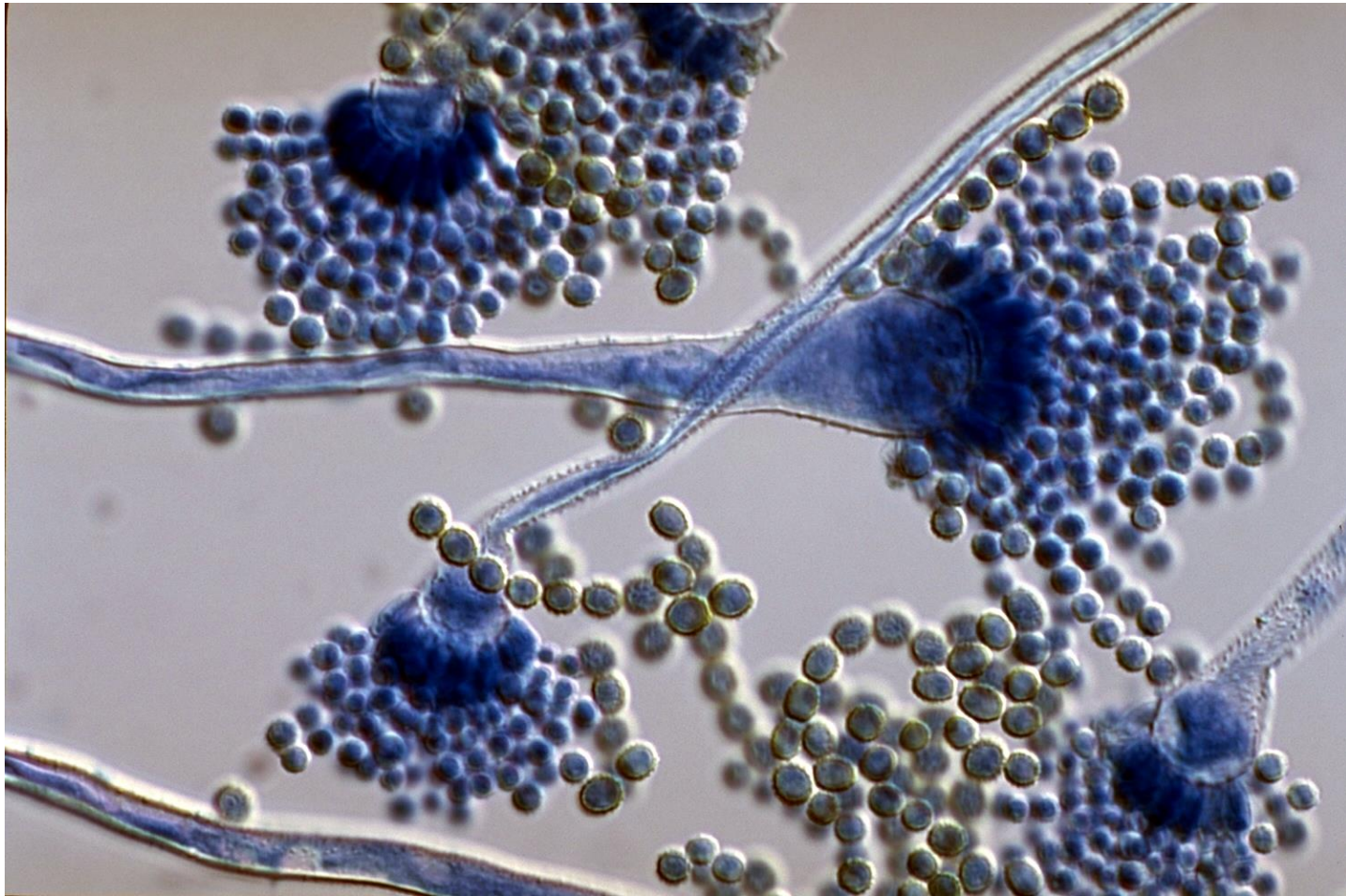
A. flavus



A. niger



Slide culture for aspergillus



Does culture alone diagnose aspergillosis?



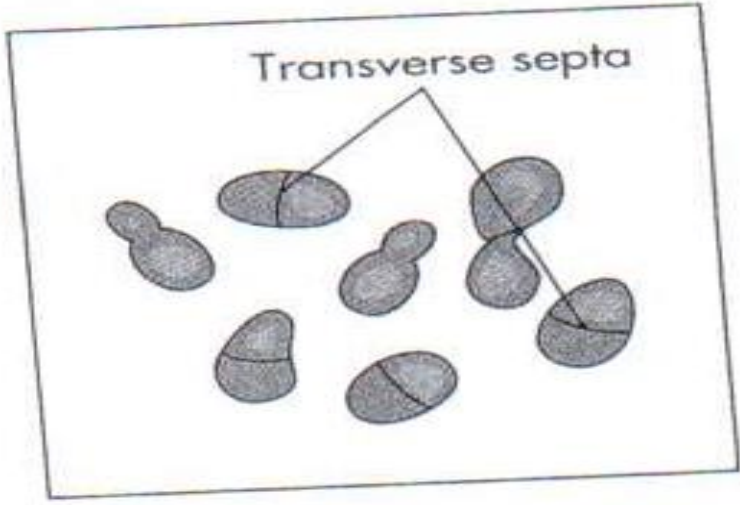
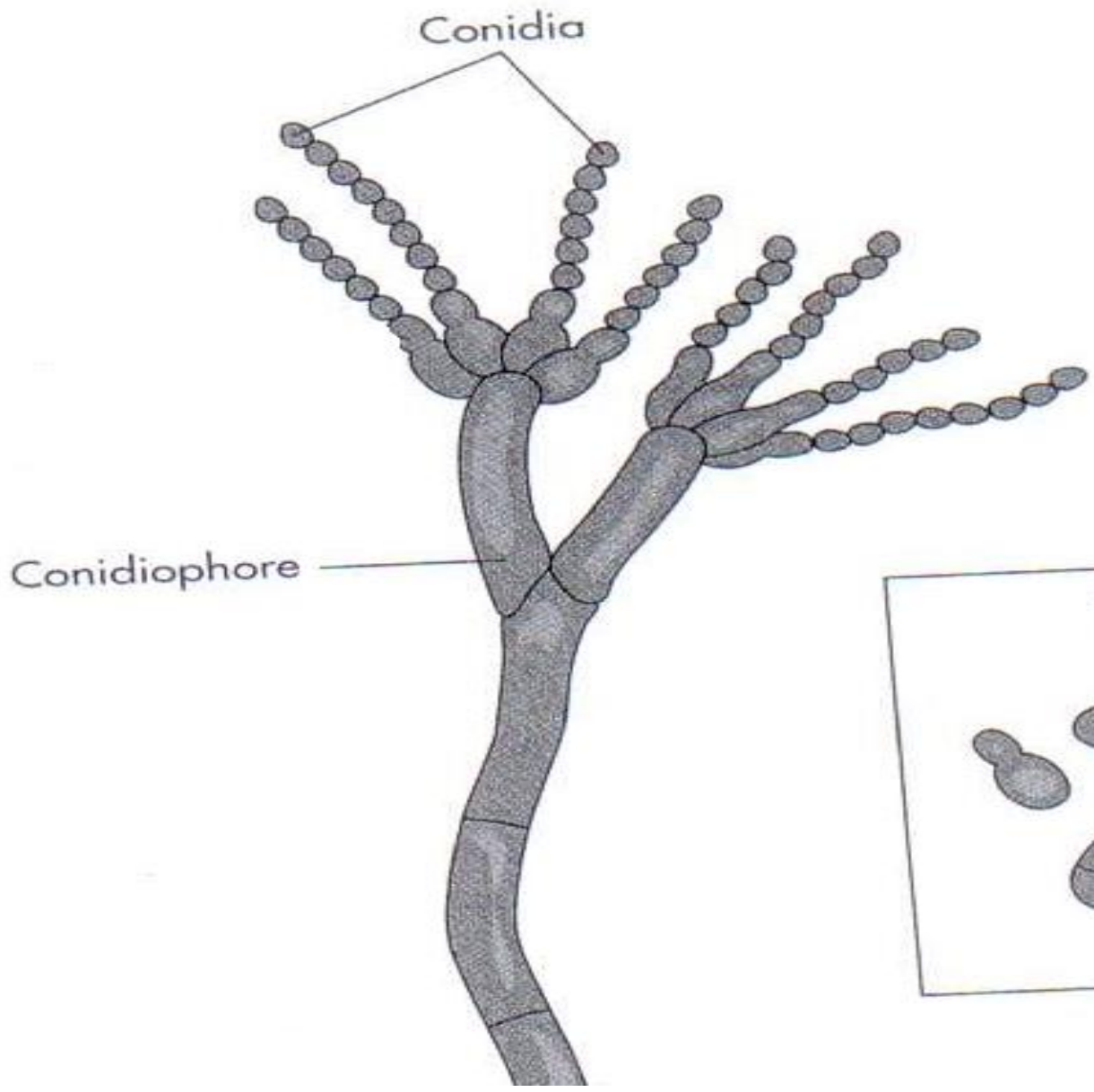
- ❑ No, because aspergillus is present everywhere and may arise in culture as contaminant.
- ❑ So, to confirm the diagnosis of aspergillosis:
 - Direct microscopic examination of sputum should reveal hyaline septate hyphae branching dichotomously.
 - Repeated isolation of the fungus from the same patient.

Treatment

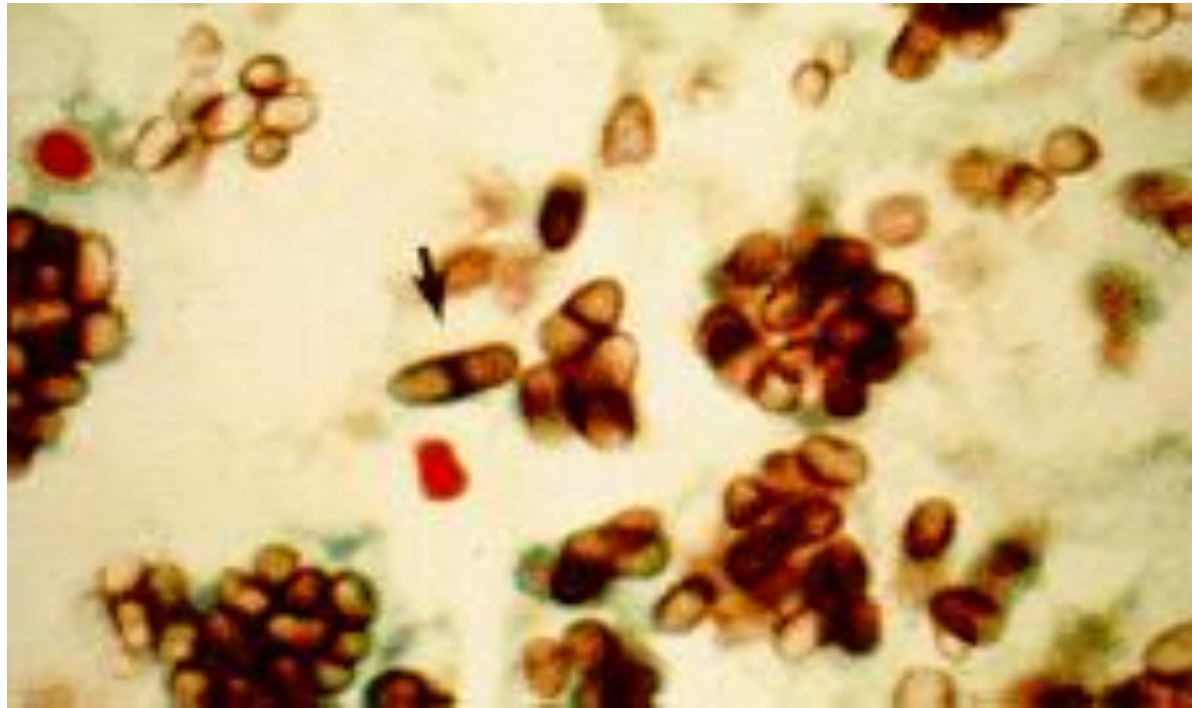
- ❑ Allergic bronchopulmonary aspergillosis: corticosteroids.
- ❑ Aspergilloma: surgical removal.
- ❑ Invasive aspergillosis: amphotericin B and itraconazole.

Penicillium marneffei

Dimorphic •

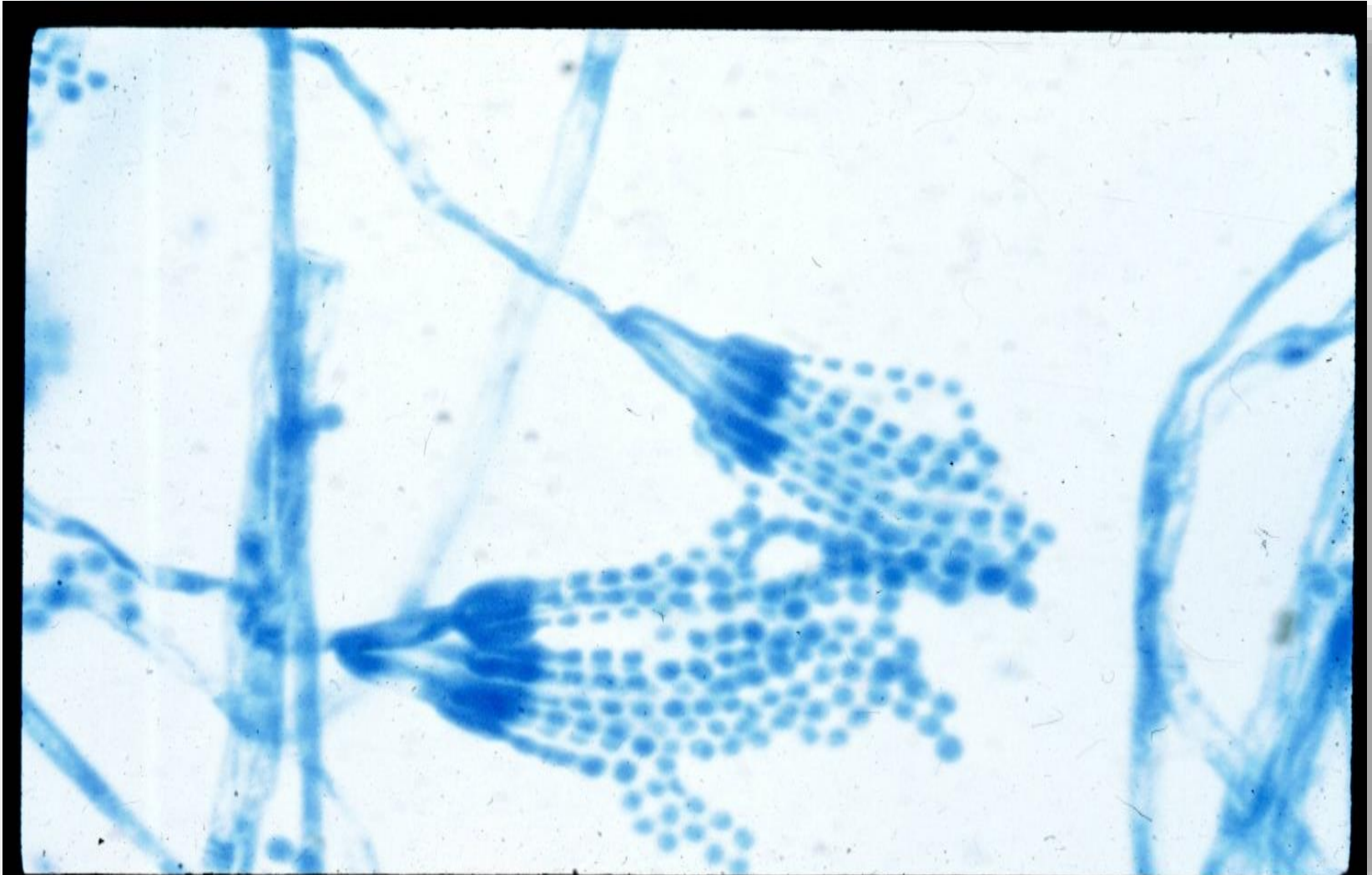


- It is present every where.
- It produces disease only in immunocompromised patients like AIDS patients.
- **It is a dimorphic fungus:**
 - ✓ At 37 degree: yeast cells that divide by transverse septum.



✓ At 25 degree: branching septate hyphae which produce:

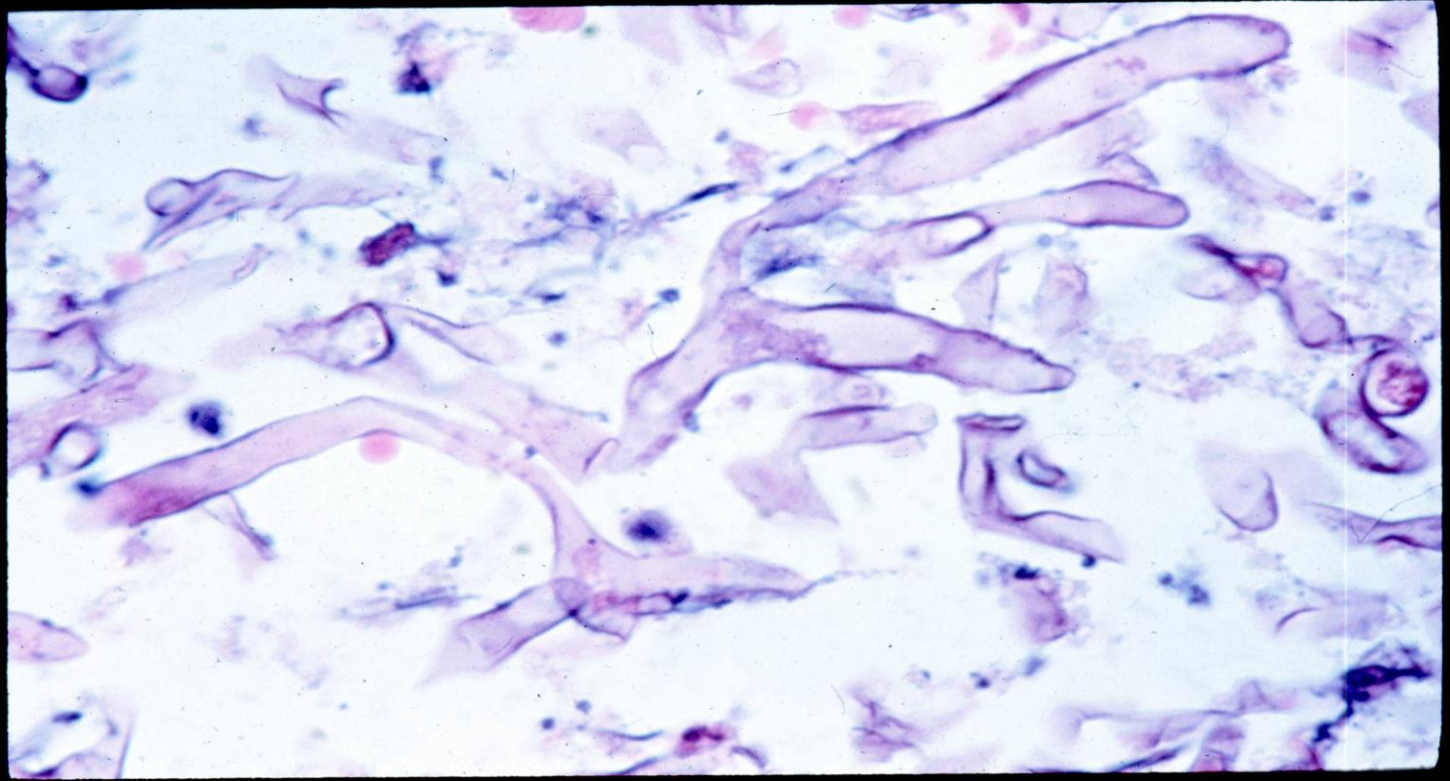
❖ Conidiophore which produces metulae which bear phialides that produce chains of microconidia.



- This fungus produces red pigment on culture.



Mucormycosis (Zygomycosis) (Phycomycosis)

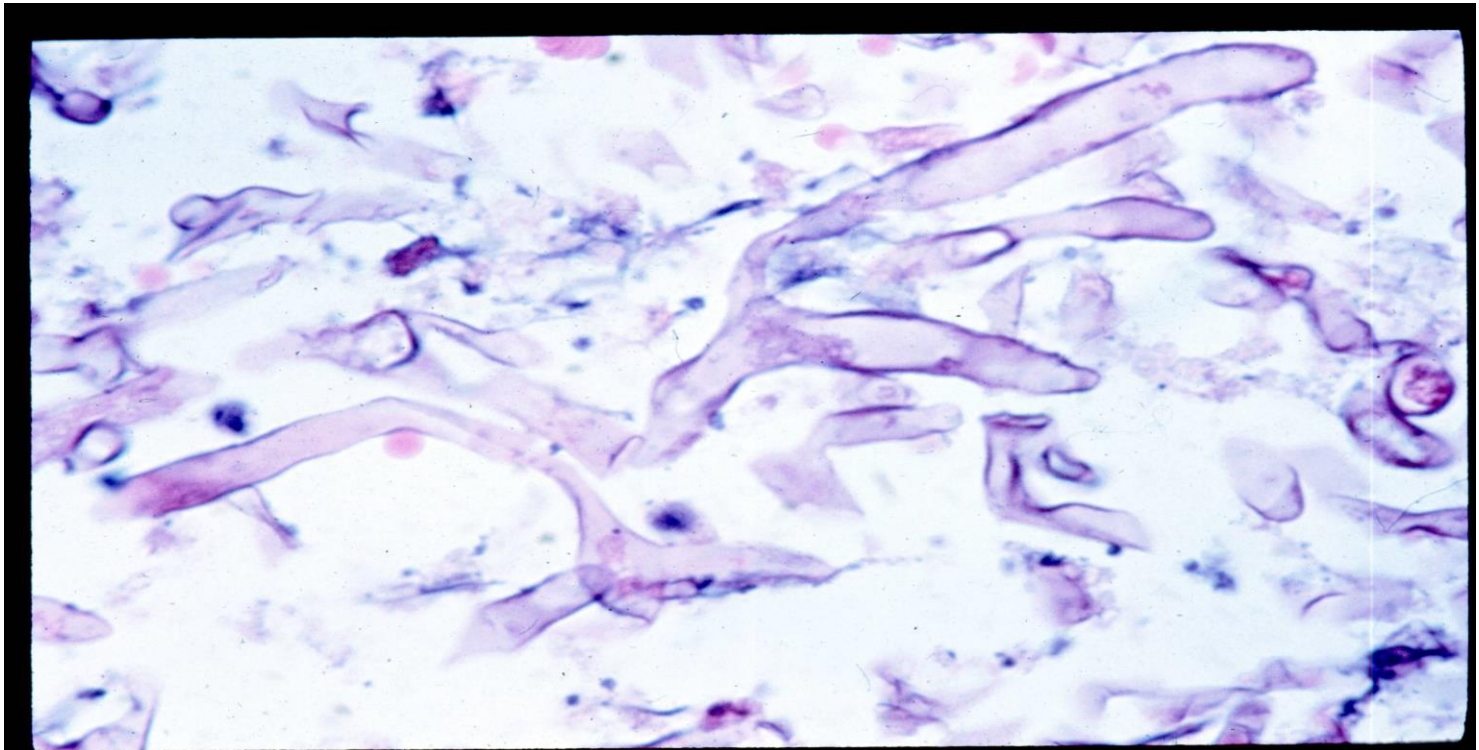


Causative fungus

- ❑ Mucormycosis is caused by the following fungi:
 - *Mucor*
 - *Rhizopus*
 - *Absidia*
- ❑ These fungi are saprophytic molds.
- ❑ These fungi produce sexual spores called zygospores. So, they are called zygomycetes.

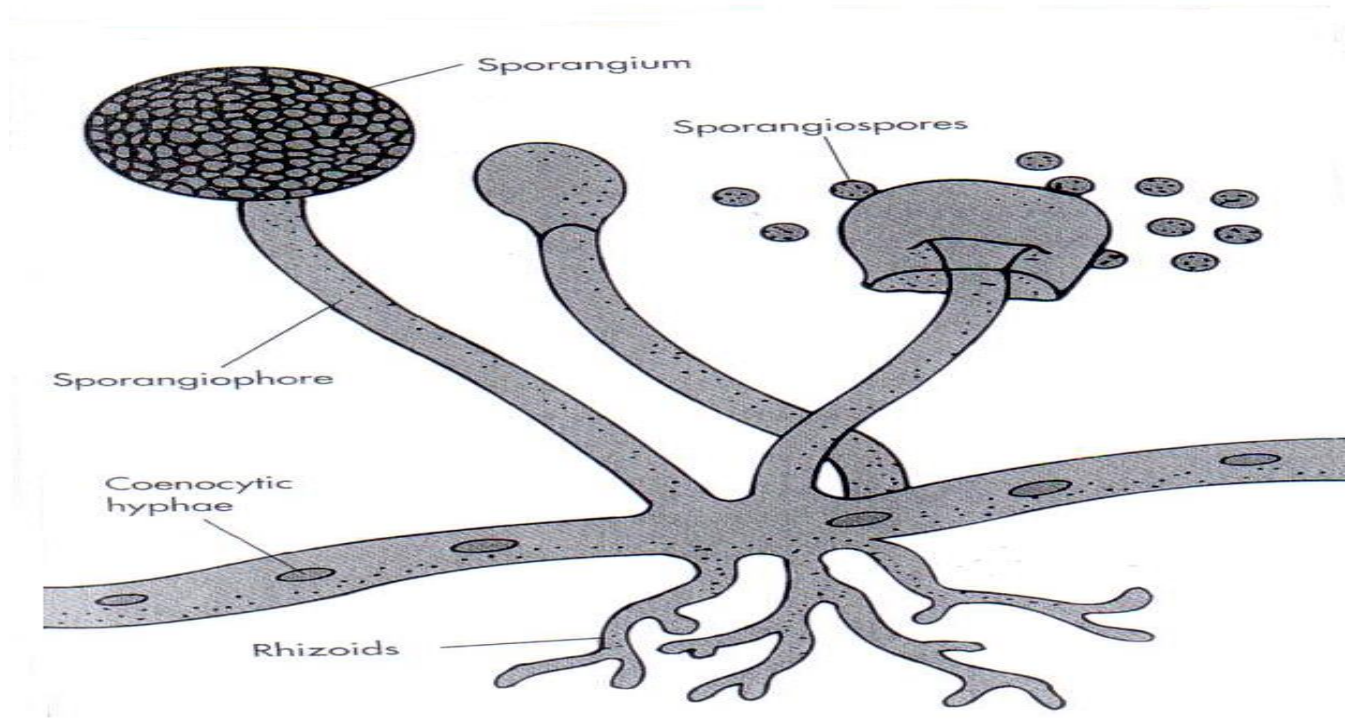
Morphology

- These fungi are molds.
- They are formed of hyphae which are: wide, aseptate (coenocytic), irregular in width.



- Hyphae produce asexual spores called sporangiospores which are formed inside a sac called sporangium.

Sporangiospores & sporangium



Pathogenesis

- ❑ *Mucor* and *Rhizopus* produce disease in the following patients:
 - 1) Patients with diabetic ketoacidosis.
 - 2) Patients with leukemia or lymphoma.
 - 3) Patients with Severe burns.
 - 4) Patients under corticosteroids.
- ❑ Hyphae invade the blood vessels causing thrombosis followed by ischemia and necrosis.

Diseases

❑ Rhinocerebral mucormycosis.

* Starts in the nasal mucosa then invades the orbit, sinuses and brain.

❑ Pulmonary mucormycosis.

❑ Contaminated wound dressing.

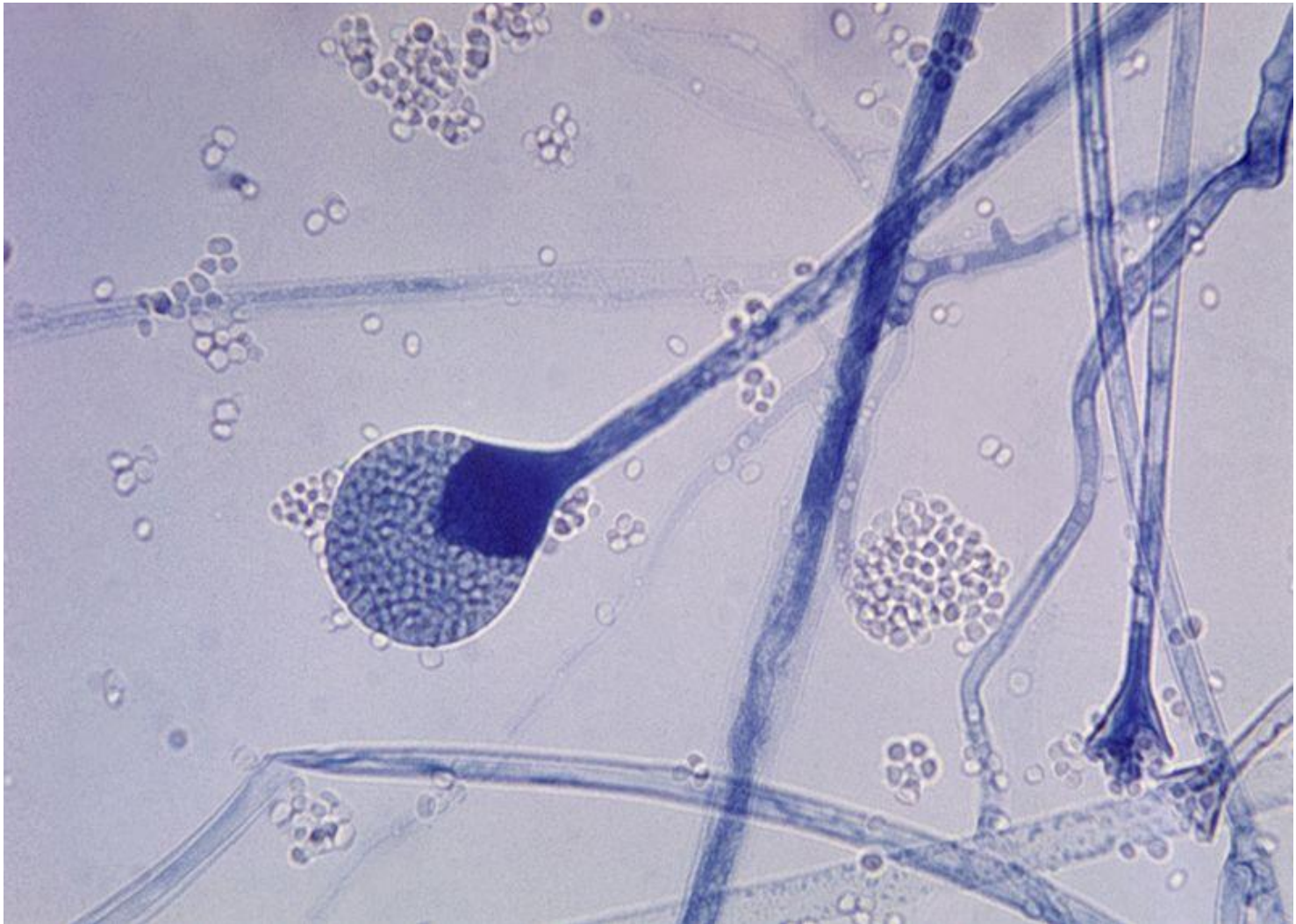
Rhinocerebral mucormycosis



Laboratory diagnosis

- ❑ **Specimen**: biopsy from the lesion.
- ❑ **Direct microscopic examination**: reveal hyphae which are wide, aseptate and with irregular width.
- ❑ **Culture**:
 - On SDA with no cycloheximide and incubated at the room temperature.
 - **Identification of the fungus is done by:**
 - ✓ **Colonial morphology**: cottony colonies
 - ✓ **Slide culture**: to see **the sporangiospores inside sporangium.**

Slide culture for mucormycosis



Does culture alone diagnose mucormycosis?



- ❑ No, because fungi causing mucormycosis are present every where and may arise in culture as contaminants.
- ❑ So, to confirm the diagnosis of mucormycosis:
 - Direct microscopic examination should reveal hyphae which are wide, aseptate and with irregular width.
 - Repeated isolation of the fungus from the same patient.

Treatment

- ❑ Amphotericin B + surgery



BEST Regards