Medical mycology

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Mycology

-Mycology - the study of fungi

-Fungi - includes molds and yeasts.

-Molds - exhibit filamentous type of growth.

-Yeasts - exhibit pasty or mucoid form of fungal growth.

- 50,000 species

- Fungi stain gram positive, and require oxygen to survive.

- Fungi are eukaryotic. (Bacteria are prokaryotes)

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Mycology

-Fungi are diverse groups of saprophytic and parasitic eukaryotic organisms.

-Human fungal diseases (mycoses) are classified by the location where the infection occurs.

-Some fungi secrete a variety of metabolic products that are highly toxic when ingested, thus fungi can produce poisonings as well as infections.

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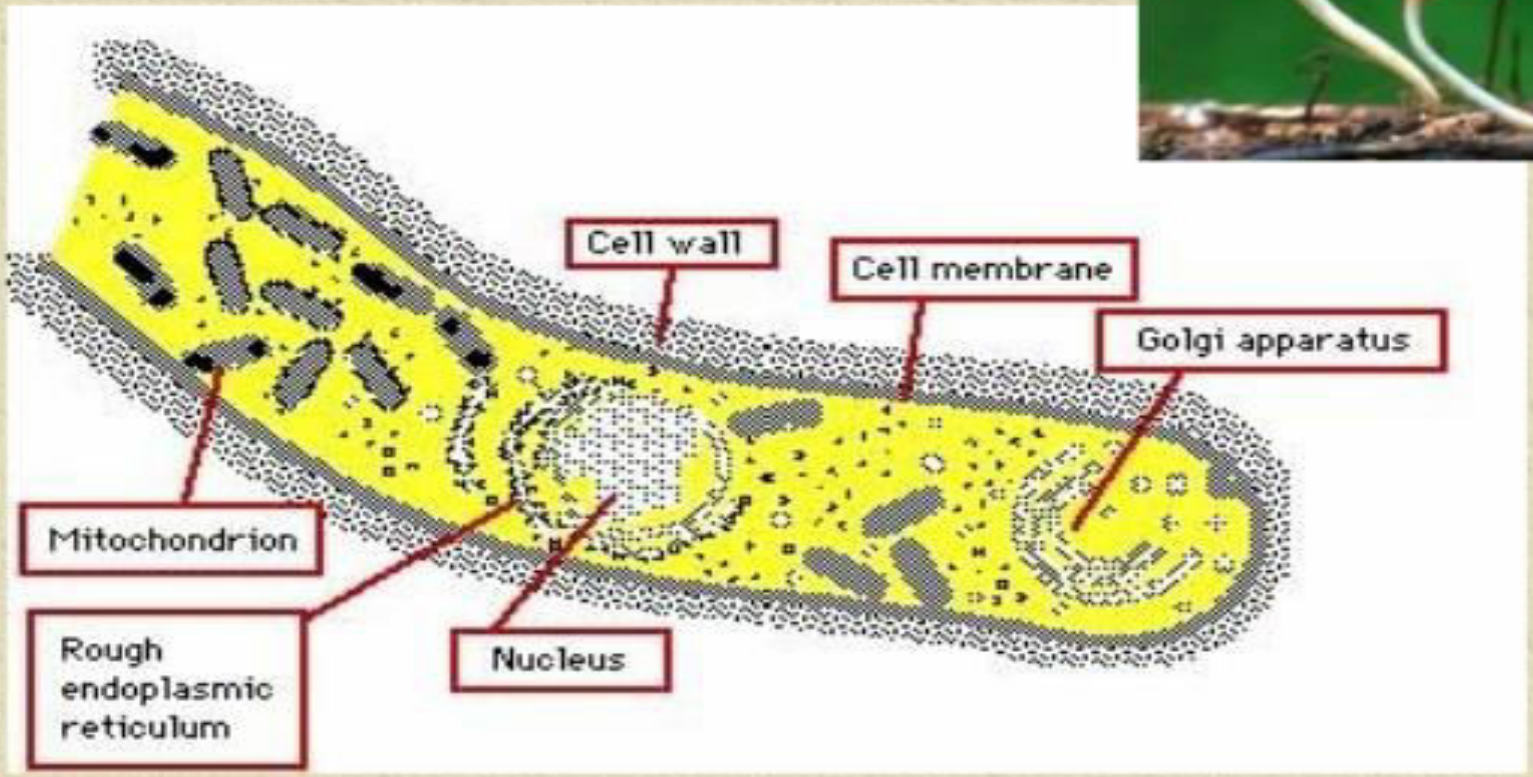
Structure of fungi



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General structure of fungi

Fungi are eukaryotic cells their structure corresponds essentially to that of the plant cell but it is devoid of chlorophyll. Fungal cells are composed of:

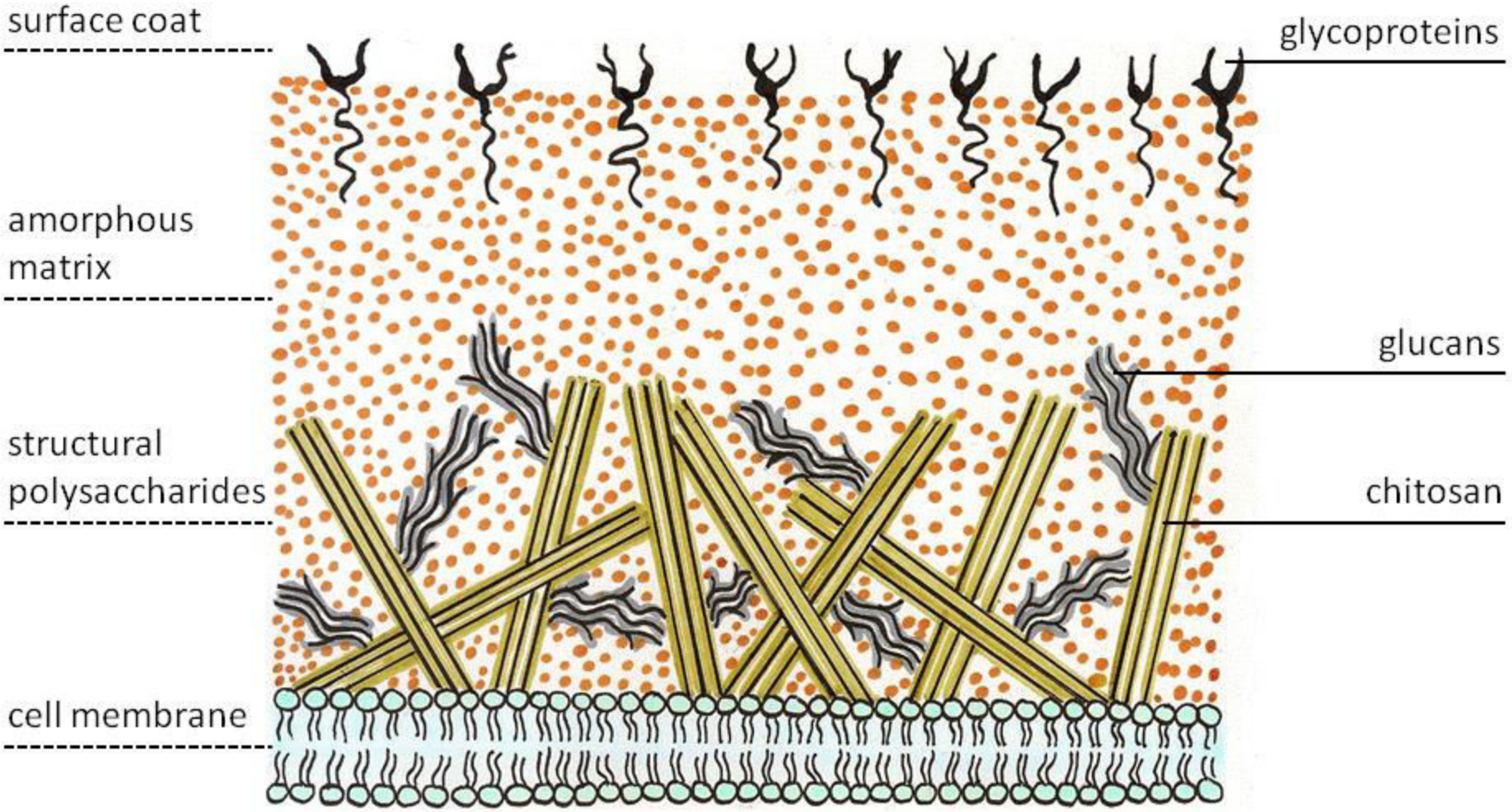
1-Cell wall:

*Chitin

*Cellulose.

* Polysaccharides as mannans and glucans.

* Glycoproteins



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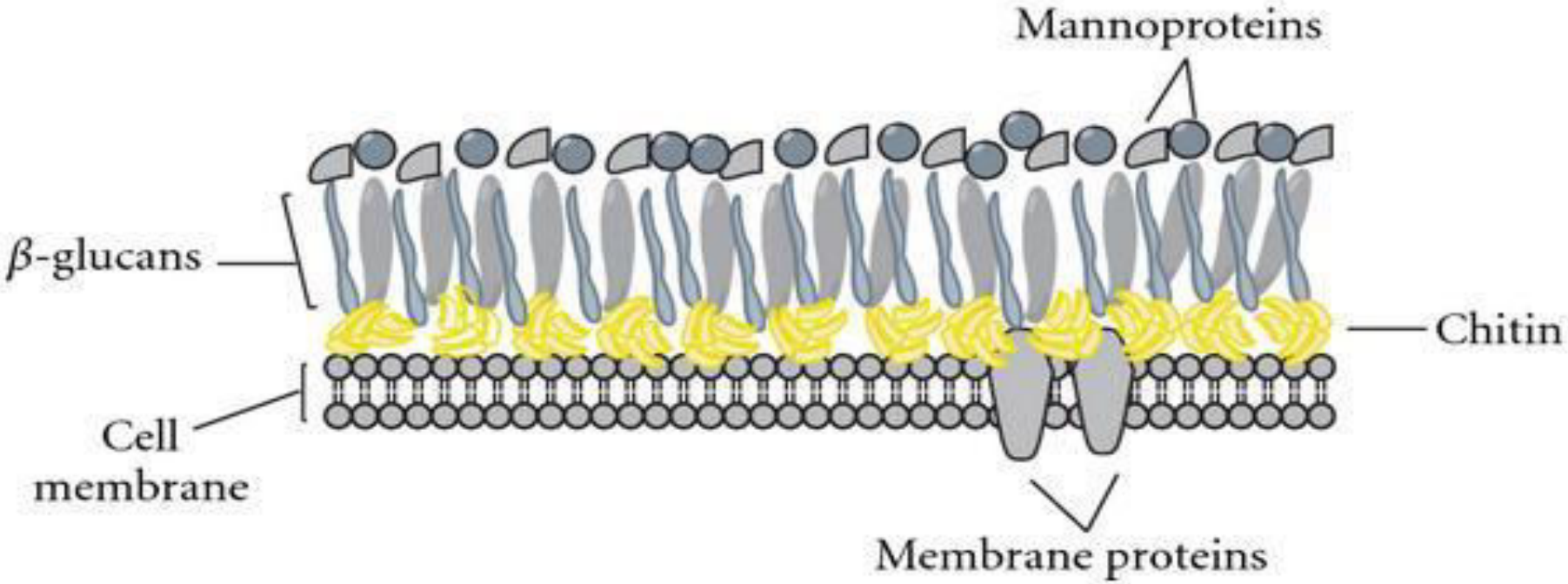

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Function of cell wall

Functions:

* It maintains the shape of the fungus.
* Osmotic strength.
* Protection.
* Antigenic determination.

The cytoplasmic membrane:

The fungal cytoplasmic membrane contains ergosterol rather than cholesterol present in mammalian membranes. Prokaryotes (except mycoplasma) do not contain sterols in their membranes.

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Cytosol

It is complex structure and contains many membrane bound organelles such as nucleus, mitochondria, vacuoles, glycogen and volutin granules, endoplasmic reticulum…etc.

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Nucleus

Fungi differ from the bacteria in containing a true nucleus which is demarcated from the cytoplasm of the cell by a nuclear membrane. The nucleus contains; as a rule; a nucleolus and always several chromosomes.

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Capsule

It helps adherence and clumping. Also it protects against phagocytosis.

Differences between bacteria and fungi.

	Bacteria	Fungi
Type	Prokaryotic	**Eukaryotic**
Size	0.2 to 8 um.	**4-15 um.**
Cell wall	*peptidoglycan.	***Cellulose. *polysaccharides as mannose and glycans. *Chitin.**
Cell membrane	No sterols	**Sterols**
Nucleus	*No nuclear membrane. *No nucleolus. *DNA has no histones. *One Chromosome	***Enclosed by nuclear membrane. *Nucleolus is present. *DNA has histones. *Many Chromosomes.**
Ribosomes	70S	**80S**
Division	Binary fission	**Budding**
Spores	**Endospores for survival, not reproduction**	**Spores for reproduction**

Classification of Fungi

I- According to morphology:

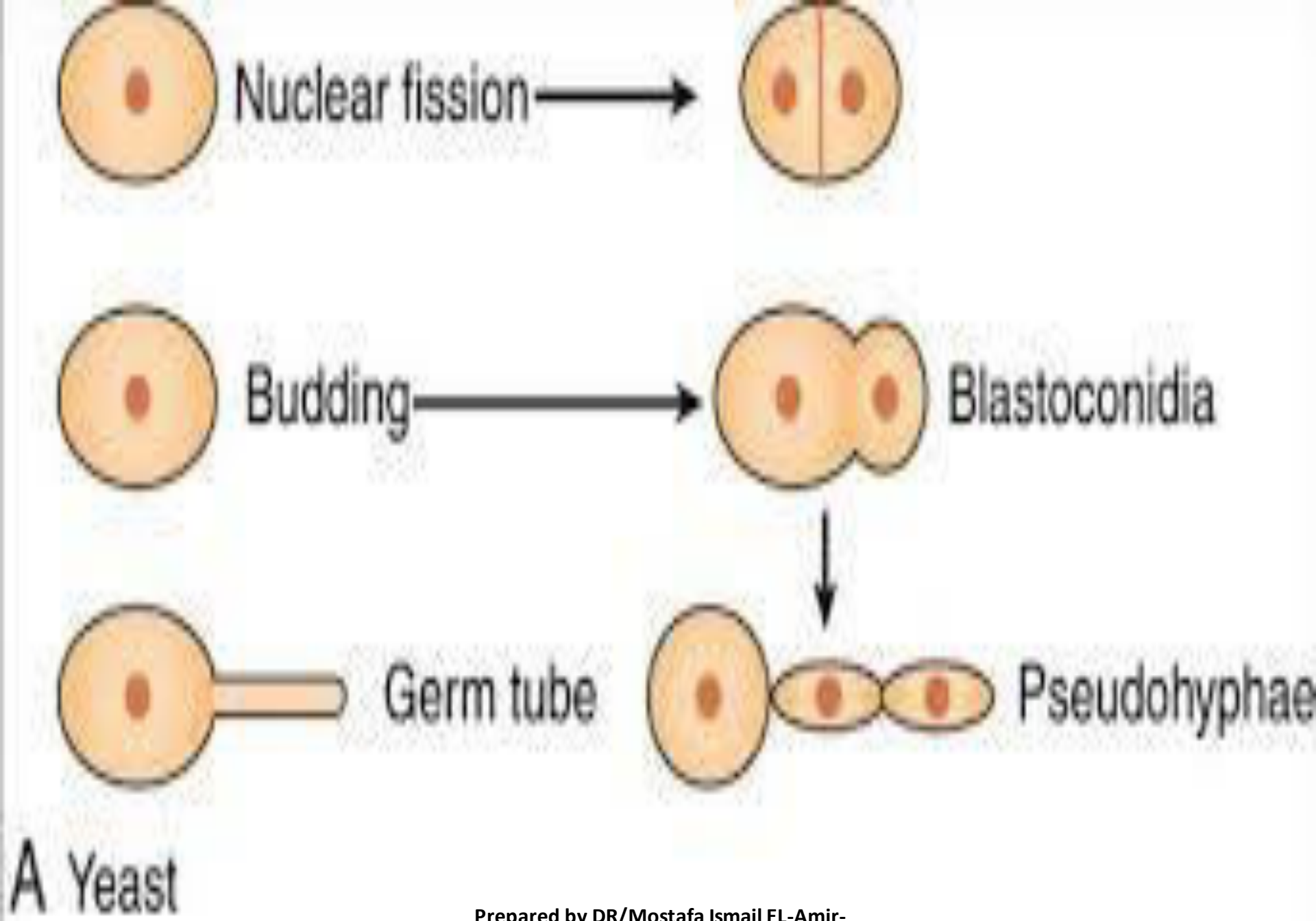
1) Yeasts:

Unicellular round or oval fungi 4-15 um in size.

Reproduce by budding (blastoconidia formation).

Sometimes buds do not separate off and extend to form pseudohyphae that show constrictions at the attachment sites. This form is known as yeast like fungi e.g. *C.albans.*

They form moist mucoid, creamy or waxy colonies.



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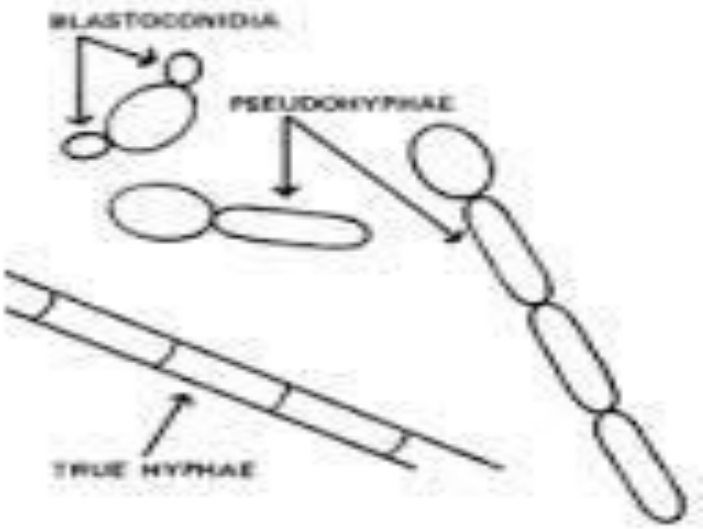

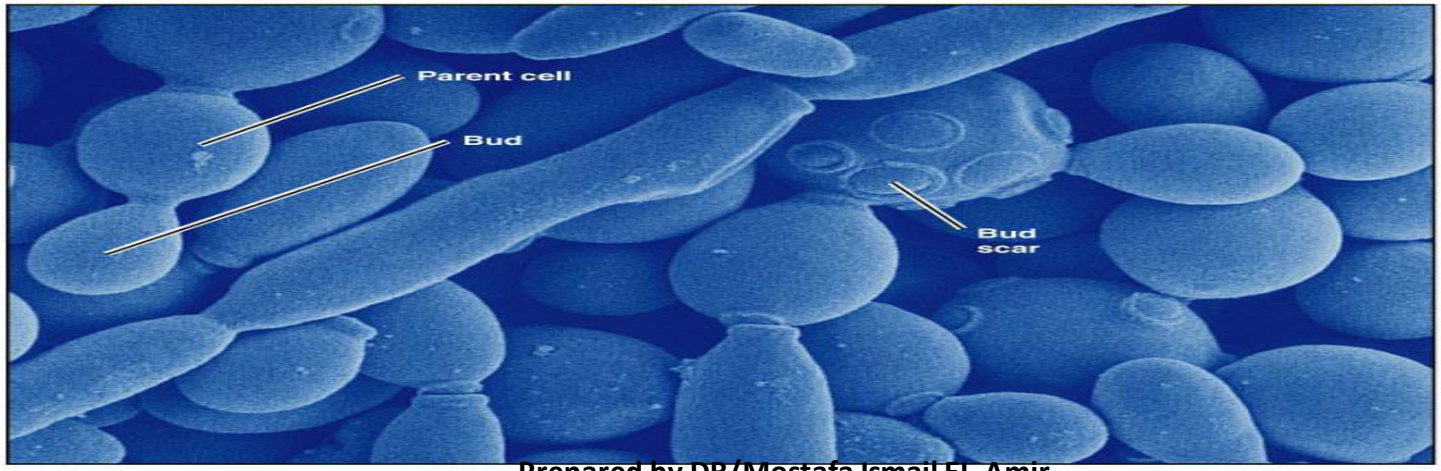

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Classification of Fungi

I- According to morphology:

2) Moulds:

Multicellular filamentous, colonies consisting of branching tubular structures called hyphae. Collection of hyphae called mycelium.

Hyphae may be septate (with transverse walls) or nonseptate

On laboratory media moulds form:

-**Vegetative hyphae** act like roots, penetrating the supporting medium and absorbing nutrients.

-**Aerial hyphae** project above the surface of the mycelium and bear the reproductive structures of the mould that often spread through the air. They give the mould colony a characteristic velvety like appearance.

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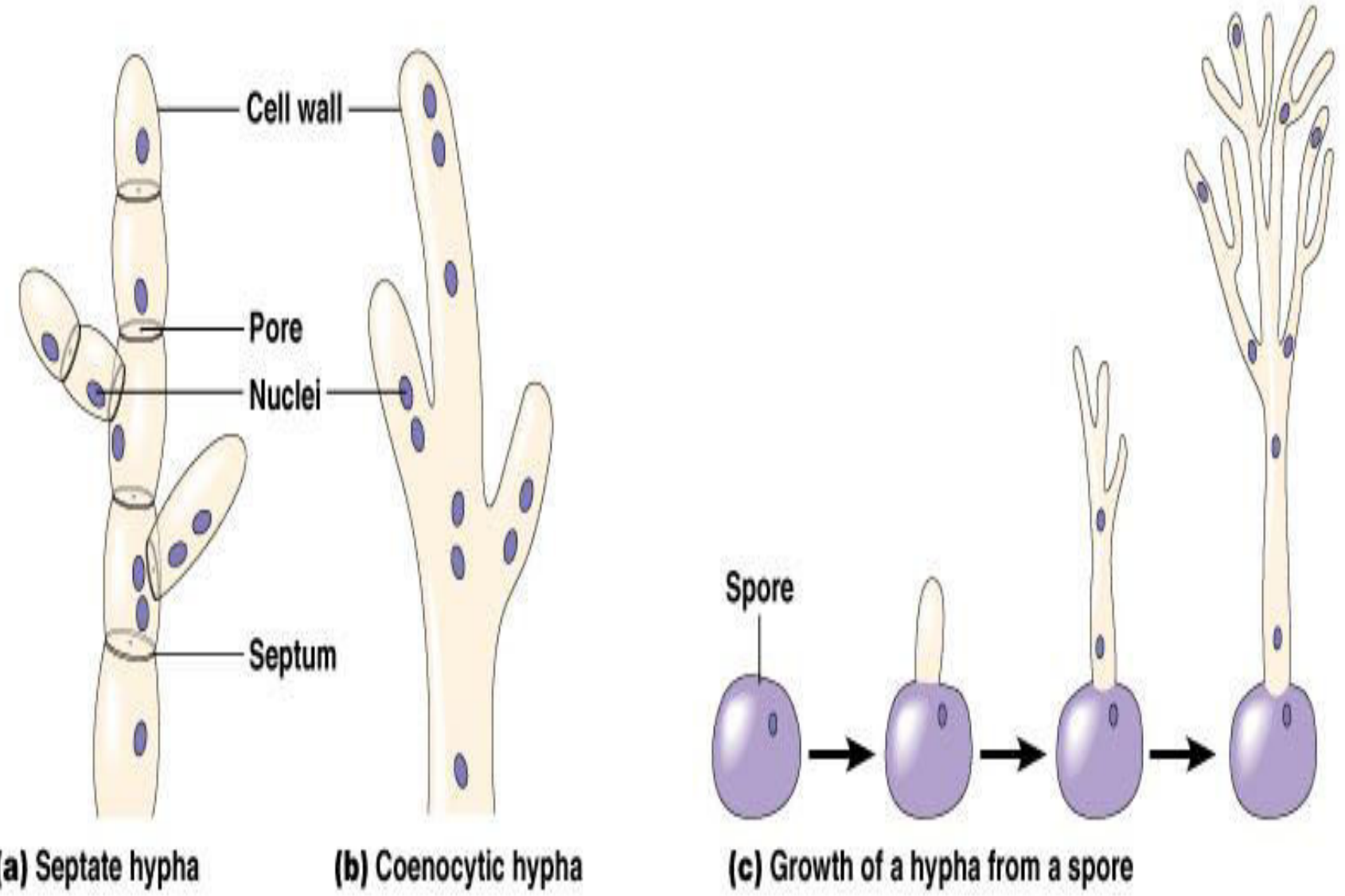

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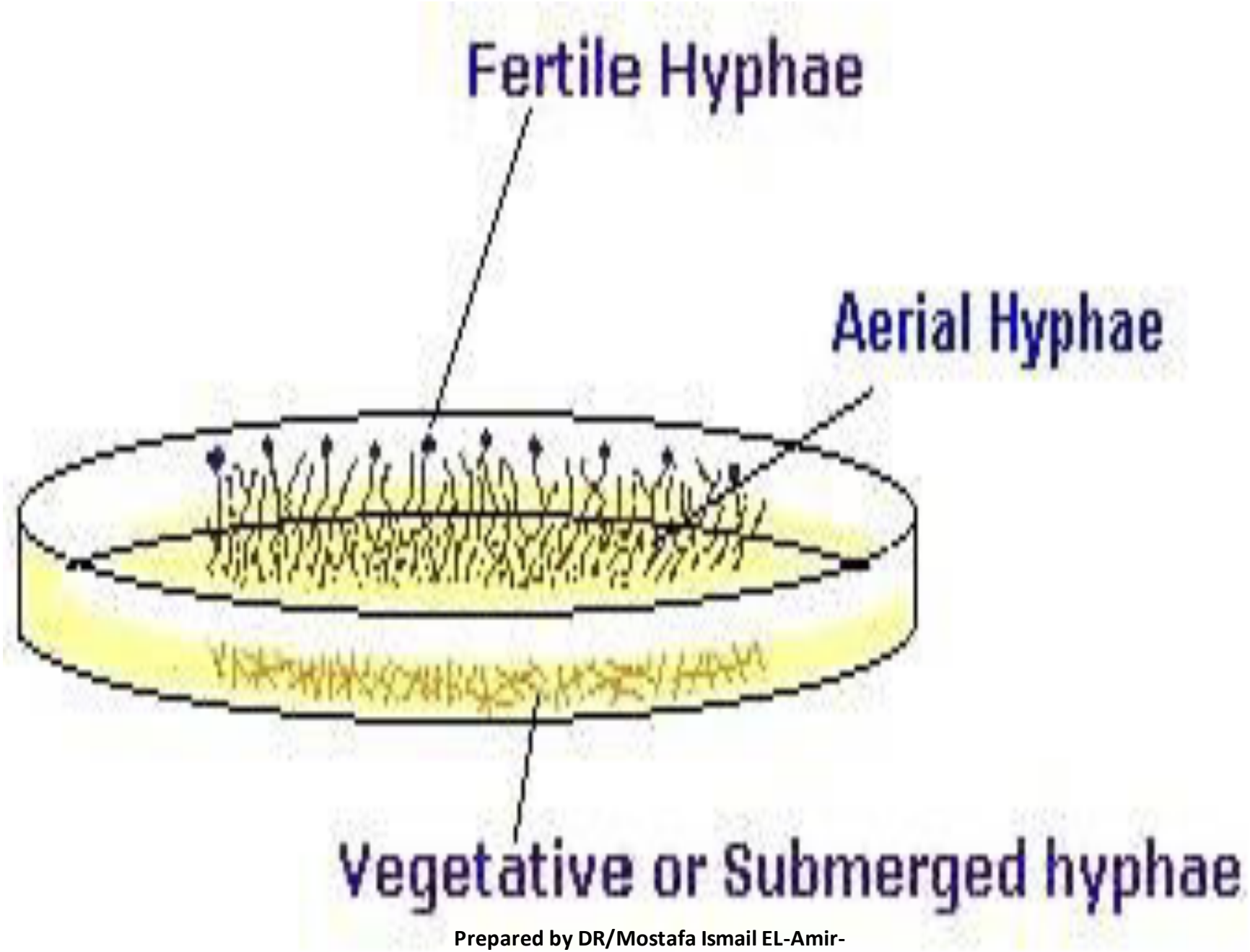

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Classification of Fungi

I- According to morphology:

3) Dimorphic Fungi:

Grow as moulds at environmental temperatures (25°C), forming reproductive spores that if inhaled by a host grow as yeasts at body temperature (37°C).

Staining of tissues shows the yeast forms and cultures at 25°C show moulds with characteristic colony appearance and microscopic morphology.

Classification of Fungi

II- According to mode of habitat:

Fungi are described according to their habitat and way of living as:

1) Saprophytic:

Saprophytic fungi live on inanimate material; in the soil, in water, in dust, in the air, on clothes or on dead bodies.

2) Parasitic:

Parasitic fungi live on or in the body of living organisms. They are subdivided into:

a- true pathogenic fungi:

Those cause disease in man or animals or plants.

b- Commensal fungi (opportunistic fungal pathogens):

Live on or in the body without exerting any harmful effect but many cause disease if the body resistance is lowered by any means (I.e. opportunistic infection).

III- According to the level of tissue involvement of fungal infections:

Type	Disease	Causative Organism	Morphology
1.Superficial Mycoses	Pityriasis versicolor	Malassezia furfur	Mould
2.Cutaneous Mycoses	Dermatophytoses	Dermatophytes (Microsporum, Trichophyton Epidermophyton)	Moulds
3.Subcutaneous Mycoses	Mycetoma	-Madurella mycetomatis.	Moulds
4.primary systemic (endemic) mycoses	Histoplasmosis. Blastomycosis. Paracoccidioido-mycosis. Coccidiomycosis.	-Histoplasma capsulatum. -Blastomyces dermatitidis. -Paracoccidioides brasiliensis. Coccidioides immitis	dimorphic
5.opportunistic mycoses	Cryptococcosis. Aspergillosis. Candidiasis.	Cryptococcus neoformans Aspergillus speies Candida albicans, candida sp.	Yeast Moulds Yeast like

Reproduction of fungi

I-Asexual Reproduction:

Asexual reproduction is the production of new individuals from a single parent. It does not involve the fusion of gametes. It occurs by one of two forms:

A-Vegetative reproduction.

B-Spore formation.

2-sexual Reproduction:

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I-Asexual Reproduction

A- Vegetative Reproduction:

It is the type of reproduction which involves the body of the fungus. It occurs by the following methods.

1- Fragmentation:

In this process, the mycelium breaks into two or more similar fragments either accidentally or due to some external force (as when a part of the colony is sub cultured). Each fragment grows into a new mycelium.

2- Budding:

The parent cell produces one or more projections called buds, which later develop necessary structures and detach to grow into new individuals. Budding is common in unicellular forms like yeast.

3- Fission:

In this process, the parent cell splits into two equal halves, each of which develops into a new individual. Fission is also common in yeast.

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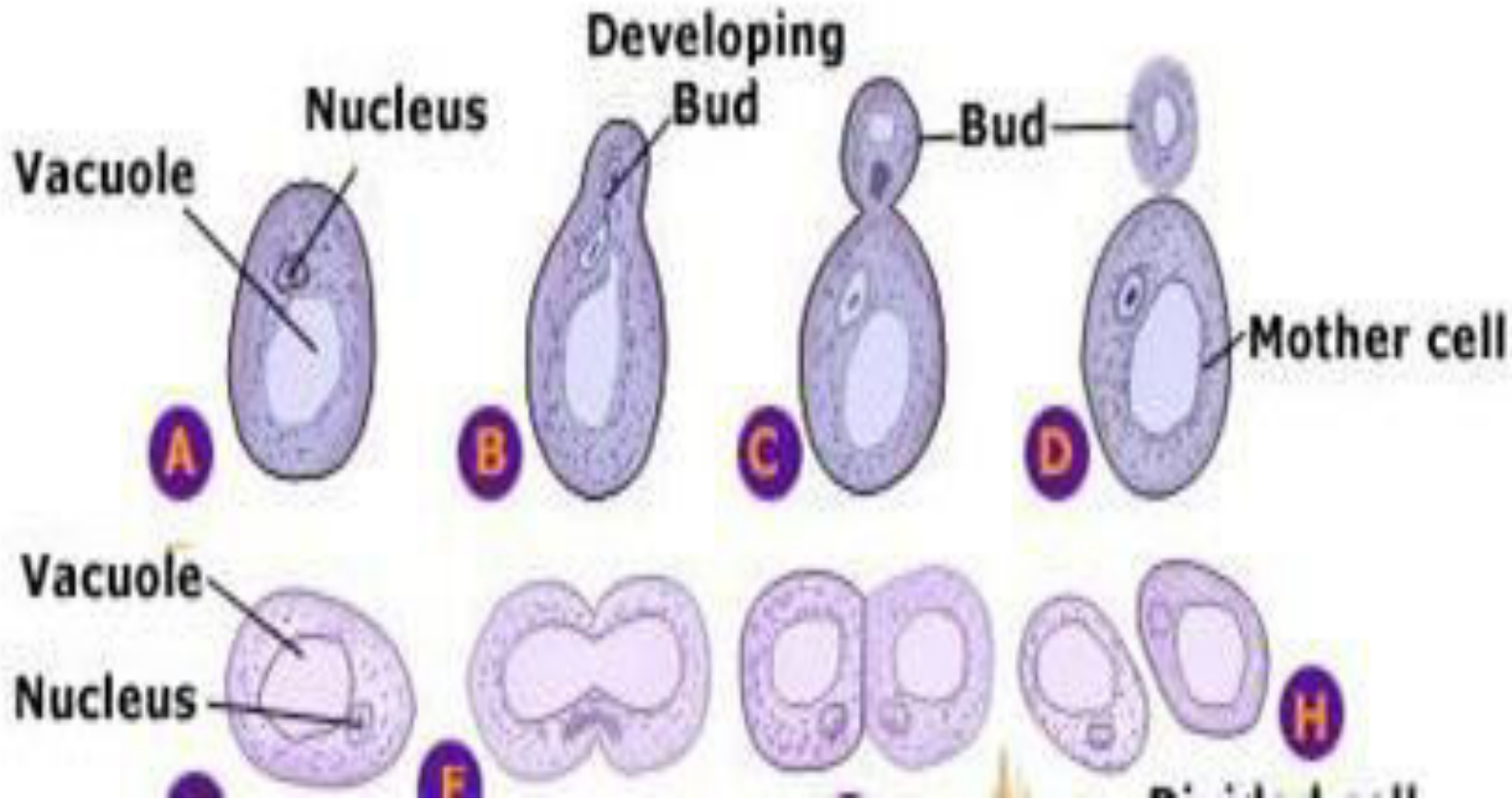
A- Vegetative Reproduction



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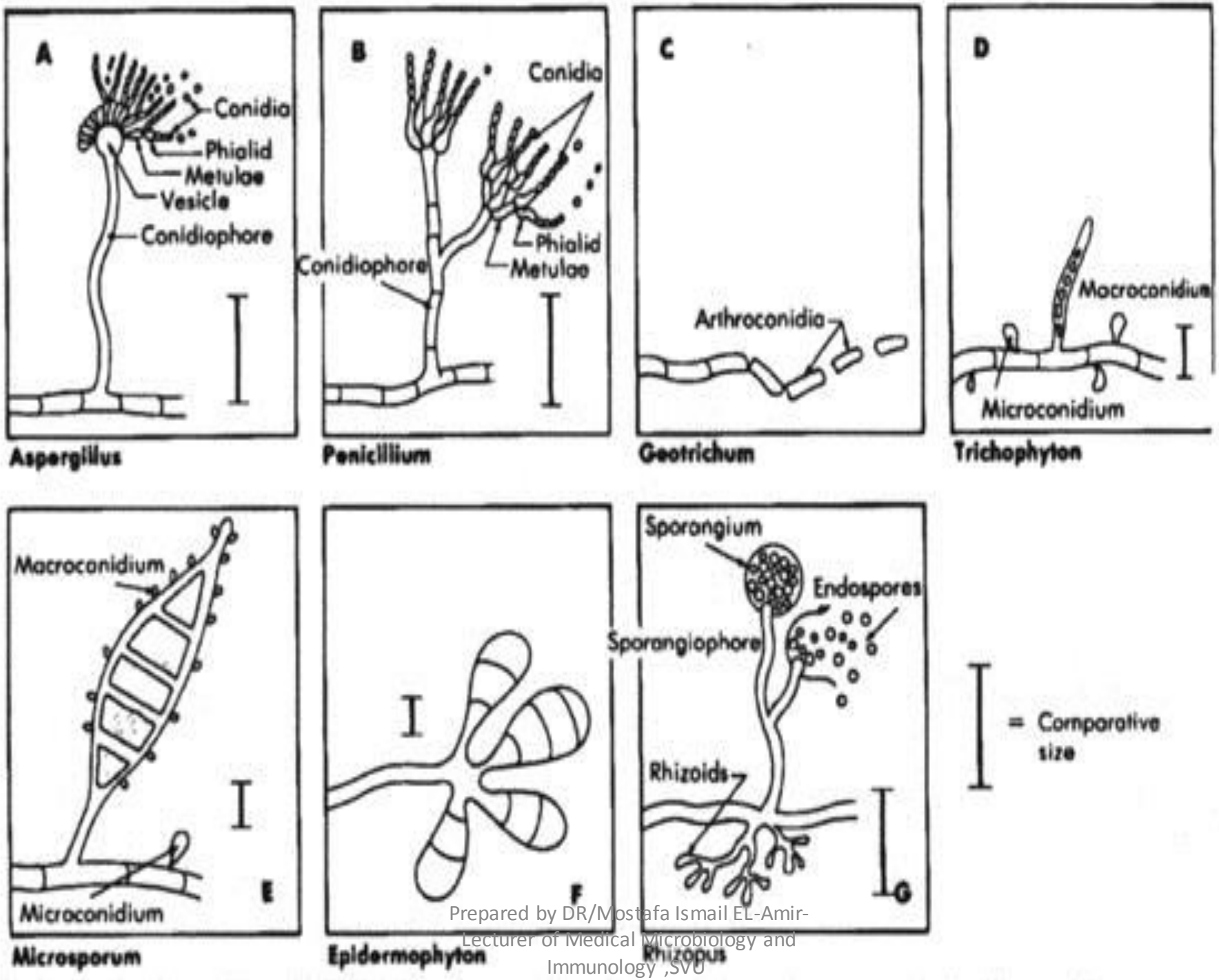
I-Asexual Reproduction

B-Spore formation(Conidia):

It is the type of reproduction in which special reproductive structures called spores are formed. These asexual spores always result from mitosis and hence are described as mitospores.

These spores are easily disseminated by air and they are more resistant to unfavorable environmental conditions.

Most medically important fungi reproduce asexually and are known as imperfect fungi.



Spore formation(Conidia)

That may be unicellular i.e. micro conidia or multicellular i.e. macro conidia.

They are formed by fragmentation or disarticulation of existing hyphae.

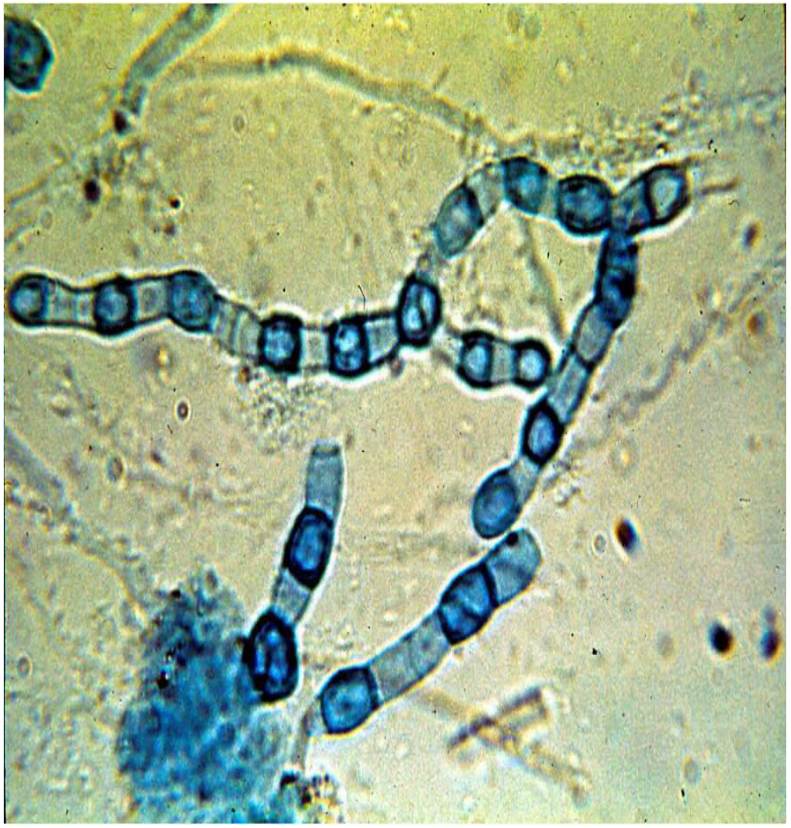
1- Arthrospores :

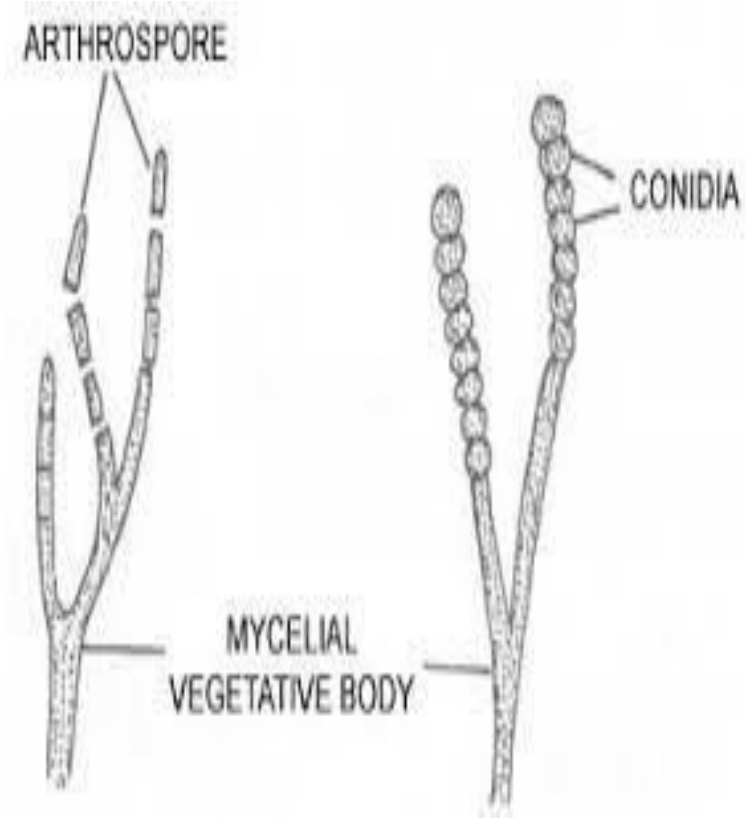
These are spore like structures formed by the breaking up of hypha cells.

2- Chlamydospores (chlamydoconidia):

These are thick walled resting spores which arise directly from hyphal cells. They store reserve food.

Spore formation(Conidia)





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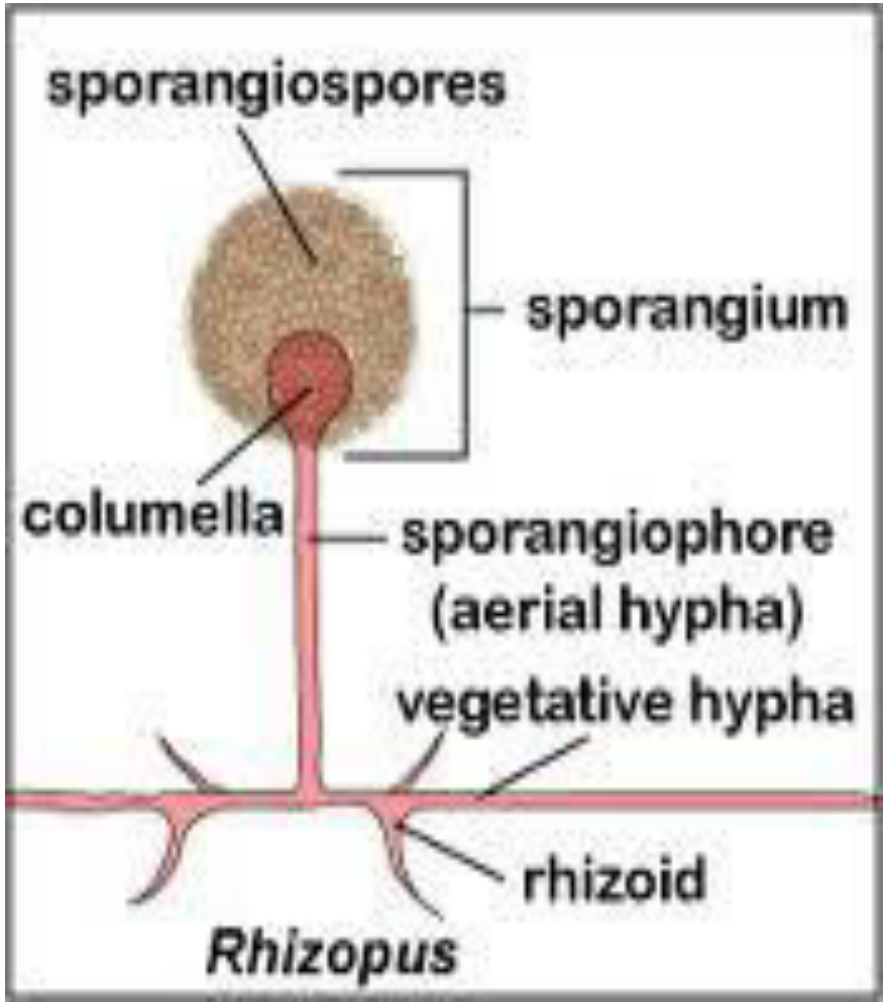
Spore formation(Conidia)

3-Sporagiospores:

The hyphal apex or cell swells. Within the sac, called a sporangium, spores are formed. Sporangiospores are non-motile and dispersed by wind.

Sporangiospores



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2-sexual Reproduction

A- Zygospores

Formed by the union of 2 cells forming spore with thick wall.

B-Ascospores

Reproduction occur in large sac called ascus containing spores.

C-Basidospores

Formed externally from the tip of basidium.

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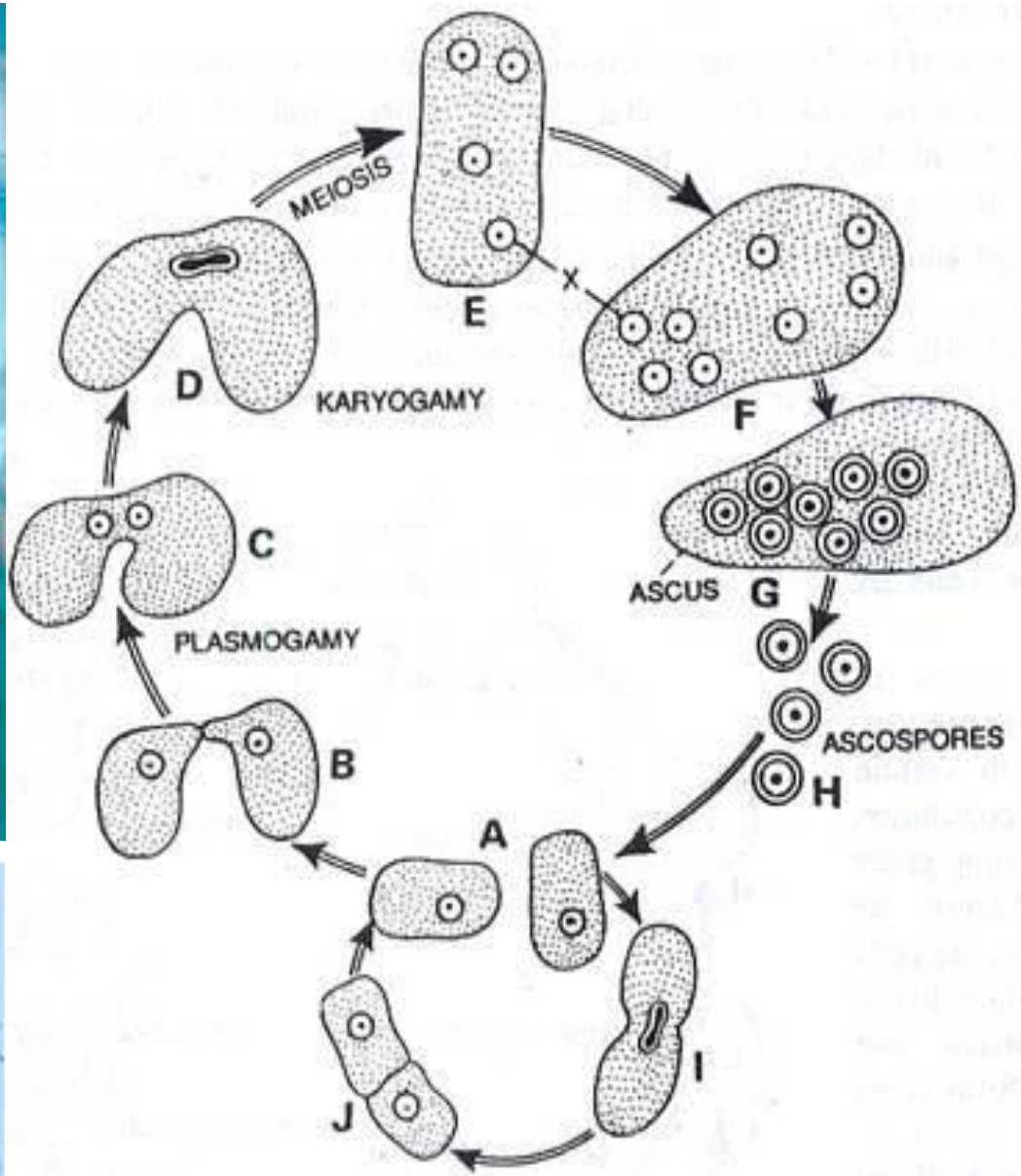

Fig. 12.21. Haplobiontic type of life-cycle in *Schizosaccharomyces octosporus*.

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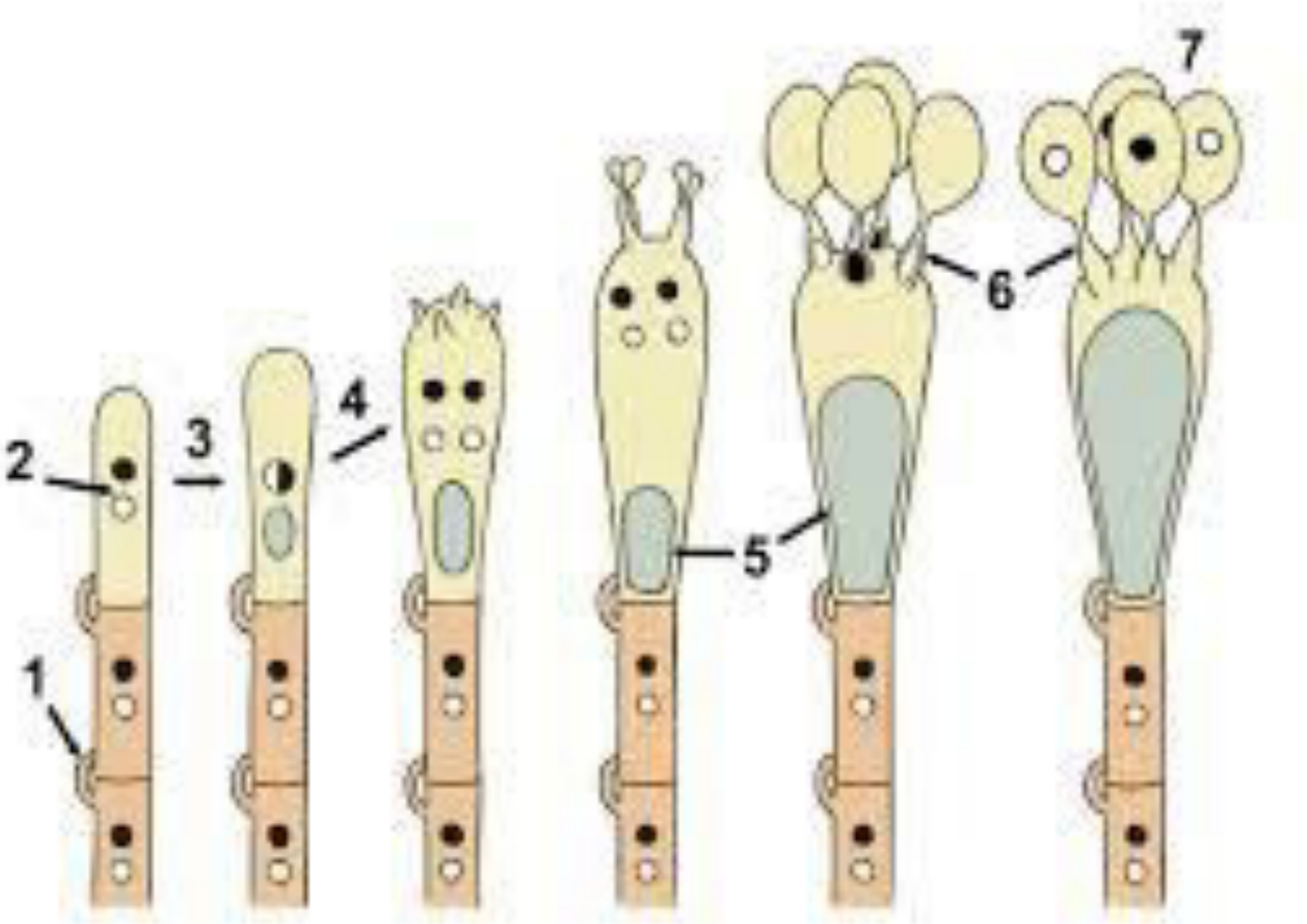

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Products of fungi(Mycotoxins)

Definition:

Mycotoxins are toxic metabolites produced by fungi. One species may produce many different mycotoxins and/or the same mycotoxin may produced by another species.

These products cause mycotoxicosis following ingestion, inhalation or direct contact.

NB: mycotoxicocis is the ingestion of food contaminated with toxin producing fungus.

Mycetismus: is the ingestion of food containing preformed toxins.

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Mycotoxins

Factors affecting the presenting symptoms of mycotoxicosis:

1-Type of mycotoxin.

2-Amount of mycotoxin.

3-Duration of exposure.

4-Route of exposure.

5-Age, sex and general health state of exposed individual.

6-Other factors e.g. malnutrition, alcohol intake ….etc.

Mycotoxins:

Major groups:

A- Aflatoxins:

Produced by: Aspergillus species such as *A. flavus* .

Aflatoxin B1 is the most toxic. Toxicity is either acute or chronic.

Acute aflatoxicosis is presented in the form of acute hepatitis that may be fatal.

Chronic aflatoxicosis is associated with increased risk of hepatocellular carcinoma due to activation of oncogenes and mutation of tumor suppressor gene P53 . Co infection with HBV increases the risk.

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Mycotoxins:

B-Ergot Alkaloids:

They are compounds produced as a toxic mixture of alkaloids.

The ingestion --- commonly in the form of bread produced from contaminated flour, cause ergotism.

There are two forms of ergotism gangrenous affecting blood supply to extremities and convulsive that affects the central nervous system.

Diagnosis and Identification of Fungi

Diagnosis of fungal infections is based on:

(1) Clinical diagnosis:

Detailed history and careful patient examination are usually required.

(2) Laboratory diagnosis:

Methods of laboratory diagnosis of fungal infections include:

I- Microscopic examination of patient samples.

II- Detection of fungal antigens in patient samples.

III- Culture and isolation of fungal pathogens.

IV- Indirect methods based on the host immune response.

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Diagnosis and Identification of Fungi

<u>I- Microscopic examination of patient samples:</u>

Is the first-line procedure in detecting the presence of fungal elements and it is perhaps the most rapid, useful, and cost-effective means of diagnosing fungal infections.

<u>A- Wet mounts:</u> Include

-Potassium hydroxide (KOH) mounts 10-20%:

breaks down the human cells and dissolves keratin of skin scrapings, hairs and nail clippings although the fungus is unaffected.

-India ink mounts of CSF sediment:

demonstrate the encapsulated yeast; *Cryptococcus neoformans* in C.S.F.

-Calcofluor white mounts:

It binds to the chitin in the fungal cell wall and fluorescence blue-white or green, thus providing a rapid and sensitive means of detecting fungi in clinical material.

-Lactophenol cotton blue mounts:

Commonly used to detect mycelia and spores specially in slide culture technique.

Diagnosis and Identification of Fungi

B- Stained smears:

Different stains can be used e.g.

Gram stain: most fungi are gram positive.

Giemsa stain.

C- Histopathological examination:

Wright's stain of blood or bone marrow smears to detect *Histoplasma capsulatmn*.

Hematoxylin and eosin rarely used as they may not stain some fungal cells.

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Diagnosis and Identification of Fungi

II- Detection of fungal antigens in patient samples:

Can be performed by many methods e.g.

-Latex agglutination as in Cryptococcus meningitis.

-ELISA.

-Direct immuonoflurescence

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Diagnosis and Identification of Fungi

III- Culture and isolation of fungal pathogens:

All methods of direct examination are less sensitive than culture, and negative results of direct examination of a clinical specimen never rule out a fungal infection.

Cultures are usually done on Sabouraud's dextrose agar medium, this is a traditional agar that encourages the growth of fungi and discourages bacterial growth as it has (5.6) and added (chloramphenicol and gentamicin). (actidione) is added to prevent saprophytic fungi.

Specimens are inoculated in two sets; one is incubated at room temperature (25°c) and the other is incubated at body temperature (37°c) to reveal dimorphism. Fungi grow slowly so cultures are incubated for 1-2 weeks.

Diagnosis and Identification of Fungi

IV- Indirect methods based on the host immune response:

a- Skin tests: e.g. candidin test, histoplasmin test and skin tests for aspergillosis. They are used only to evaluate immunity of the patient and to construct an exposure index in epidemiological studies.

b- Serologic tests:

Tests to detect specific serum antibodies:

Most conventional serologic tests designed to detect specific serum antibodies (as CFT and latex agglutination test) are ineffective because of the following:

-Many patients who are at risk for fungal disease are not capable of mounting a specific antibody response to infection.

-Cross-reactions among different species.

-Presence of antibodies to common environmental or commensal fungi.

Superficial Mycoses

General Characteristics:

Affect outermost layer of skin and hair.

Generally cause no cellular response to the infection.

It has primarily cosmetic symptoms.

It includes pityriasis versicolor, and tinea nigra

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Pityriasis Versicolor (Tinea versicolor):

Etiology:

It is caused by *Malassezia furfur* which is not a member of dermatophytes but only infects the skin.

Clinical manifestations:

It is a fungal infection of the stratum corneum that manifests as hypo-or hyper pigmented skin patches, usually on the trunk of the body. (Color of patches varies with pigmentation of skin i.e. it appears as hypo pigmented in dark skin persons while appears as hyper pigmented in light skin persons).

These skin patches may be associated with itching.

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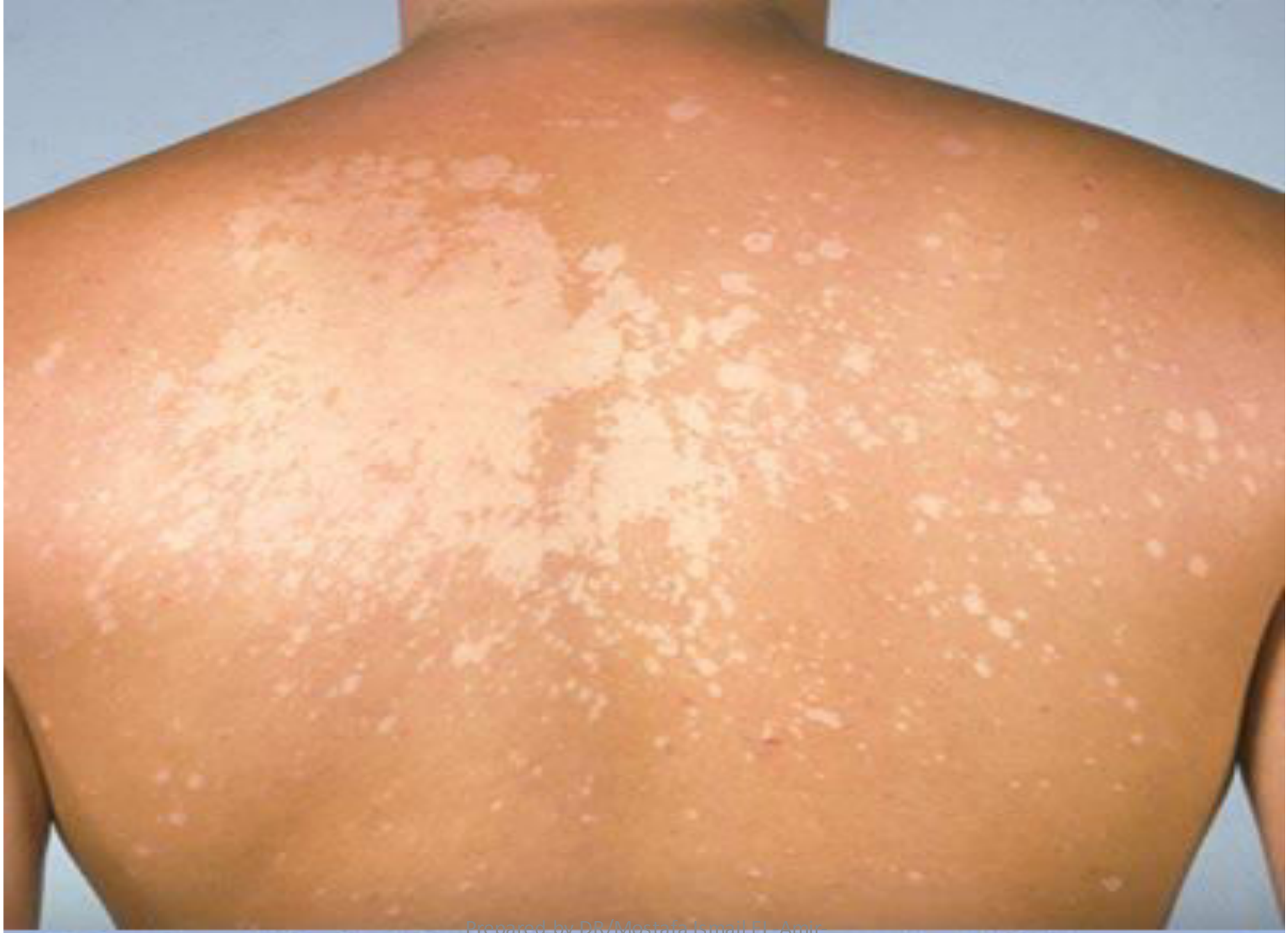

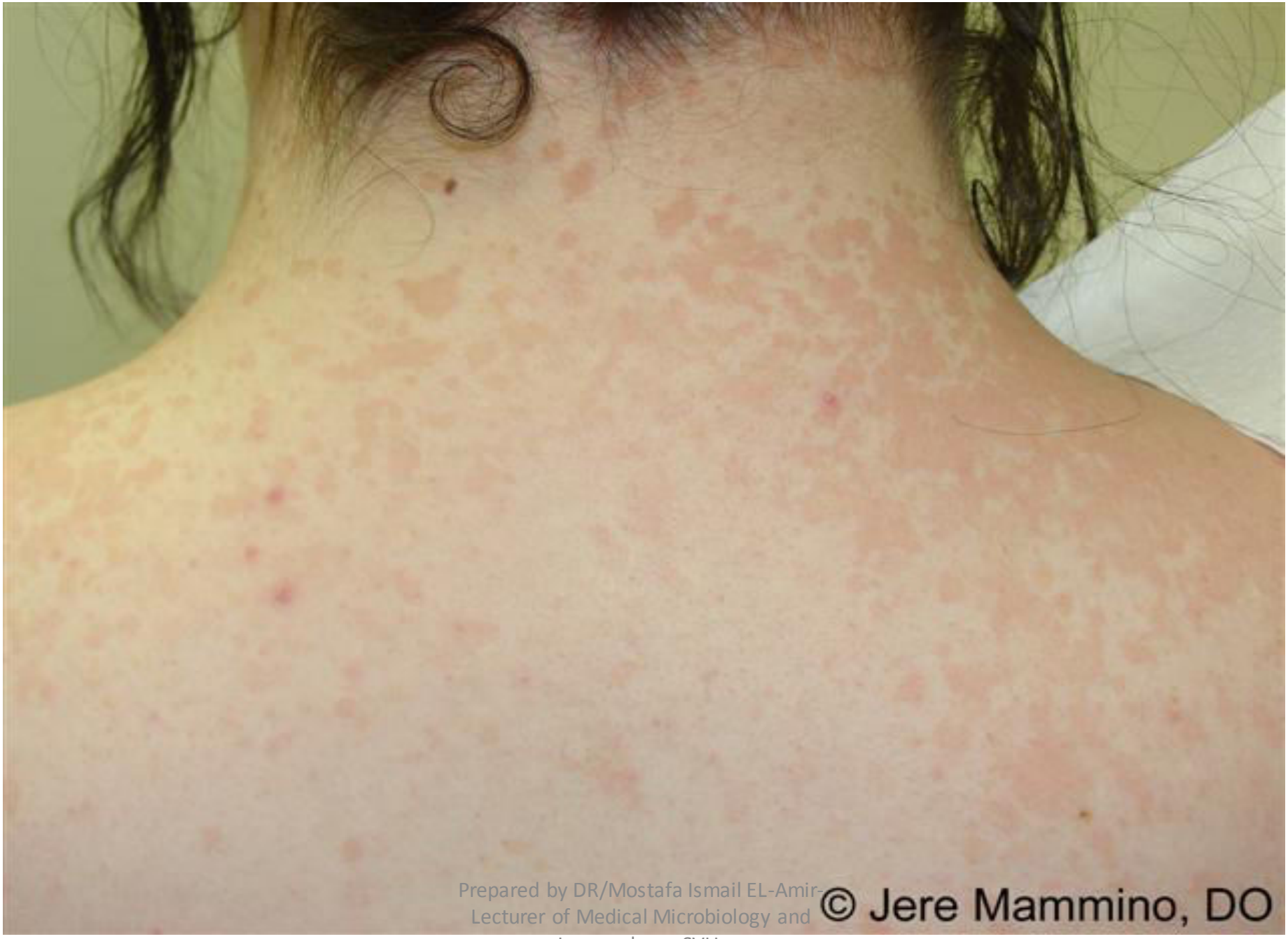
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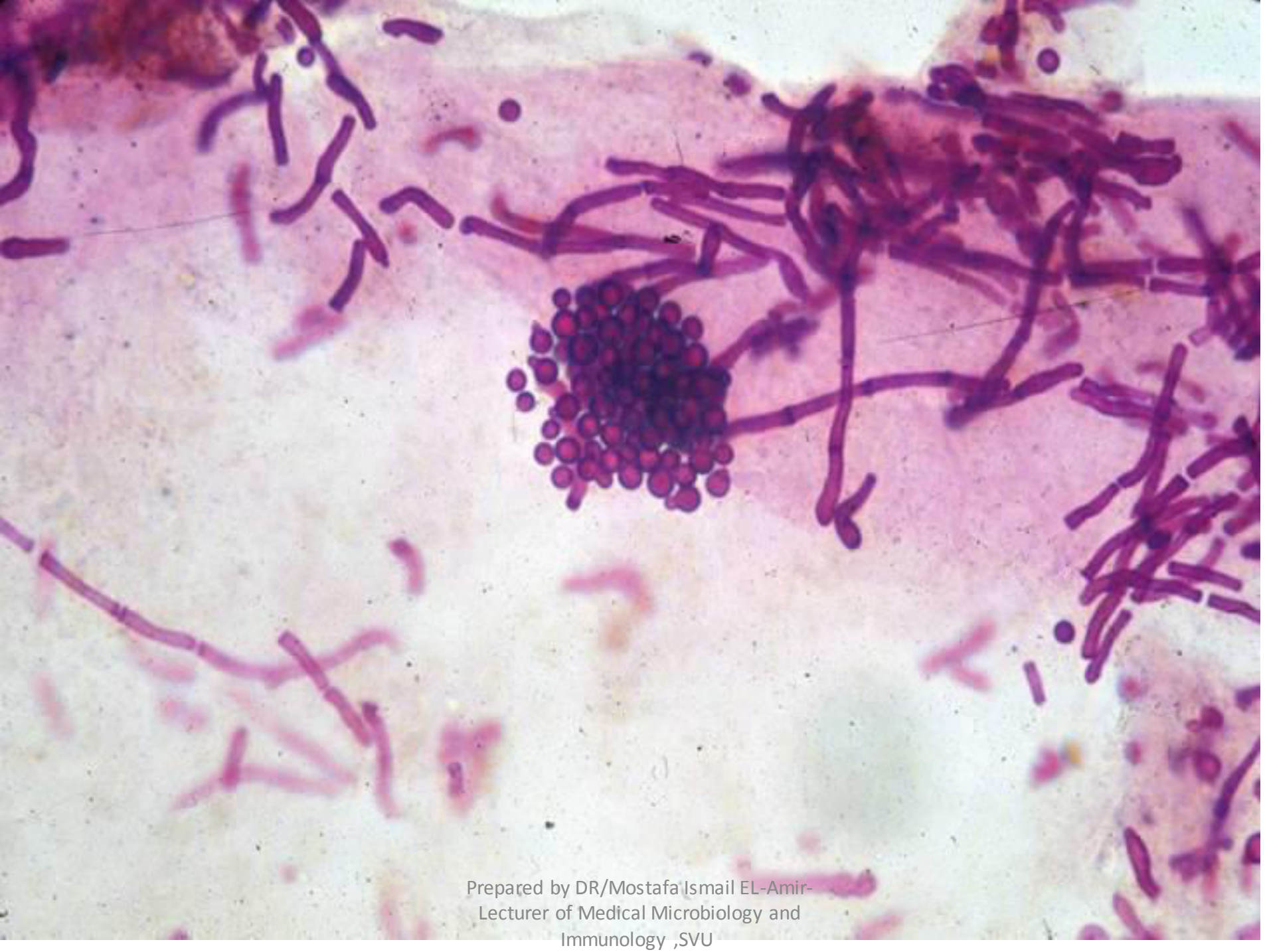
Pityriasis Versicolor (Tinea versicolor):

Laboratory diagnosis:

It is diagnosed by KOH mount of skin scales that show the fungus as short curved septate hyphae and yeast-like cells.



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II- Cutaneous Mycoses (dermatophytoses)

Involve the skin, hair, or nails.

Caused by any of the dermatophytes; a homogeneous group of filamentous fungi with three genera:

A- *Trichophylon:* infect skin, hair and nails and occasionally cause subcutaneous infections in immunocopromised individuals.

B- *Microsporum:* infect skin and hair but not nails.

C- *Epidermophyton floccosum:* infect skin and nails but not hair.

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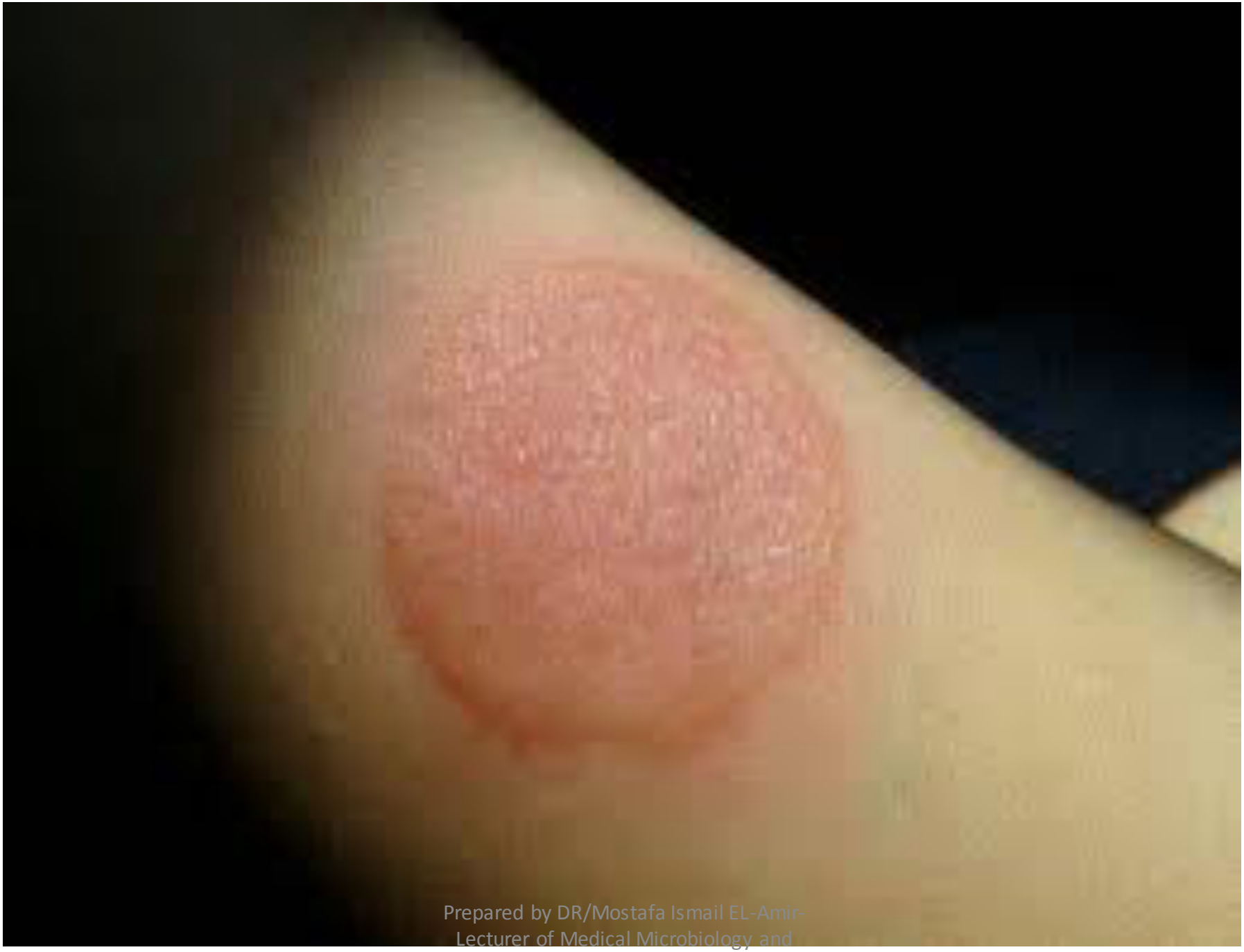
II- Cutaneous Mycoses (dermatophytoses)

Epidemiology:

Dermatophytes may acquired from animals by close contact,from soil,or from humans by close contact or via contaminated objects.

Clinical manifestations:

Dermatophytes affect the keratinized tissues and spread peripherally from initial foci to produce a ring like lesions. Hence the name ringworm or tinea (Tinea is the Latin name for a growing worm).



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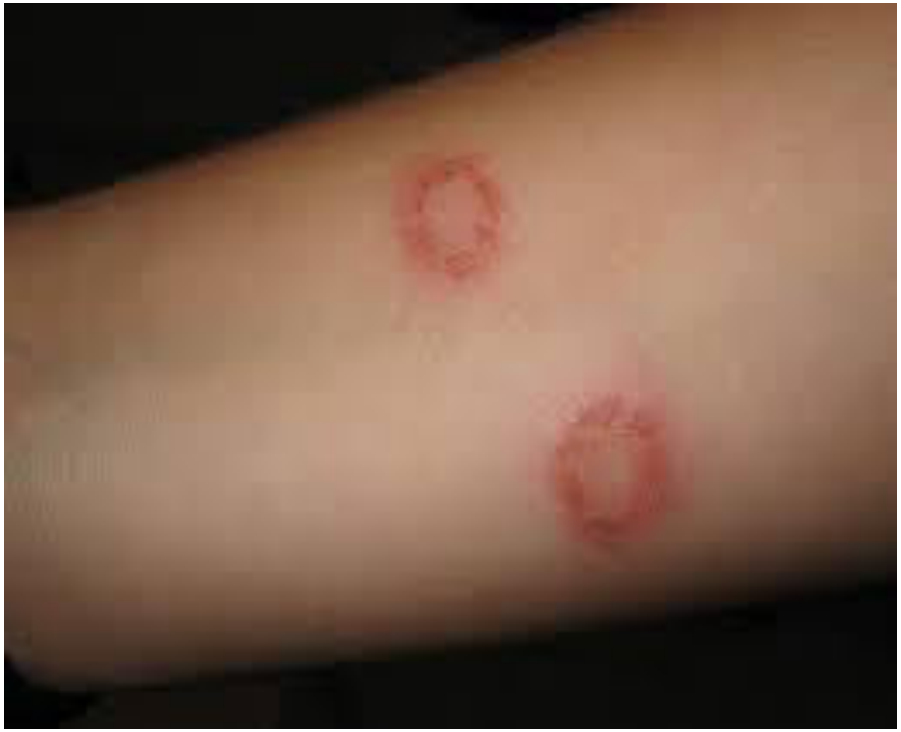

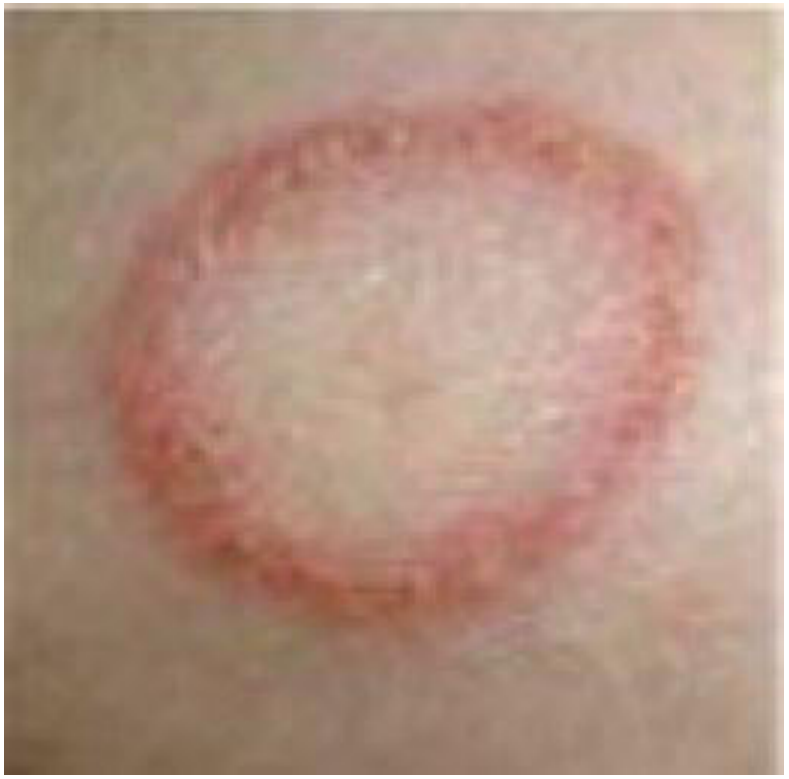

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Skin disease	Location of the lesion
Tinea corporis	smooth skin of the body. Circular itchy patches with advancing red border and central scaling.
Tinea pedis	Interdigital spaces of feet.
Tinea cruris	Groin, perineum or perianal area.
Tinea capitis	Scalp hair. Endothrix: fungus inside hair shaft. Ectothrix: fungus on the surface of hair.
Tinea barbae	Beard hair.
Tinea unguium (Onychomycosis)	Nail. Nails are thickened,and discolored. Usually associated with tinea pedis

II- Cutaneous Mycoses (dermatophytoses)

Laboratory Diagnosis:

1. Wood's light examination:

The tissues infected with Microsporum species fluorescence green when exposed to U.V.

2. Direct microscopic examination:

Few hairs and some of the skin scales are put in a drop of 10-20% potassium hydroxide solution on a slide ,a cover slip is added ,and heat gently but not to boil. the preparation is examined for the presence of hyphae or spores and for the type of hair invasion; ectothrix or endothrix.

3. Culture:

For the isolation of dermatophytes, the medium universally used is Sabouraud's dexstrose (SDA) agar. To inhibit bacterial contaminants, antibiotics are added as chloramphenicol. Also actidione is added (0.5 gm/L) as an inhibitor for contaminating moulds. Hairs and skin scrapings are inoculated on the surface of the medium and incubated at 30°C for 2-8 weeks.

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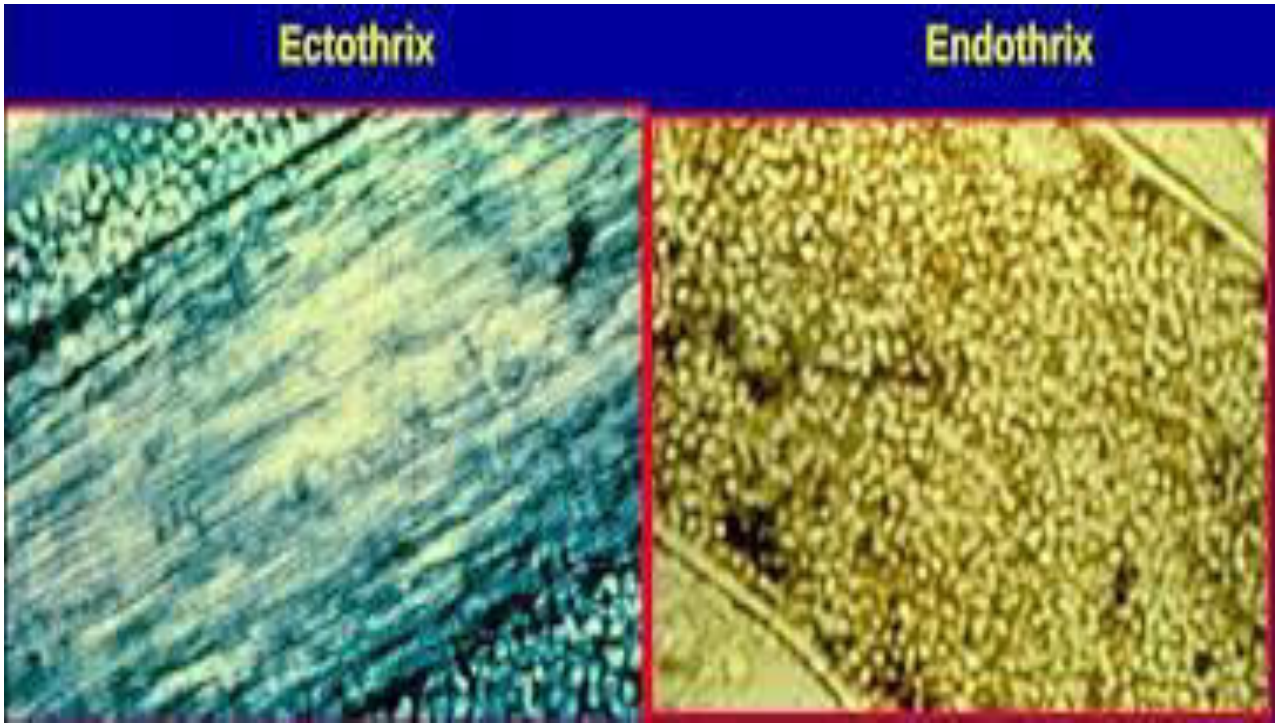

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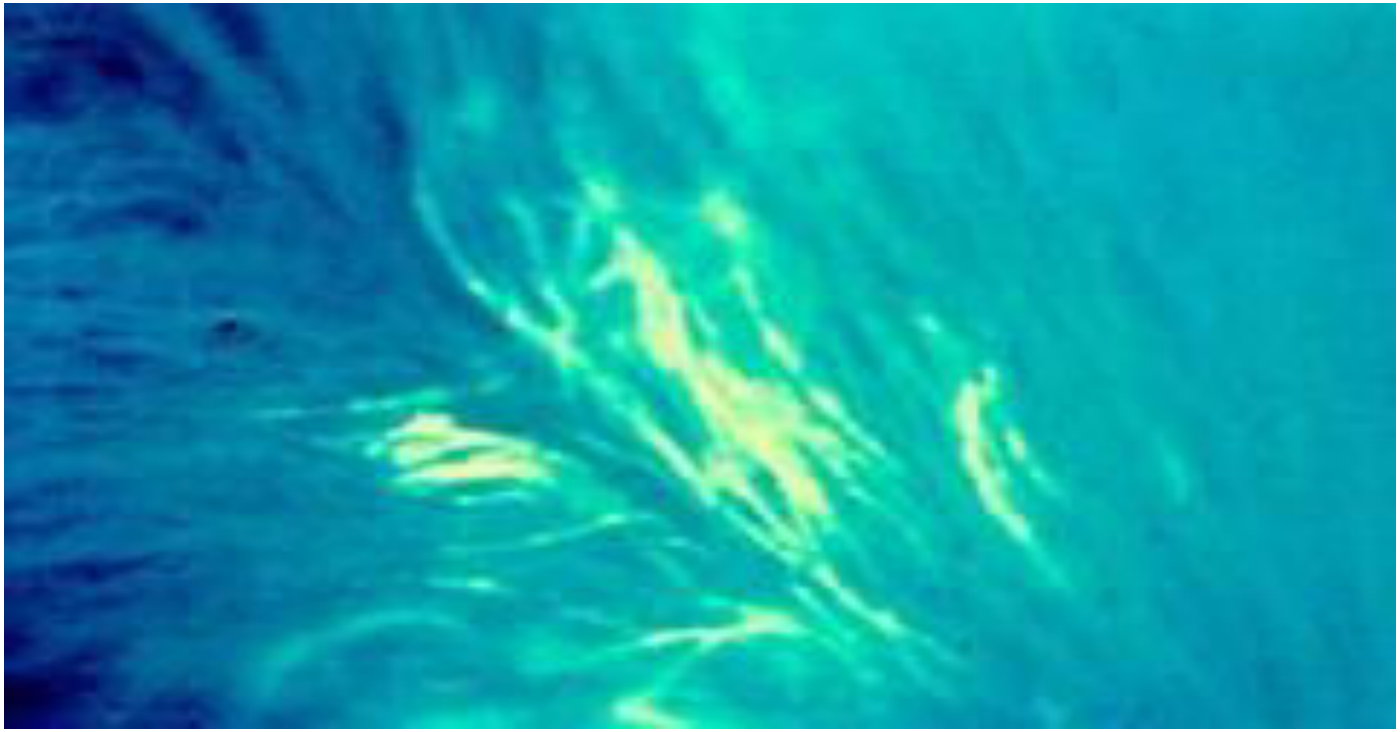

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II- Cutaneous Mycoses (dermatophytoses)

Treatment:

Topical therapy:

clotrimazole and miconazole are usually successful for eradicating dermatophytoses.

Oral therapy:

Oral antifungal agents are indicated in extensive infections or infections refractory to topical therapy. For some infections particularly those involving the nails the drug of choice is griseofulvin that must be administrated for several months.

III- Subcutaneous Mycoses

Eumycotic Mycetoma (Madura foot)

It is caused by filamentous fungi living in soil. The most common fungal cause is *Madurella mycetomatis* that enters the body via wounds; usually occurs in rural area in agricultural workers in the tropics.

IV- Systemic Mycoses (Deep Mycoses)

They are mycoses that affect internal organs and may disseminate to multiple sites of the body. It is subdivided into:

A-Primary systemic mycoses (endemic mycoses).

B-Opportunistic mycoses.

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A- Primary systemic mycoses (endemic mycoses):

They are caused by true pathogenic dimorphic fungi that are found in the human body at 37°C as the yeast form and grow in lab cultures at 25°C or lower as filamentous forms.

They are found in sand, soil, decaying organic material, bird or bat feces and all produce airborne spores.

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A- Primary systemic mycoses (endemic mycoses):

1- Blastomycosis

2- Histoplasmosis

3- Coccidioidomycosis

4- Paracoccidioidomycosis:

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Histoplasmosis

Etiology:

It is a granulomatous fungal infection caused by *Histoplasma capsulatum*; a dimorphic fungus found in soil enriched with bat or bird (particularly chicken) .

Histoplasma capsulatum can be isolated from most areas around the world; the major endemic region lies in the drainage areas of Mississippi rivers.

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Mode of transmission &Pathogenesis

Mode of transmission:

Inhalation of the aerosolized particles contaminated with the organism is the most common mode of transmission.

Pathogenesis:

After inhalation *Histoplasma capsulatum* is taken by alveolar macrophages and behave as a facultative intracellular parasite circulating throughout the reticuloendothelial system (RES) such as bone marrow, liver and spleen. Cell mediated immunity develops against this fungus which is evident by positive skin testing.

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Clinical manifestations

Histoplasmosis may remain mild or asymptomatic. Manifest disease is presented as pulmonary, fulminant or ocular histoplasmosis.

Pulmonary histoplasmosis

The severity of the disease depends largely on the general health state, and immune system of the host and the dose of the inoculum.

It can be presented as acute pneumonia but more commonly as chronic pulmonary disease. There is cough which is initially dry productive or bloody, anorexia, weight loss and night sweats; a picture similar tuberculosis but the chest x-ray shows bilateral interstitial infiltrates that is not seen with tuberculosis.

Disseminated histoplasmosis :

presented with hepatosplenomegally. The disease is fatal.

Ocular histoplasmosis:

It is not true retinal infection. It occurs as a hypersensitivity reaction to undetected infection with *histoplasma capsulatum* elswhere.

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Clinical manifestations

Pneumonia is self-limited in immunocompetent patients, but disseminates in immunocompromised (very young, very old, HIV+) to liver, spleen, bone marrow, nodes, lung, rarely to skin

- Disseminated disease: strongly associated with AIDS; patients have fever, weight loss, splenomegaly; variable cutaneous lesions

- Cutaneous lesions are nodules, papules, ulcers; less commonly macules, pustules or vesicles

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Laboratory diagnosis

Type of the samples depends on the presentation of the patient. Commonly sputum samples. Sometimes blood, bone marrow smear, or liver biopsy can be used.

1-Microscopic examination:

-The organism can be detected by histopathological examination as intracellular yeast cells 5-6µm in diameter in tissue phagocytes or circulating polymophonuclear leucocytes or monocytes in the peripheral blood.

-Bone marrow smear, or liver biopsy can be stained with Wright's or Giemsa stain. In tissue sections stained with Giemsa stain the inflammatory reaction is generally granulomatous with giant cells. Necrotic centers and calcifications may be detected.

Laboratory diagnosis

Culture:

Cultures of *Histoplasma capsulatum* is the definitive method for diagnosis.

At 25°C; it produces filamentous, white to brown colonies, and hyphae

At 37°C; it produces creamy white yeast colonies with a narrow neck between buds and mother cells. Definitive identification of cultures is based on:

Microscopic examination:

It is done by conversion from yeast form to the filamentous form with microscopic demonstration of typical sporulation.

Nucleic acid probes:

It is highly specific and sensitive method to confirm the diagnosis.

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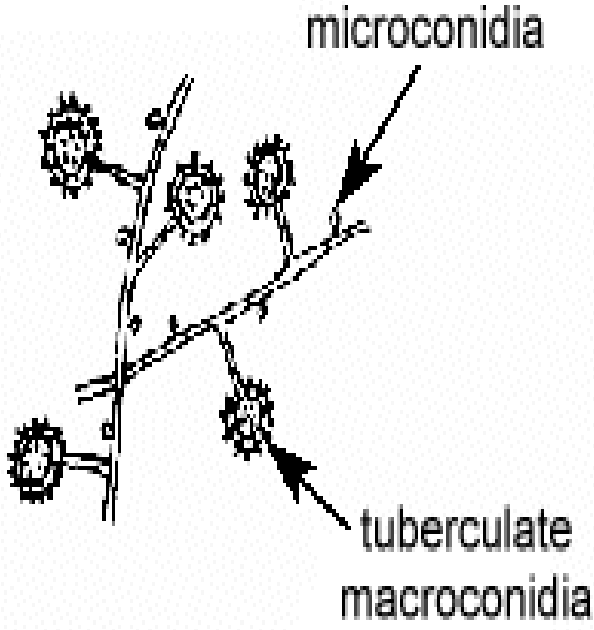
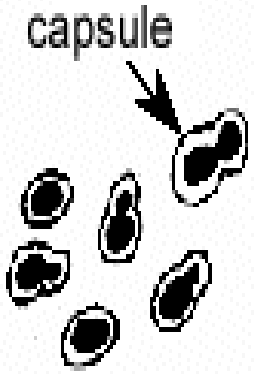
Histoplasma capsulatum





mycelial form in the soil → inhalation of conidia by the human → grows as an encapulated yeast in the body

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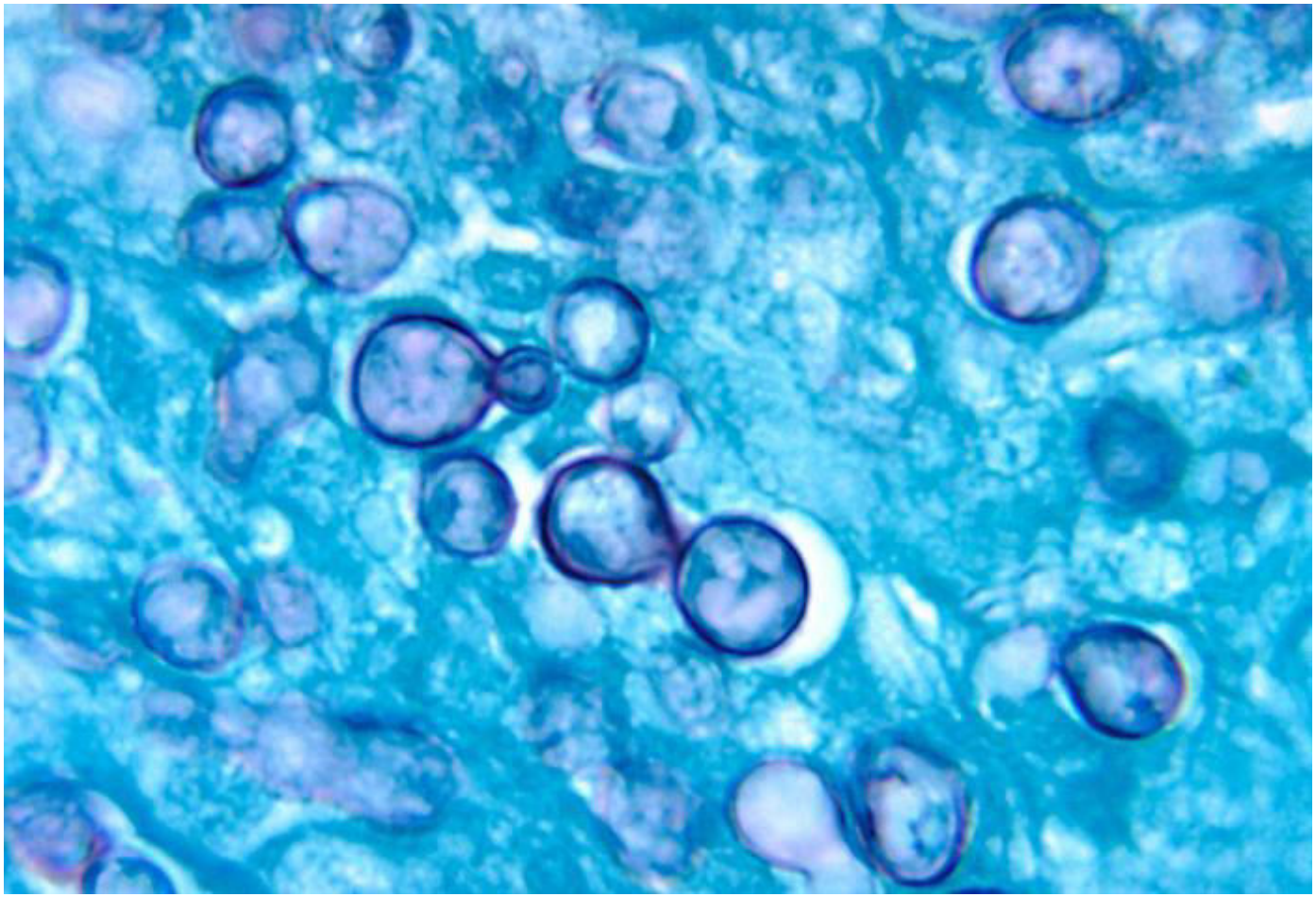

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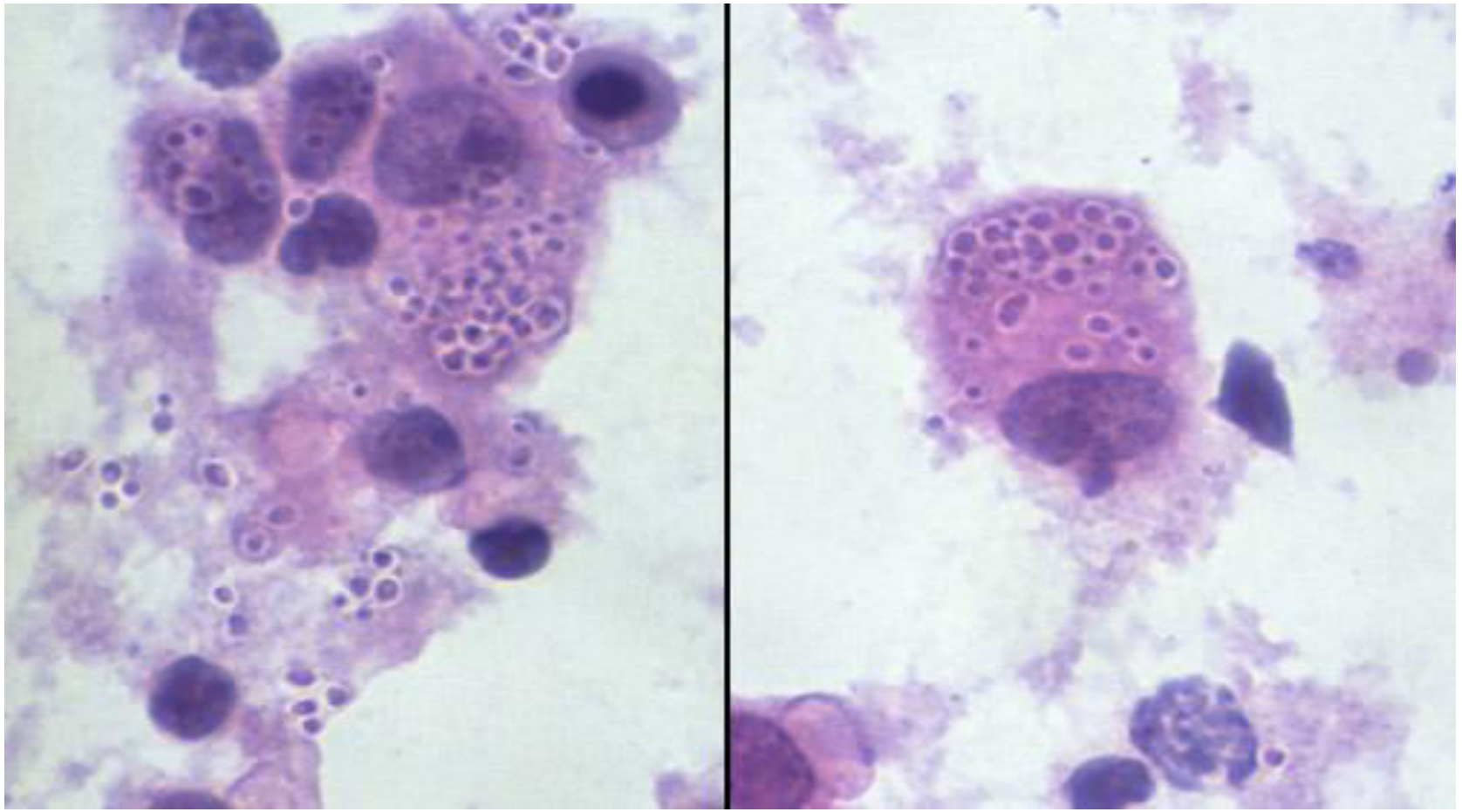

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Serology

1- Latex agglutination test:

Latex particles coated with fungal antigen extract are used to detect IgM antibodies.

2- Complement fixation test:

-Disadvantages: cross reactivity with other fungal pathogens.

-Advantages: the results allow evaluation of patient response to therapy.

3- Skin test (histoplasmin test):

It can not be used to diagnose active disease. It can be used to demonstrate previous exposure to the antigen.

A negative test is a poor prognostic sign in a patient with known active histoplasmosis

B- Opportunistic Systemic Mycoses:

Opportunistic pathogens have low virulence and can invade and cause disease only in immunocompromised patients.

Opportunistic mycoses are caused by saprophytic (i.e. from the environment) or endogenous (i.e. commensal) fungi. Commonly isolated organisms from these patients include *Candida* species, *Cryptococcus neoformans,and Aspergillus* species.

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Risk factors of opportunistic mycoses

Any factor that suppresses the patient immunity predisposes to opportunistic mycoses e.g.

-Drug therapy:

Anti-neoplastic drugs, steroids, and immunosuppressive drugs. 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.

-Severe illness or chronic debilitating diseases:

Diabetes, tuberculosis, malignancies, AIDs and severe burns.

Invasive procedures:

-Indwelling catheters, prosthetic valves…etc.

Candidiases

This term refers to infections caused by different Candida species the commonest of them is *Candida albicans* which is a part of normal flora of the skin, mucous membranes, and gastrointestinal tract.

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Candidiases

Predisposing factors to Candida infections are:

-Extremes of age.

-Nutritional disorders.

-Excessive moisture.

-Pregnancy.

Candidiases

I- Superficial candidiases: include the following forms

1. Oral thrush:

It is infection of the oral mucous membranes manifested as white like patches. It commonly occurs in children with prolonged antibiotic therapy, immunosuppressed patients e.g. AIDS patients.

2. Vulvovaginitis or vaginal thrush:

It is infection of the vagina manifested as a thick yellow-white discharge, burning sensation, patches on the vaginal mucosa and inflammation of the perineum. It is commonly seen in diabetic patients and during pregnancy.

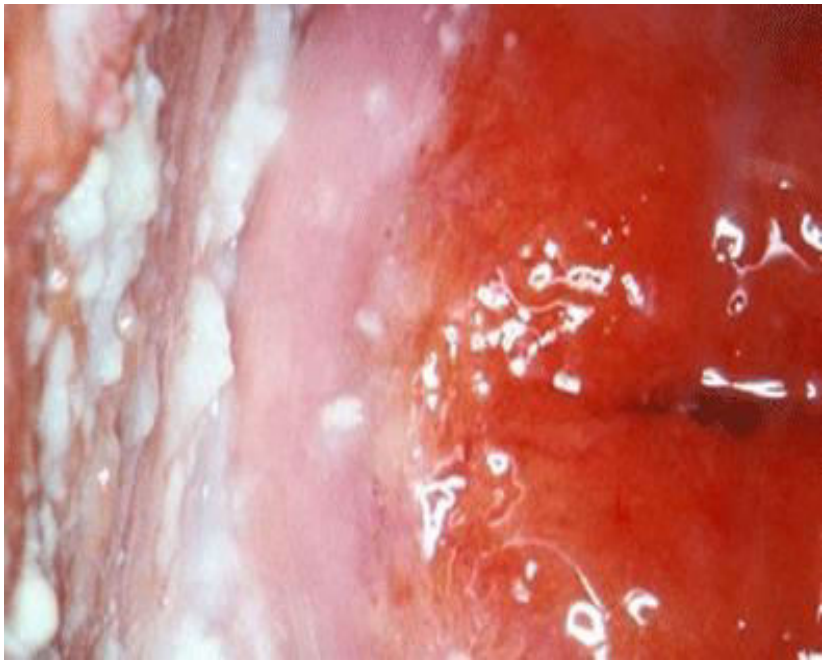
3. Cutaneous Candidiasis:

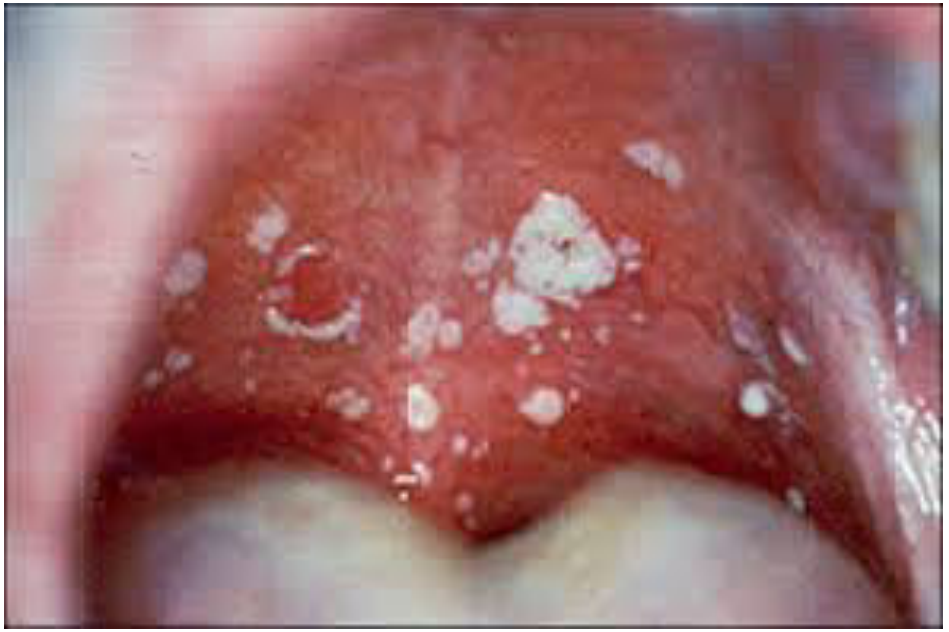
It involves nails, skin folds, or groin region.

4. Alimentary tract disease, including esophagitis:

It is usually an extension of oral thrush found in AIDS patients and other immunosuppressed patients, particularly those on long-term antibiotics.

Candidiases





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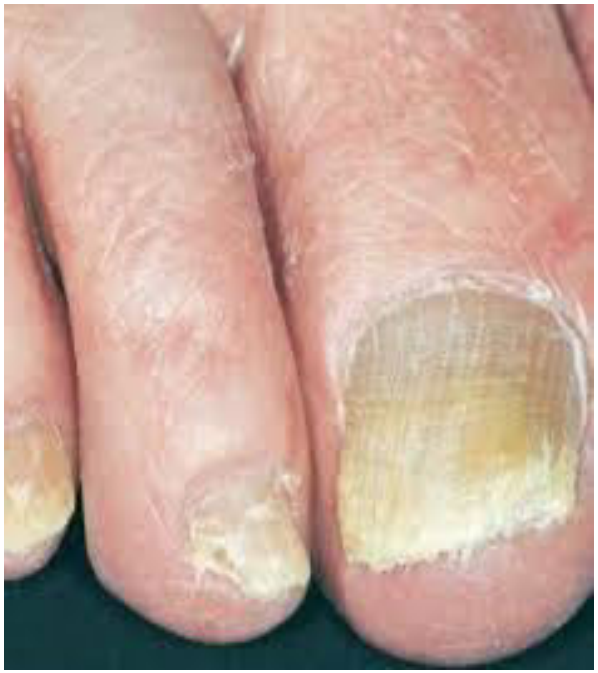
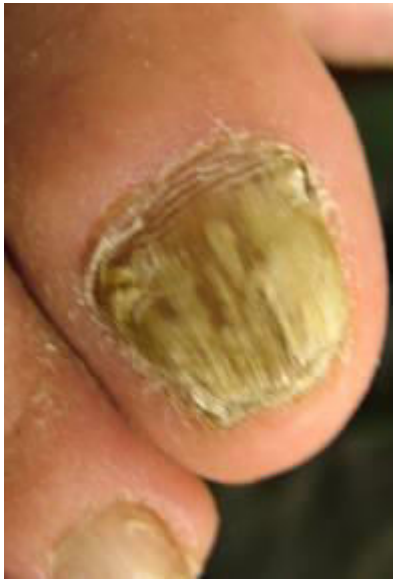
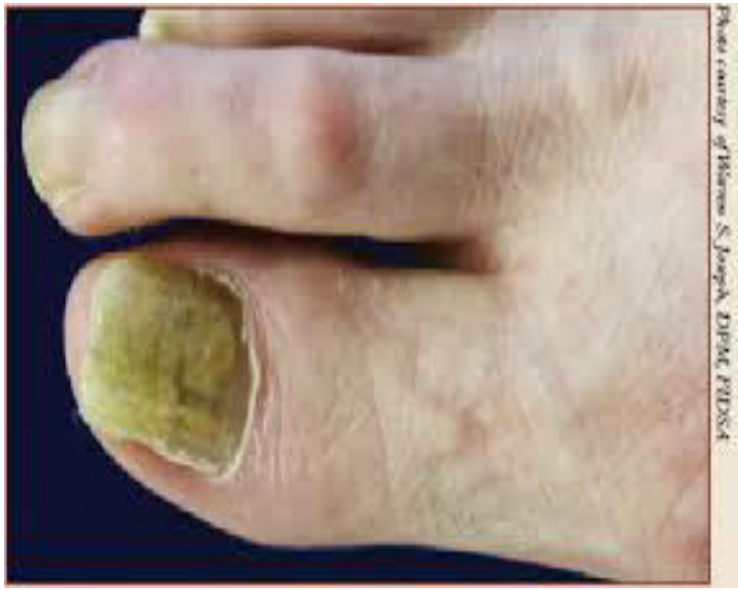
Candidal paronychia







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Candidiases

II- Systemic candidiasis

may occur almost anywhere in the body

-Candidemias or blood-borne infections occur most commonly in patients with indwelling intravenous catheters.

-Endocarditis occurs in patients who have manipulated or damaged valves or in intravenous drug abusers.

-Bronchopulmonary disease (usually manifested by persistent cough) occurs in patients with chronic lung disease.

Diagnosis of candidiases

I- Superficial candidiases:

Diagnosis is mainly clinical and confirmed by detection of yeast cells in Gram stained film or KOH mount of the lesion.

II- Systemic candidiasis:

Samples differ according to the site of infection and include urine, sputum, bronchial washings, cerebrospinal fluid, pleural fluid, blood and tissue biopsies from various visceral organs.

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Diagnosis of candidiases

1-Direct microscopy:

large oval gram positive budding yeast cells with pseudohyphae.

Demonstration of the presence of pseudohyphae and yeast cells is diagnostic when samples are obtained from normally sterile site e.g. blood or when it is obtained from a site where candida is normally present as a part of body flora and the clinical presentation is consistent with candidiases.

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Diagnosis of candidiases

2-Culture:

on SDA or corn meal agar

Colonies are typically white to cream colored with a smooth surface and yeasty odour. The organism is identified by:

-The formation of pseudohyphae (oval budding gram positive yeast like cells) and chlamydoconidia *on corn meal agar.

-Carbohydrate assimilation tests*, C.albicans ferments glucose and maltose with acid and gas production.

-Germ tube test*:

When yeast isolates are incubated in serum at 37°C germ tubes (drum stick non septate elongation of yeast cells) are formed within 2-3 hours.

*This test is positive only with *c.albicans*.

NB: germ tubes are non septate and show no constriction at the point of attachment while pseudohyphae may be septate and show constriction at the point of attachment.

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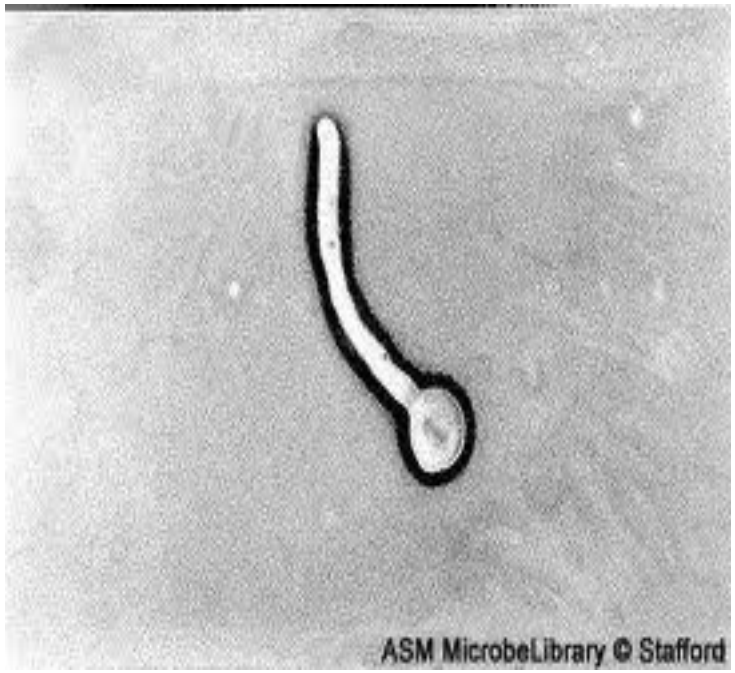
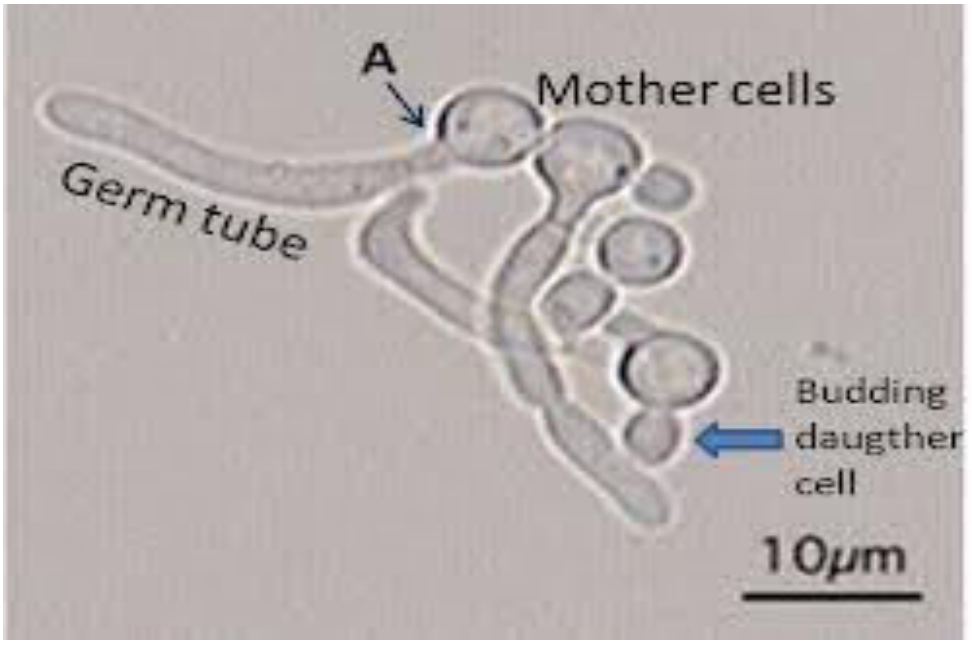
Diagnosis of candidiases





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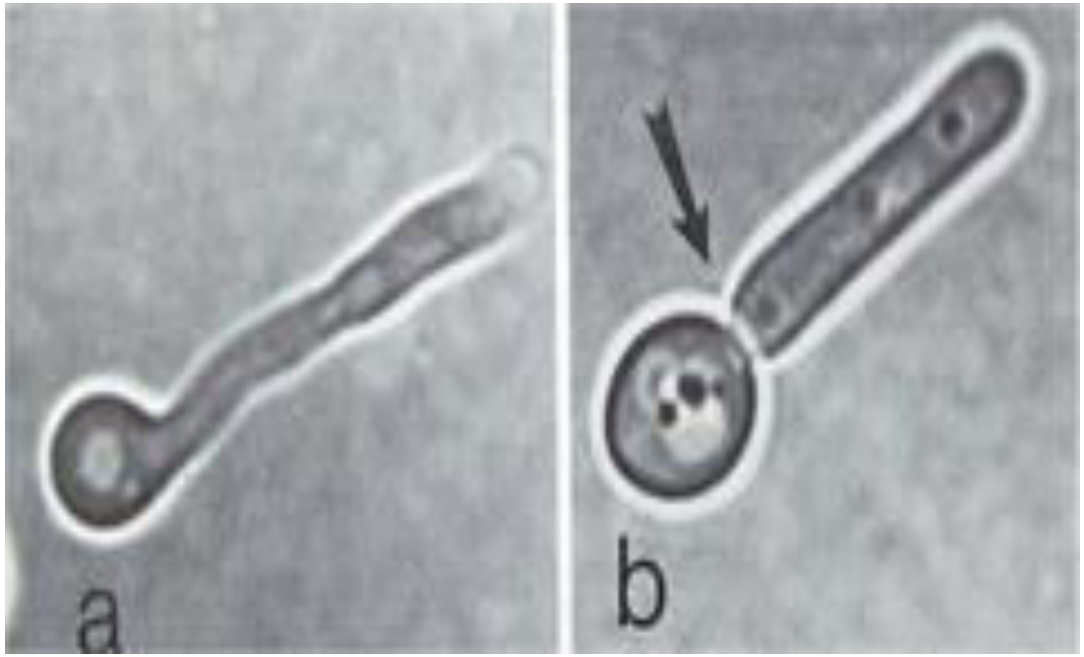
Diagnosis of candidiases

Serology:

Serological tests for detection of antibodies against candida antigens are usually unuseful because they can not discriminate infection and normal colonization also immunocomprimised patients who are at risk of infection have weak antibody response.

A skin test; candidin test can be also used but of limited value.



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2- Cryptococcosis

Etiology:

It is caused by *Creptococcus neoformans* which are yeast cells possessing an antigenic polysaccharide capsule. It is present in soil and associated with pigeon excreta; an occupational hazard to pigeon handlers.

2- Cryptococcosis

Mode of transmission:

Infections occur commonly in immunocomprimised patients by inhalation where it causes subclinical lung infection or pneumonia. Infection spreads systemically to the meninges causing meningitis.

Pathogenesis:

The antiphagocytic polysaccharide capsule is the major virulence factor.

Clinical manifestations:

1. Creptococcal meningitis:

It is the most common clinical presentation of cryptococcosis. It presents as headache of increasing severity (over a period of several months), usually with fever followed by typical meningitis signs.

2. Pulmonary cryptococcosis:

It is usually asymptomatic and self-resolving. Fulminant forms are highly variable but may resemble pneumococcal pneumonia.

Laboratory diagnosis:

1- Examination of CSF:

Physical examination:

CSF is turbid and under tension.

Chemical examination:

Protein level is increased, glucose level is decreased and the number of leukocytes is increased (mainly mononuclear cells).

Microbiological examination:

India ink wet mount of CSF sediment for demonstration of encapsulated yeast cells. This has been greatly replaced by latex agglutination test for detection of the capsular antigen in CSF (can be also performed on serum samples).

Diagnosis is confirmed by isolation of *C. neoformans* by culture of CSF.

Specimen collection for CSF Examination

- Lumbar puncture to collect the CSF for examination to be collected by Physician trained in procedure with aseptic precautions to prevent introduction of Infection.

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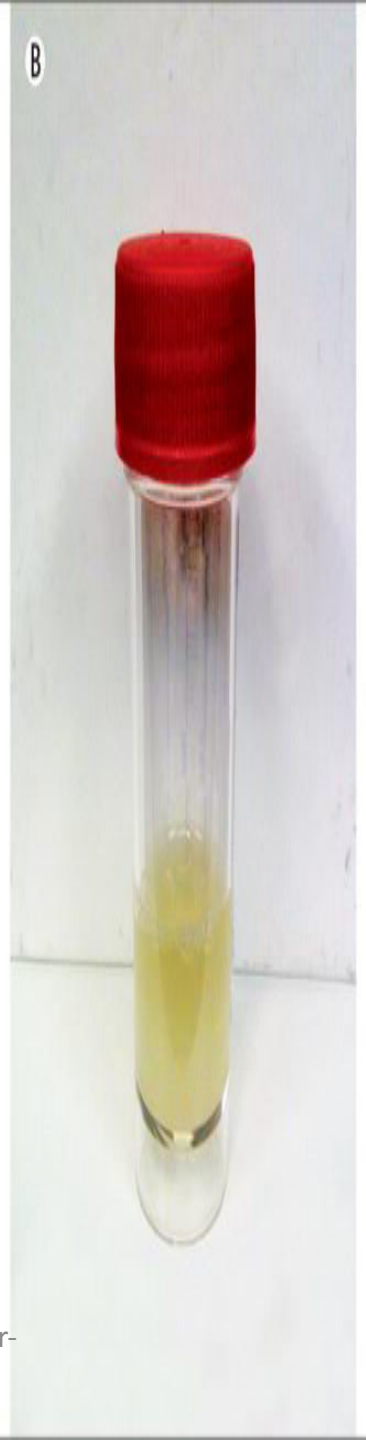
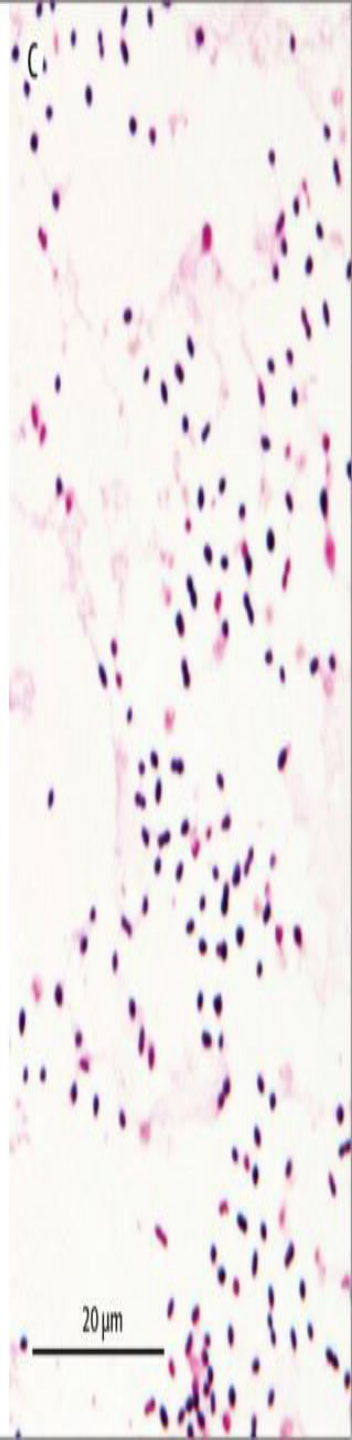
Collecting the CSF



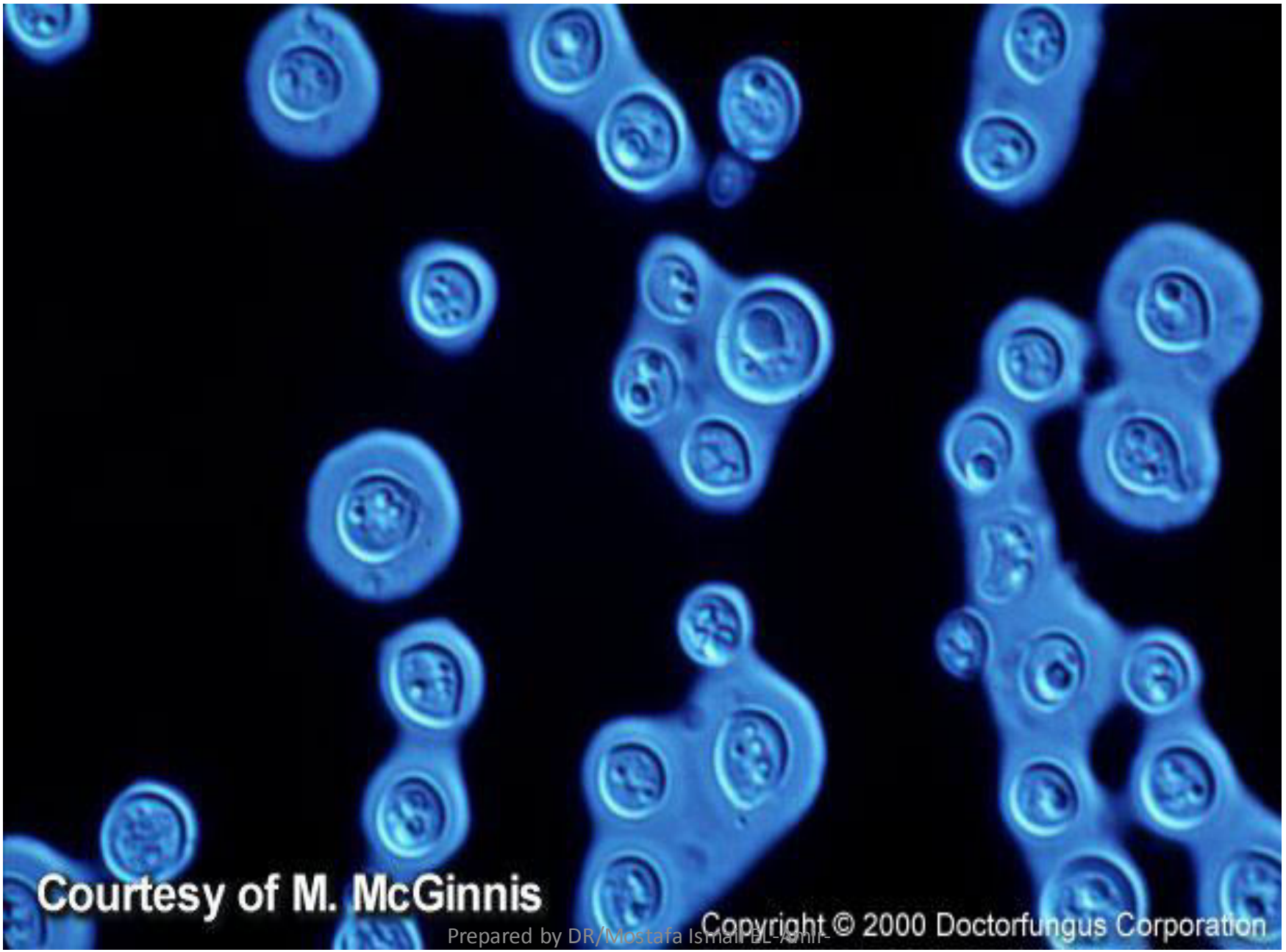


2- Serology:

Indirect immunoflourescence for antibody detection but it does not discriminate between active infection and past exposure.

3- culture:

Performed on cyclohexamide free media. Colonies are creamy mucoid (because of the capsule). Organism is identified by urease production, carbohydrate assimilation or direct immunoflourescence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3- Aspergillosis

Etiology:

Aspergillosis is the name given to a wide variety of diseases caused by fungi of the genus Aspergillus. The most common are *A.niger* and *A.flavus*.

Aspergillosis develops mainly in immunocompromised individuals.

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Clinical manifestations

Asprigillus species can invade any part of the body but commonly cause disease in the respiratory system. There are 3 form of pulmonary aspergillosis:

Allergic bronchopulmonary aspergillosis: due to allergy to inhaled fungal spores.

Pulmonary aspergilloma: A fungus ball in the lungs (commonly in the cavities of previous TB infection) may cause no symptoms and may be discovered only with chest X-ray , or it may cause repeated coughing of blood.

Invasive aspergillosis: propagation of the fungus through the lung parenchyma. It is seen in immunocompromised individuals particularly children

Laboratory diagnosis:

1-Microscopic examination:

Aspergillus species are reliably demonstrated by silver stains . Aspergillus hyphae tend to branch that is progressive and primarily at acute angles of about 45°.

2-Culture:

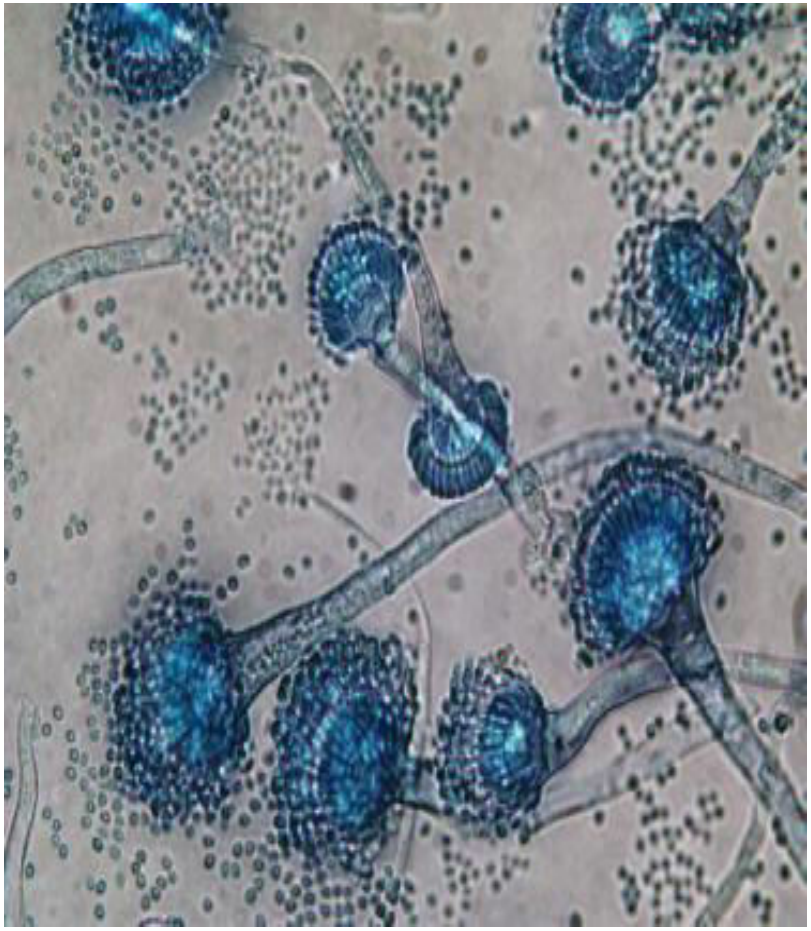
At 25°c colonies appear after 2-3 days with yellow or greenish coloration..

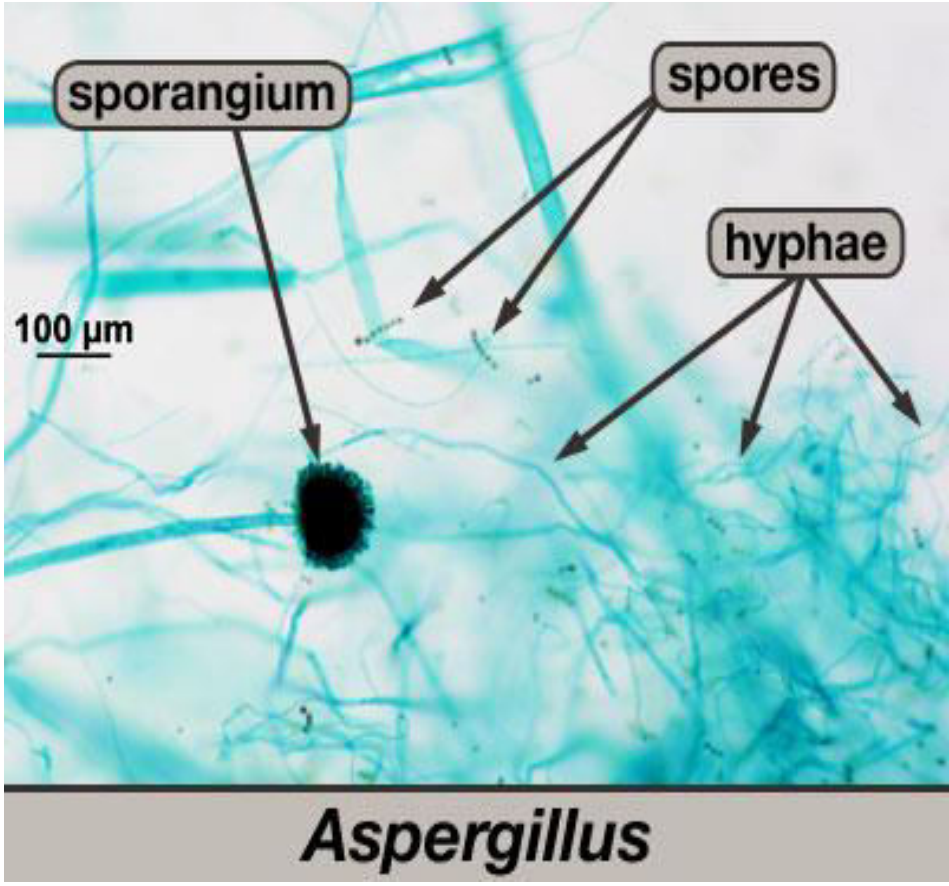
3-Serology:

Detection of rising specific IgG in patients with aspergilloma.

Detection of IgE in patients with allergic bronchopulmonary aspergillosis.

Skin test; aspergillin test.





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Antifungal Drugs

Selective toxicity is very limited in antifungal drugs due to the fact that fungi, like human cells are eukaryotic.

The available drugs are those which bind to ergosterol in the cell membrane or inhibit its synthesis.

Others act by inhibiting chitin synthesis in the cell wall.

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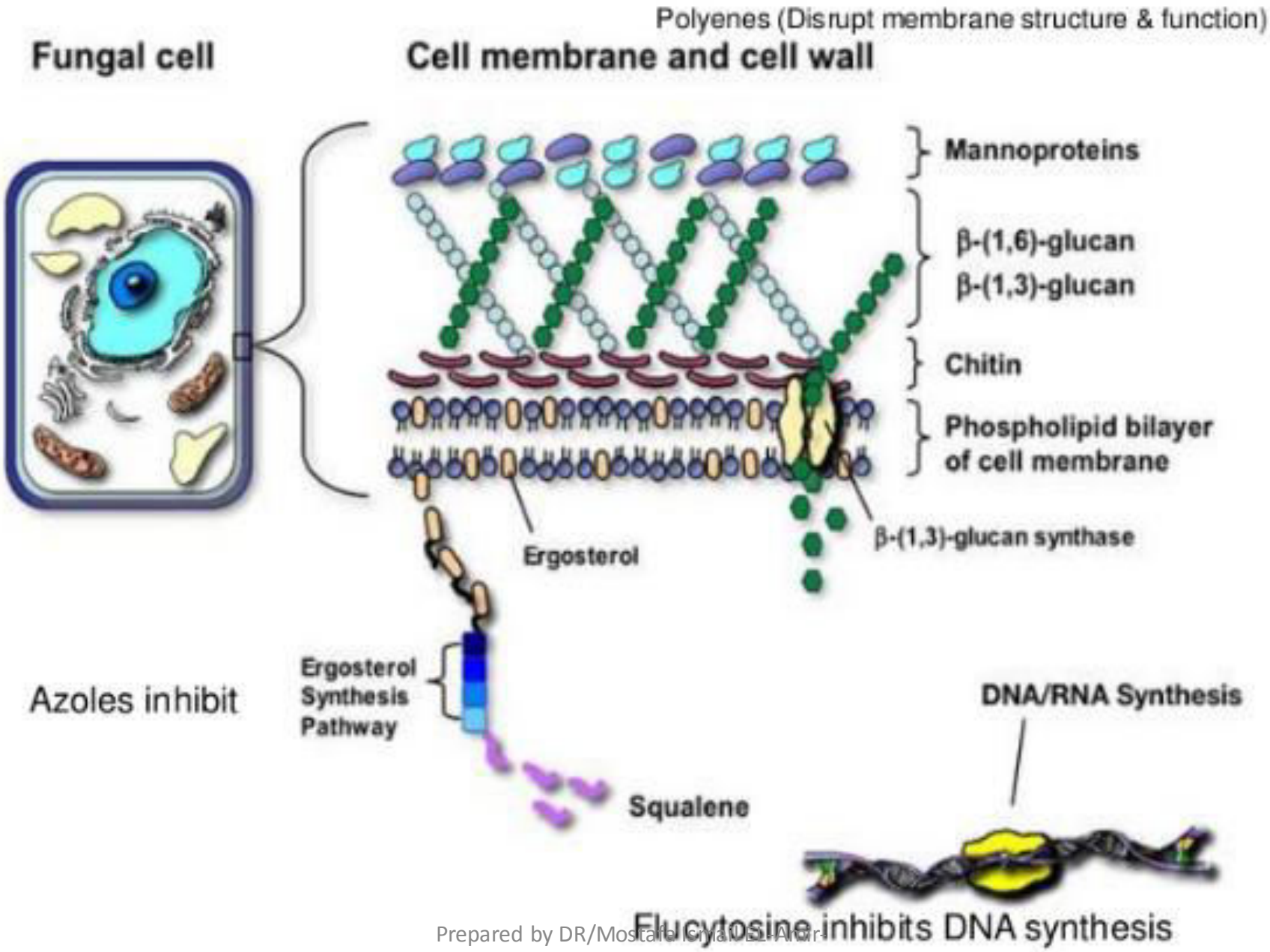
Polyenes (Disrupt membrane structure & function)
Fungal cell
Cell membrane and cell wall
Mannoproteins
β-(1,6)-glucan
β-(1,3)-glucan
Chitin
Phospholipid bilayer of cell membrane
Ergosterol
β-(1,3)-glucan synthase
Azoles inhibit
Ergosterol Synthesis Pathway
Squalene
DNA/RNA Synthesis
Flucytosine inhibits DNA synthesis

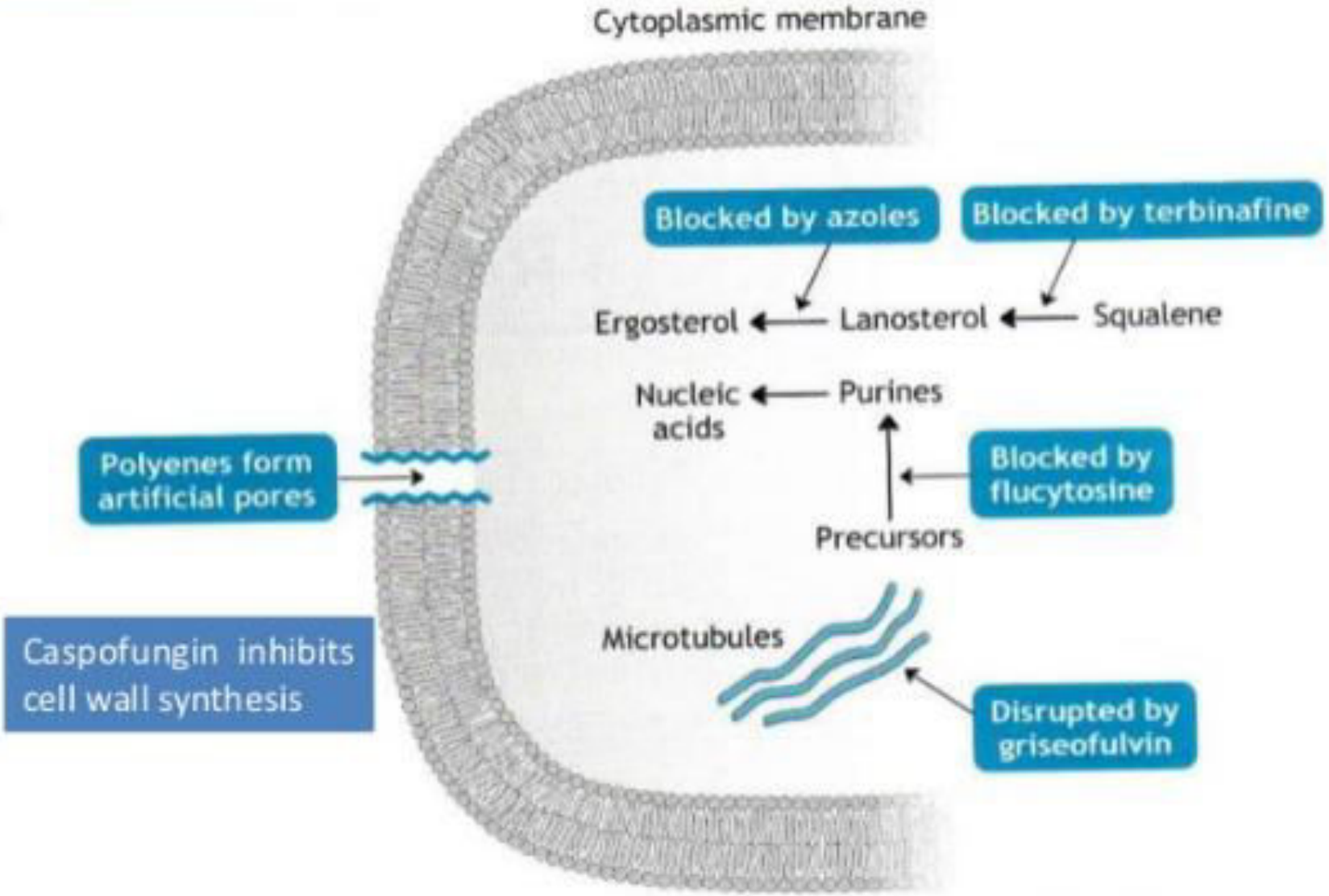

Figure 8–1. Sites of action of some antifungal drugs. The cell cytoplasmic membrane shown is that of a typical fungus. Because ergosterol is not a component of mammalian membranes, significant selective toxicity is achieved with azole drugs.

Classification based on mechanism of action

1. Fungal cell wall synthesis inhibition: Caspofungin.
2. Bind to fungal cell membrane ergosterol: Amphotercin–B, Nystatin.
3. Inhibition of ergosterol + lanosterol synthesis: Terbinafine, Naftifine, Butenafine.
4. Inhibition of ergosterol synthesis: Azoles
5. Inhibition of nucleic acid synthesis: 5–Flucytosine.
6. Disruption of mitotic spindle and inhibition of fungal mitosis: Griseofulvin.

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I- Polyenes “causing pores”

A- Amphotericin B:

Mechanism of action:

It binds with sterols in the fungal cell membrane, principally ergosterol leading to disruption of its functions with subsequent leakage of cell contents and cell death. (Fungicidal)

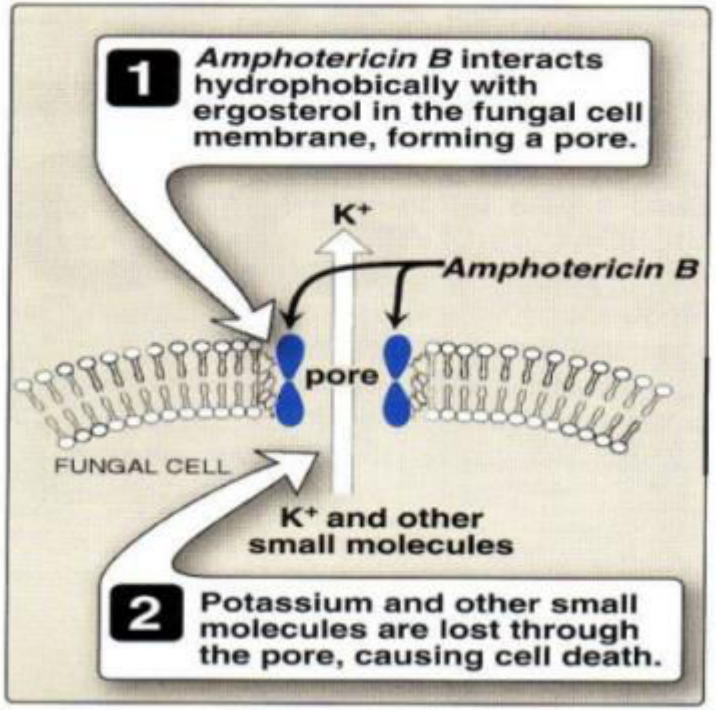
Human cells contain cholesterol instead of ergosterol and so they are much less susceptible. However, at therapeutic doses, some amphotericin B may bind to human membrane cholesterol, increasing the risk of toxicity.

Side effects:

It is nephrotoxic and bone marrow suppression.

Amphotericin B:

Mechanism of action



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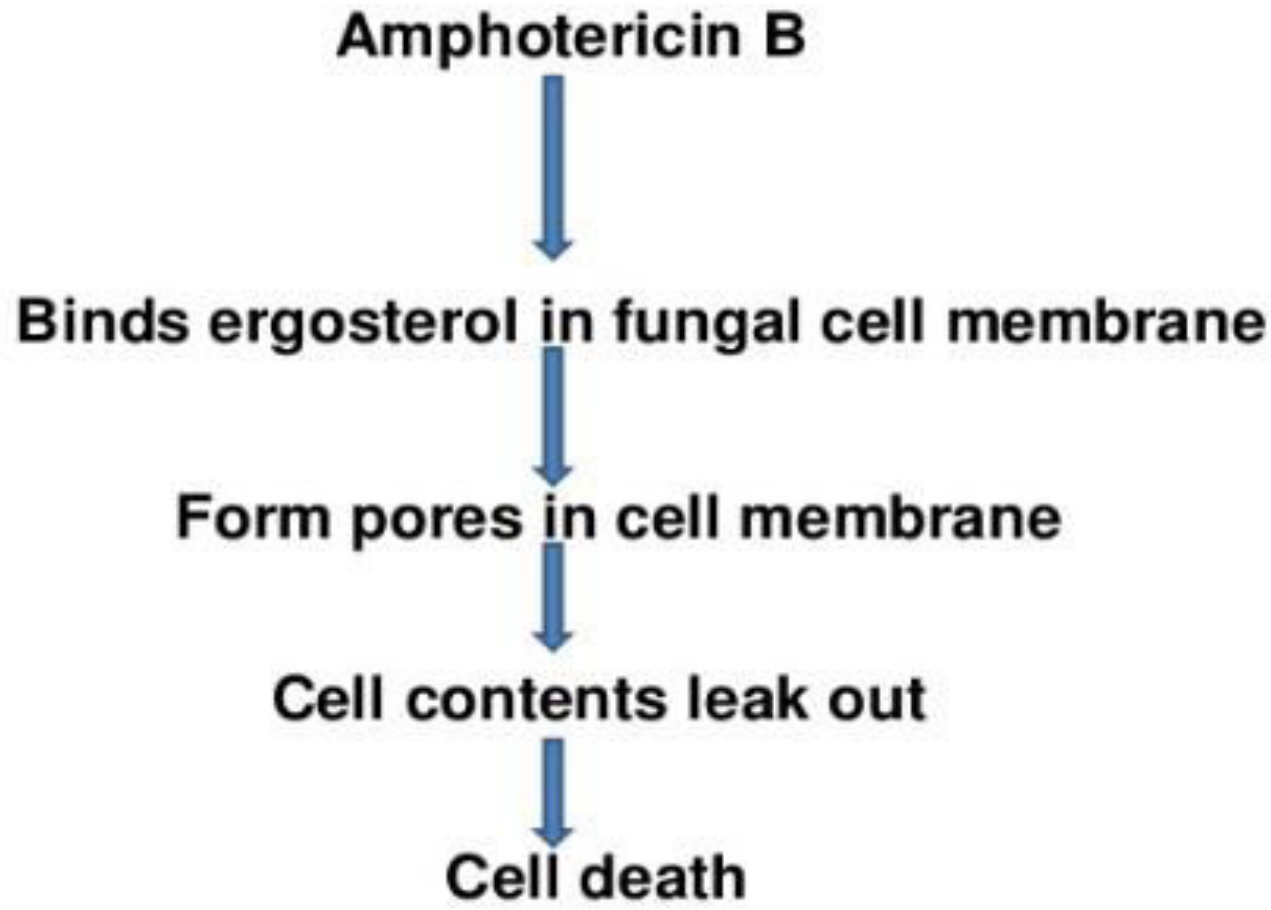
Mechanism of action



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I- Polyenes

B- Nystatin:

Mechanism of action:

The same as amphotericin B but effective only against *candida albicans*.

Administration:

It is used to treat candidiasis topically or to reduce candida growth in the gastrointestinal tract of compromised patients.

Nystatin is not absorbed after oral administration and can not be given IV so not used for treatment of systemic infections.

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II- Azoles

Mechanism of action:

It inhibits the enzyme necessary to convert lanosterol to ergosterol. Depletion of ergosterol in fungal membrane disrupts the structure and many functions of fungal membrane leading to inhibition of fungal growth. azoles are fungistatic.

Azoles include:

A- Imidazoles e.g. Ketoconazole, clotrimazole, econazole and miconazole.

B- Triazoles e.g. fluconazole, itraconazole.

C- Thiazoles e.g. abafungin.

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II-Azoles

Indications:

These drugs are effective for treatment of mucocutaneous candidiasis, dermatophytosis (particularly useful in grisofulvin-resistant cases) and some systemic fungal infections.

Administration:

These drugs are used topically as creams and ointments for localized infections and orally for disseminated skin infections and systemic infections.

Side effects:

Azoles are less toxic than amphotericin B.

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III- Grisofulvin

Indications:

It is used for dermatophytes infection of skin, hair and nails.

Fungistatic,systemic drug for superficial fungal infection

Mechanism of action:

-inhibiting fungal mitosis.

It binds to keratin in keratin precursor cells and makes them resistant to fungal infections. The drug reaches its site of action only when hair or skin is replaced by the keratin-griseofulvin complex.

Administration:

It is administered orally (up to 1 year for nail infections) and localizes in the stratum corneum.

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III- Grisofulvin

Interact with microtubules ,disrupt mitotic spindle s--→arrest fungal mitosis

IV- Fluocytosine (5FC):

Mechanism of action:

It is converted by fungal cytosine deaminase into 5- fluorouracil that inhibits both RNA and DNA synthesis.

Animal cells lack cytosine deaminase.

Administration:

It is administered orally.

Indications:

Development of drug resistance is the main problem with flucytosine so
it is used primarily in combination with amphotericin B(synergism).

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Advantages of combination:

–Entry of 5 FC
–Reduced toxicity
–Rapid culture conversion
–Reduced duration of therapy
–Decreased resistance

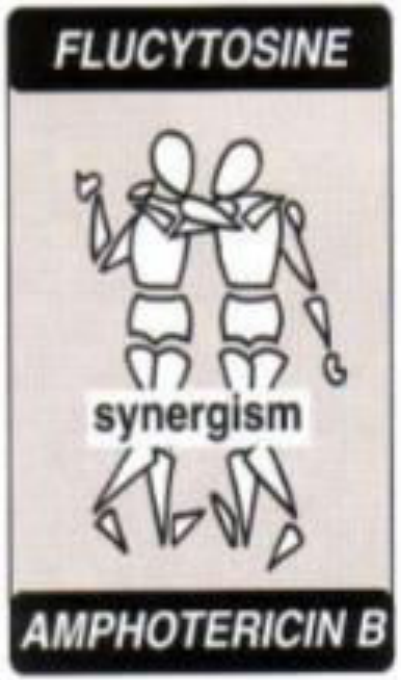
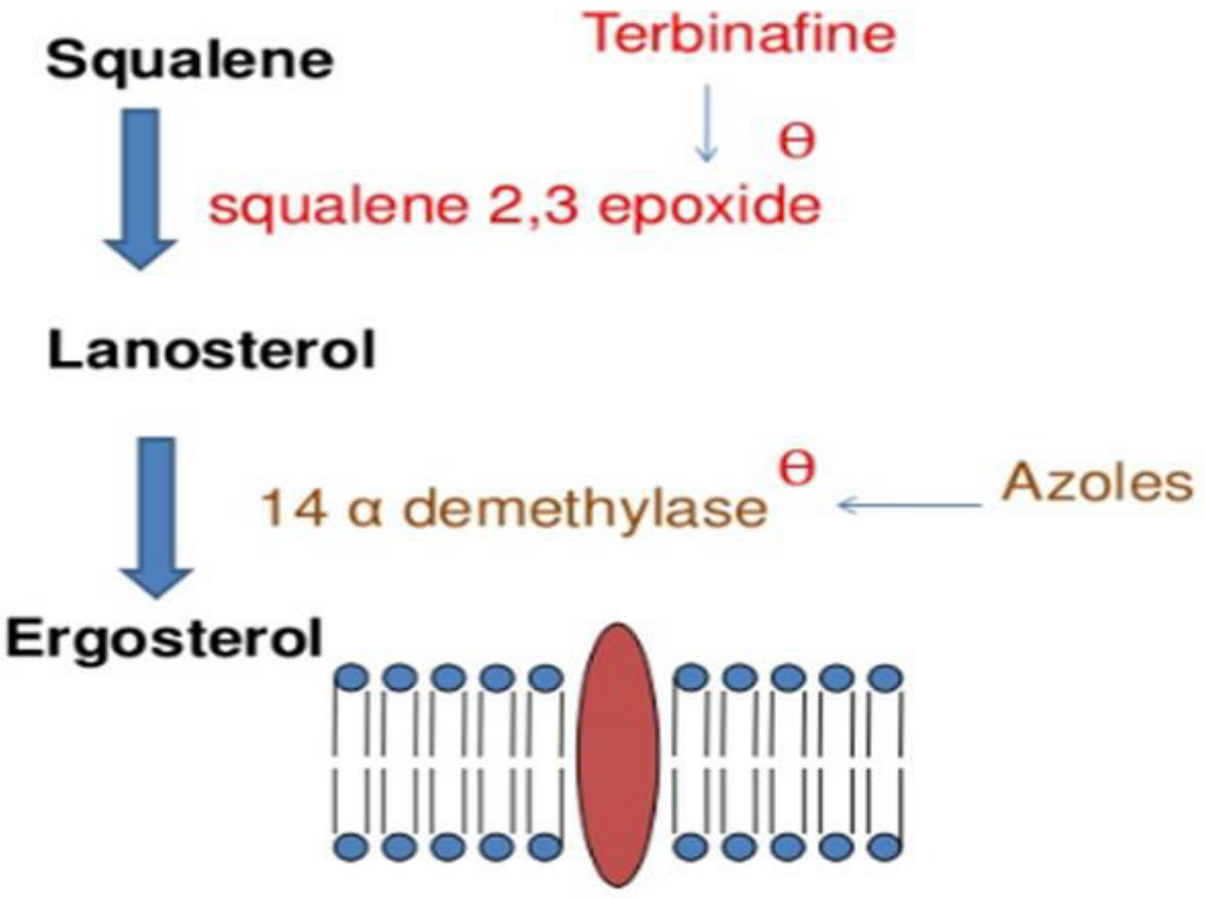


Figure 35.7
Synergism between *flucytosine* and *amphotericin B*.

V- . Terbirafine):

Mechanism of action:



VI- Caspofungin

They are used for systemic fungal infections in immunocompromised patients.

They inhibit the synthesis of glucan in the cell wall via the enzyme 1, 3- β glucan synthase.

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