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ZAPORIZHZHYA STATE MEDICAL UNIVERSITY
THE CHAIR OF MICROBIOLOGY, VIROLOGY, IMMUNOLOGY

CAUSATIVE AGENTS OF MYCOSES
BASIC PRINCIPLES
OF LABORATORY DIAGNOSTICS OF MYCOSES

The methodical manual for medical students

Zaporizhzhya
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REVIEWER: *Popovich A.P.*, docent of the Chair of Medical Biology

AUTHORS:

Yeryomina A.K., senior lecturer of the chair of microbiology, virology and immunology, candidate of Biological Sciences.

Kamyshny A.M., the head of the chair of microbiology, virology, and immunology, doctor of medicine.

Voitovich A.V., assistant of the chair of microbiology, virology and immunology, candidate of Biological Sciences.

Sukhomlina I. E., senior lecturer of the chair of normal physiology, candidate of Medicine.

Kirsanova E.V., assistant professor of the chair of hygiene and ecology, candidate of Medicine.

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The independent practical work of students is an important part of the syllabus in the course of microbiology, virology, immunology. It helps students to study this fundamental subject.

The systematic independent work enables to reach the final goal in the students' education. It is also important while preparing the students for their future clinic work with patients. These theoretical material, questions and tests help students to get ready for examination.

The methodical manual for practical lessons on microbiology, virology, immunology for the medical students of 2 -3 year of the study are approved by the Central Methods Board of ZSMU as a methodical manual on practical lessons for students of the medical faculty.

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FUNGI. FUNGAL INFECTIONS

Fungi and yeasts constitute eumycetes. They are eukaryotes lacking chlorophyll pigments. They possess differentiated nuclei surrounded by nuclear membrane and reproduce either by budding or by forming spores.

They have rigid chitinous cell walls but lack differentiation of root, stem and leaves. Morphologically the fungi may be either simple oval cells or long tubular septate hyphae showing true lateral branching.

The yeasts and fungi need organic compounds as nutrients. Their role in nature appears to be as scavenger i.e., breaking down the complex carbohydrates and proteins of dead bodies of other organisms. Needless to mention that only a few of them are pathogenic. In many ways, fungi have been of service to man, as in the making of bread, fermented drinks, cheese, antibiotics etc.

MORPHOLOGICAL CLASSIFICATION OF FUNGI

Fungi can be divided into four groups each of which have some human pathogenic species.

1. Moulds: They are filamentous and fungi. They grow as long filaments or hyphae which branch and interlace to form a meshwork or mycelium. They reproduce by forming various kinds of spores.

The part of the mycelium which grows on and penetrates into the substrate, absorbing nutrients for growth is called vegetative mycelium.

On artificial medium they are seen as filamentous mould colony which may be dry and powdery. The pathogenic members are trichophyton, microsporurn and epidermophyton.

2. Yeasts: They are unicellular occurring as spherical or ellipsoidal cells. They reproduce by budding. On solid media they form moist, Hum.

The part of mycelium which protrudes into the air is called aerial compact, creamy, mucoid colonies resembling to those of staphylococci. *Cryptococcus neoformans* is the only important pathogen.

3. Yeast like fungi: They grow partly as yeasts and partly as long filamentous cells joined end to end forming a pseudomycelium. On solid medium moist creamy colored colonies are produced. *Candida albicans* is the example.

4. Dimorphic fungi: They grow in mycelial form at low temperature 22 °C and in soil whereas growth at 37°C and in animal body occurs in yeast form. The pathogenic members are *Histoplasma capsulatum*, *Sporotrichum*, *Blastomyces* and *Coccidioides immitis*.

SYSTEMIC CLASSIFICATION

Based on sexual spore formation fungi are kept in 4 classes as described below:

Phycomycetes: They are fungi having non-septate hyphae. They form endogenous asexual spore (sporangio spore) contained within sac like structure called sporangia.

Sexual spores are also found & are of two varieties oospore and zygosporangium.

1. **Ascomycetes:** They form sexual spores (ascospores) within a sac. This sac is called ascus. They include both yeasts and filamentous fungi. They form septate hyphae.

2. **Basidiomycetes:** They reproduce by means of sexual reproduction. Basidiospores are borne at the tip of basidium. These basidia are sometime quite large leaf like structure as in mushroom. They form septate hyphae.

3. **Fungi imperfecti:** They consist of group of fungi whose sexual phases have not been identified. Fungi of medical importance belong to this group e.g. *Sporothrix schenckii*.

Mycology is discussed in this section in following scheme: superficial mycosis, subcutaneous mycosis, systemic mycosis, opportunistic fungus and miscellaneous.

I. INTRODUCTION

1. Study of fungi is called *Mycology*.
2. Name is derived from *Mykos* meaning mushroom.
3. All fungi are eukaryotic.
4. Water, soil and decaying organic debris are natural habitat.
5. Fungi are obligate or facultative aerobe.
6. They are chemotrophic organisms i.e. obtaining their nutrients from chemicals in nature.

II. DIFFERENCES OF FUNGI FROM BACTERIA

1. They possess rigid cell walls containing chitin, mannan and other polysaccharides.
2. The cytoplasmic membrane contains sterols.
3. The cytoplasm contains true nuclei with nuclear membrane, mitochondria and endoplasmic reticulum.
4. Fungi may be unicellular or multicellular.
5. They divide asexually, sexually or by both process.

III. CLASSIFICATION OF FUNGI

Taxonomical Classification

Fungi are placed in the phylum *Thallophyta*. There are four classes of fungi. Their characteristics are described in flow chart' shown below.

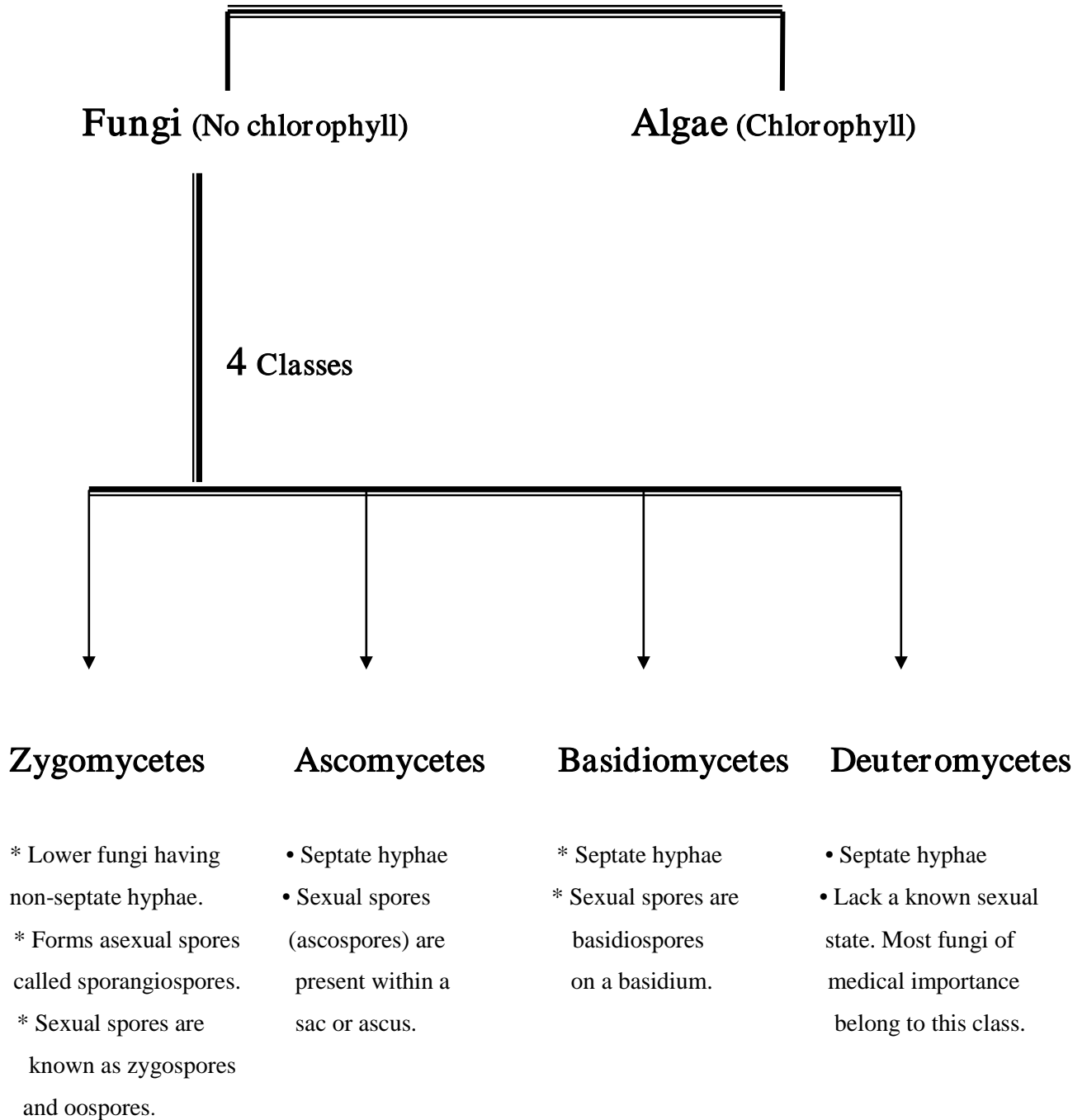
Morphological Classification

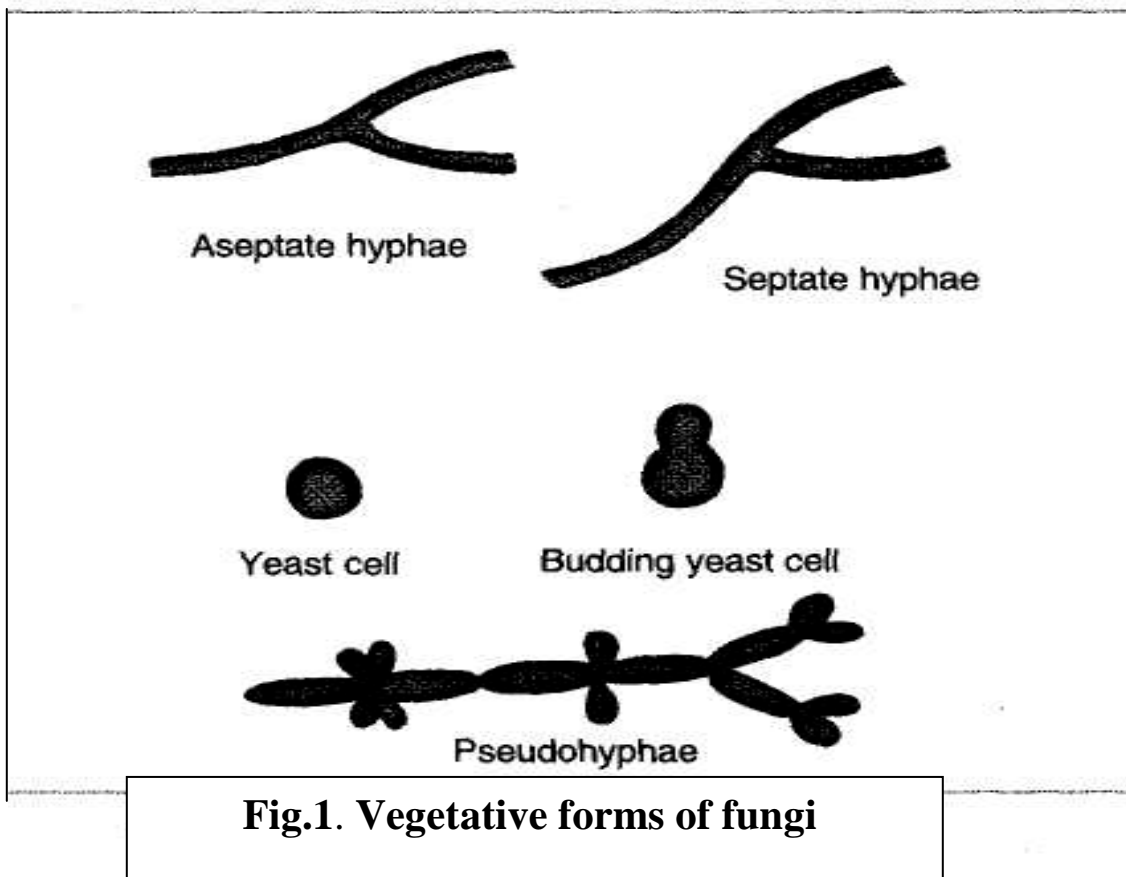
Based on the morphology, there are four main groups of fungi (Fig.1).

1. Yeasts
2. Yeasts like fungi
3. Moulds
4. Dimorphic fungi

Thallophyta

(Irregular plant masses lacking definite root, stem and leaf structures)





1. Yeasts

- (i) Round to oval unicellular fungi.
- (ii) Reproduce by budding.
- (iii) Form creamy mucoid colonies on culture media.
- (iv) The important pathogenic yeast is *Cryptococcus neoformans*.

2. Yeasts Like Fungi

These yeasts grow partly as yeasts and partly as chains of elongated budding cells joined end to end forming *pseudohypha*. Example is *C. albicans*.

3. Moulds

- (i) They grow as branching filaments called hyphae. Hyphae may be septate or non-septate.

(ii) The hyphae continue to grow and branch to form tangled mass of growth known as *mycelium*.

(iii) Dermatophytes, *Aspergillus*, *Penicillium* and *Rhizopus* are few examples of moulds.

4. *Dimorphic Fungi*

(i) Dimorphic fungi exist as yeasts in the host tissue and in the cultures at 37°C and as hyphal (mycelial) forms in the soil and in the cultures at 22-25°C.

(ii) *Blastomyces dermatitidis*, *Para-coccidioides brasiliensis*, *Coccidioides immitis*, *Histoplasma capsulatum* and *Sporothrix schenekii* are examples of dimorphic fungi.

IV. REPRODUCTION AND SPORULATION

1. Sexual spores are of four types — oospore, ascospore, zygospore and basidiospore (Fig.2).

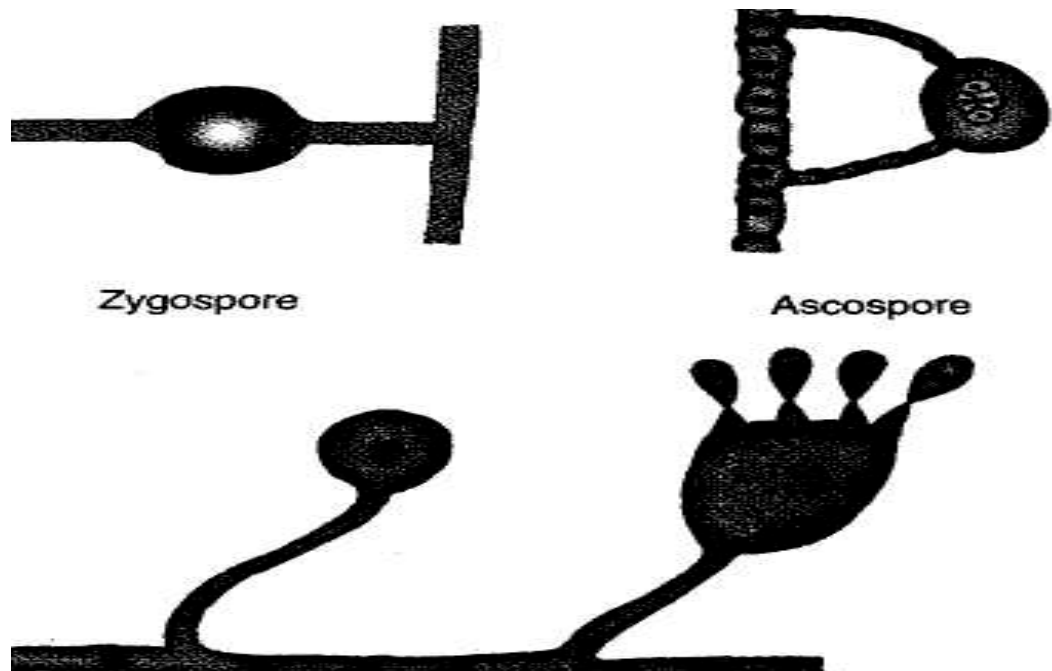


Fig.2. Sexual spores

2. Vegetative spores (Fig.3)

Blastospores: These are formed by budding from parent cell, as in yeasts.

Arthrospores: These are formed by the production of cross-septa into hyphae resulting in rectangular thick-walled spores.

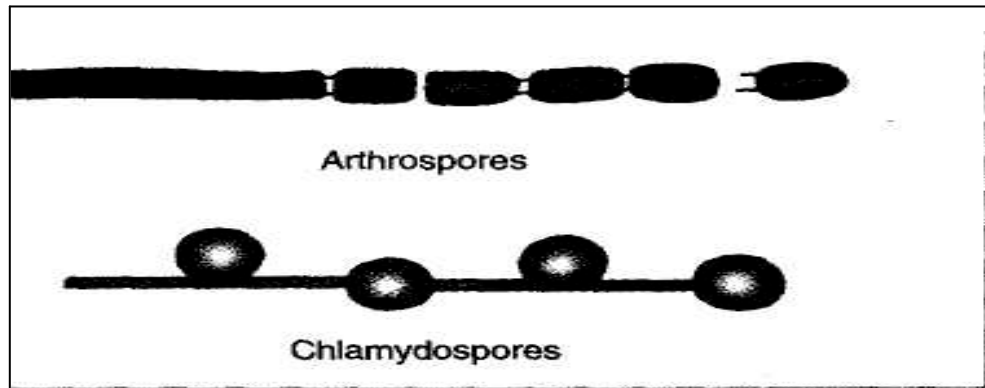


Fig.3. Vegetative spores

Chlamydospores: These are thick-walled resting spores developed by rounding up and thickening of hyphal segments.

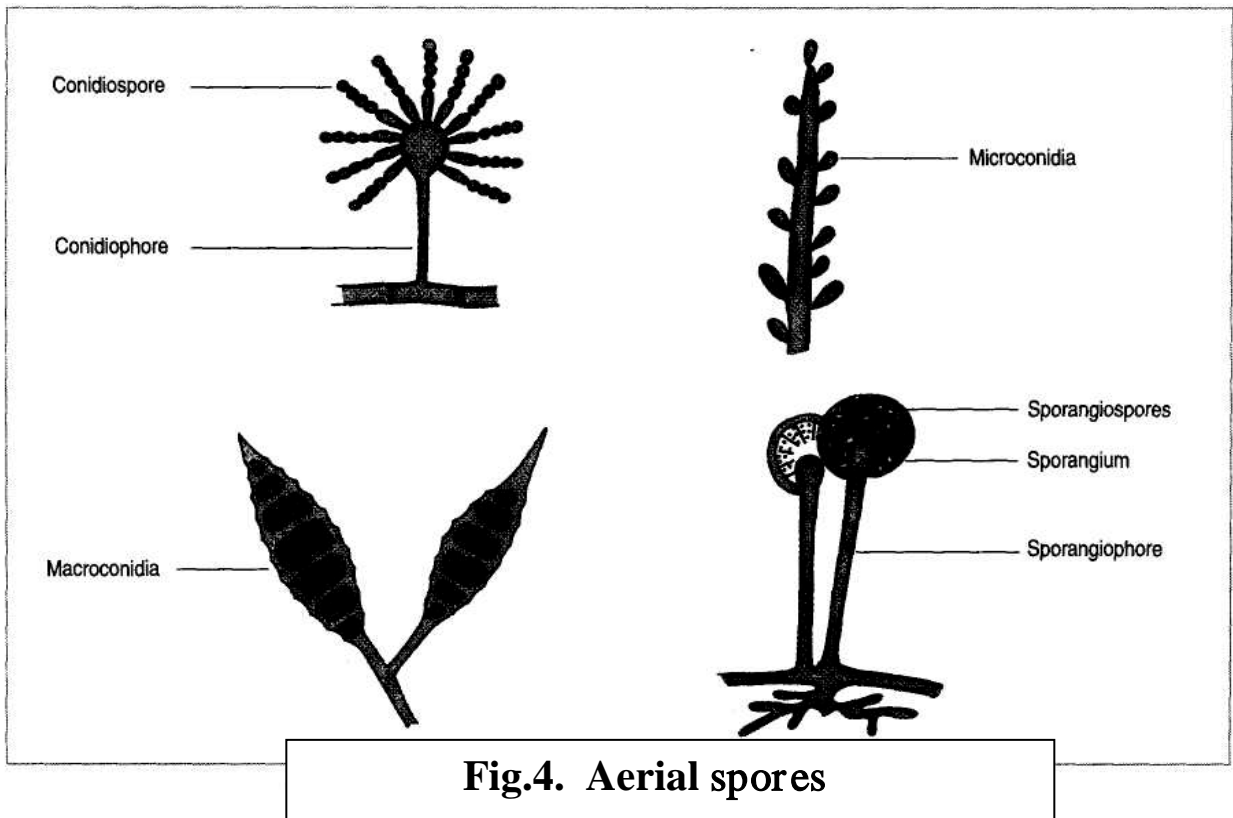
3. Aerial spores (Fig. 52.4)

Conidiospores: Spores borne externally on sides or tips of hyphae are called *conidiospores* or simply conidia.

Microconidia: When conidia are small and single, these are called *microconidia*.

Macroconidia: These are large and septate conidia and are often multicellular.

Sporangiospores: These are spores formed within the sporangium. Examples are *Mucor* and *Rhizopus*.



V. LABORATORY DIAGNOSIS

Direct Microscopy

1. *Potassium Hydroxide (KOH) Preparation*

Specimen is placed in a drop of 10% KOH on a microscopic slide and covered with a coverslip. It is heated gently and examined under microscope. Yeasts cells and hyphae may be observed (Fig. 5).

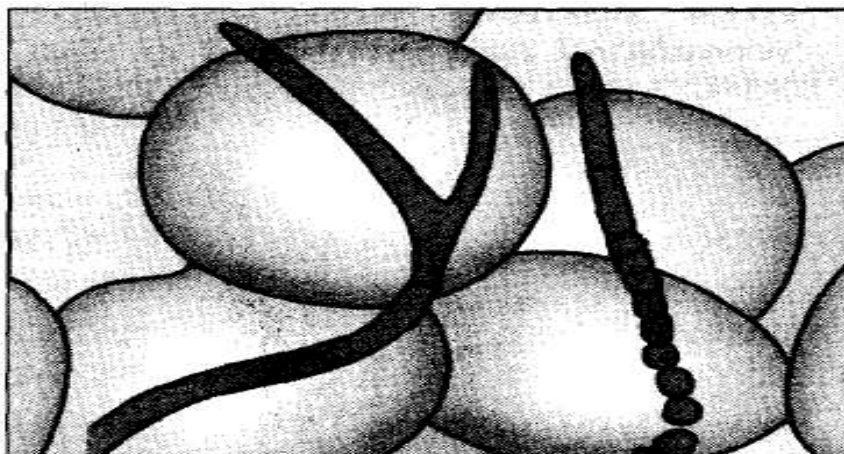


Fig. 5. KOH preparation showing hyphae

Hyphal diameter, presence or absence of septa and of special structures help in diagnosis. Special hyphal structures frequently found are spring like helical coils (*spiral hyphae*), resembling tennis racquets (*racquet hyphae*) and numerous short branches appearing at the ends of hyphae (*favic chandelier*) (Fig. 6).

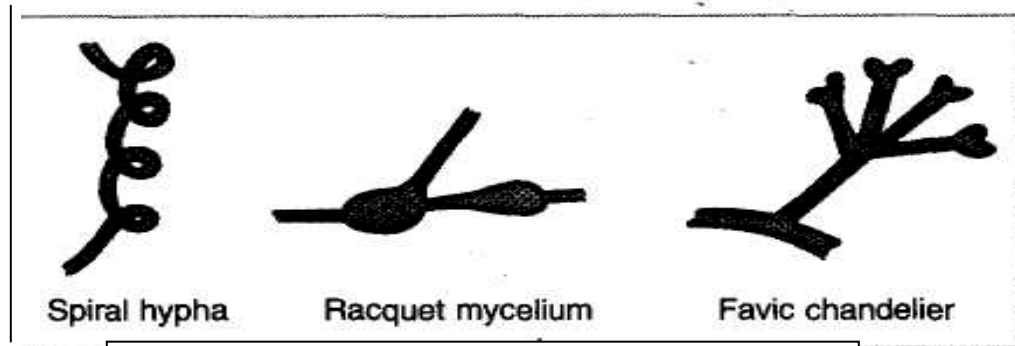


Fig. 6. Different forms of hyphae

2. KOH with Calcofluor White

A drop of calcofluor white solution can be added to the KOH preparation before covering it with the coverslip. Fungal elements fluoresce due to binding of calcofluor white to the fungus.

3. Gram Staining

It is done to observe Gram positive yeasts as in case of *Candida* species.

4. India Ink Preparation

Indian ink preparation may be used for detection of capsulated yeast such as *Cryptococcus neoformans* in cerebrospinal fluid (CSF).

Culture

1. Sabouraud's dextrose agar (SDA) and SDA medium with antibiotics are inoculated and incubated at 25°C and 37°C for three weeks. Chloramphenicol is added in the culture medium to suppress the growth of contaminating bacteria while cycloheximide (actidione) is incorporated to

suppress the contaminating fungi.

Brain heart infusion (BHI) agar with blood and antibiotics is another medium used for primary isolation of fungi.

2. Microscopy is performed from fungal colony (in teased mounts or slide cultures) to study the morphology of hyphae, spores and other structures. Teased mounts are prepared in lactophenol cotton blue (LCB) which contains lactic acid, phenol and cotton blue.
3. Slide culture is done for studying the exact morphology of the fungus.

Tissue Sections

Fungal elements in tissue can be identified by methenamine silver stain and Periodic" Acid Schiff (PAS) stain.

VI. CLASSIFICATION OF FUNGAL DISEASES

Infection caused by fungus is known as mycoses.

Fungal infections are of three principal clinical types :

- (i) superficial mycoses,
- (ii) subcutaneous mycoses,
- (iii) systemic mycoses.

A. Superficial Mycoses

1. These are strictly surface infections involving skin, hair, nail and mucosa.
2. These include infections by dermatophytes, *Pityrosporum orbiculare* (Pityriasis versicolor), *Exophiala werneckii* (tinea nigra), *Piedria hortae* (black piedra) and *Trichosporon beigeli*.

B. Subcutaneous Mycoses

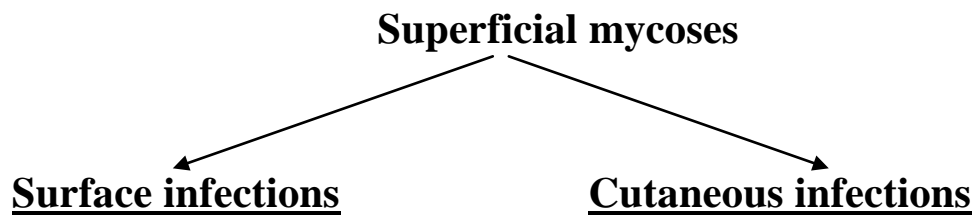
1. Saprophytic fungi of soil or decaying vegetation are usually introduced into

2. subcutaneous tissue and produce a progressive local disease.
3. Subcutaneous mycoses include mycetoma, chromoblastomycosis, sporotrichosis and rhinosporidiosis.

C. Systemic Mycoses

1. These are caused by fungi of soil and is acquired by inhalation.
2. Fungus may disseminate to CNS bones and other internal organs.
3. Systemic mycoses include blastomycosis, paracoccidioidomycosis, coccidioidomycosis, histoplasmosis and cryptococcosis.
4. Systemic mycoses and subcutaneous mycoses collectively are also named as: *Deep mycoses*.

Superficial Mycoses



e.g. Tinea versicolor, Tinea nigra, Piedra

e.g. Dermatophytes

Dermatophytes

- (i) Dermatophytes are a group of fungi that infect only superficial keratinised tissue (skin, hair and nails) without involving the living tissue.
- (ii) They break down and utilise keratin.
- (iii) They are incapable of penetrating subcutaneous tissue.
- (iv) They cause *dermatophytoses*, also known as tinea or ringworm.

Classification

Dermatophytes are classified into three genera as follows :

Genus	Infection of
1. <i>Trichophyton</i>	— Hair, skin, nail
2. <i>Microsporum</i>	— Hair, skin
3. <i>Epidermophyton</i>	— Skin, nail

Clinical types

Clinically, ringworm can be classified depending on the site involved. These include *Tinea capitis* (scalp), *Tinea corporis* (non-hairy skin of the body), *Tinea cruris* (groin), *Tinea pedis* (foot) or athlete's foot and *Tinea barbae* or barber's itch (bearded areas of the face and neck). Favus is a chronic type of ringworm involving the hair follicles. It leads to alopecia and scarring.

- In favus, there is sparse hyphal growth and formation of air spaces within the hair shaft.
- Two types of hair infection may be present, ectothrix and endothrix. In ectothrix, a sheath of arthrospores is present on the surface of hair shaft, while the arthrospore formation occurs entirely within the hair shaft in endothrix (Fig. 7).

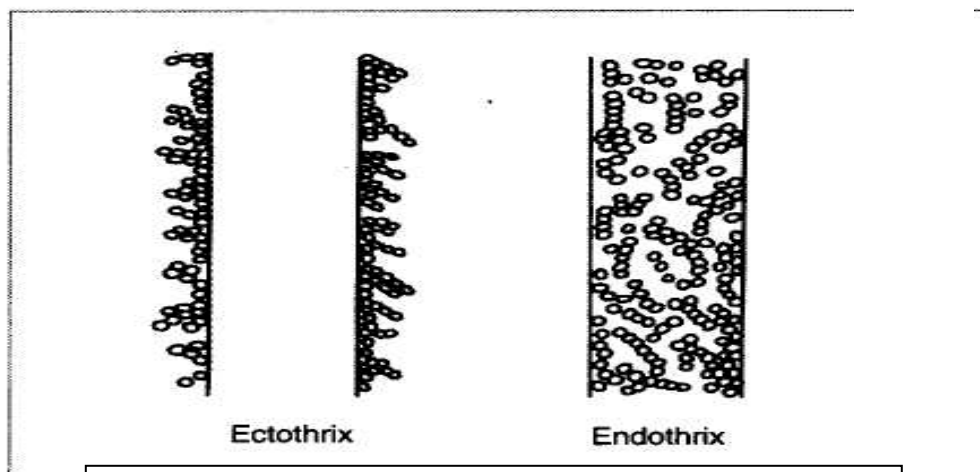


Fig. 7. Ectothrix and endothrix

Laboratory diagnosis

Specimens: Skin, hair and nail

Direct microscopy

- Direct 10% KOH mount may show fungal hyphae

Culture

- * SDA and SDA with antibiotics are used.
- * Culture media are incubated at 25-30°C for three weeks.
- * Identification of dermatophytes is based on
 - colony morphology
 - pigment production
 - microconidia and macroconidia
- * Colony characters
 - white to creamy, cottony growth
 - reverse of media is red in *T. rubrum*.
- * Microscopy
 - lactophenol cotton blue preparation from colony reveals microconidia, macroconidia or both.

The following are the characteristics of three genera :

- Genus *Trichophyton* — More microconidia, very few macroconidia
- Genus *Microsporum* — Predominant macroconidia
- Genus *Epidermophyton* — Macroconidia

Treatment of dermatophytoses

Topical antifungal agents are generally used for treatment. Oral griseofulvin is the drug of choice.

Subcutaneous Mycoses

1. Mycetoma

- (i) Mycetoma is a chronic granulomatous infection of the subcutaneous tissue, usually affects the foot and rarely the other parts of body.
- (ii) The disease was first described from Madurai, South India. It is therefore commonly referred to as *Madura foot* or *Maduramycosis*.
- (iii) It is seen mainly in the tropical countries.
- (iv) The disease is quite common in Tamil Nadu.

Aetiology

* Mycetoma is caused by a number of actinomycetes and filamentous fungi (Table 1).

Table 1. **Important Causative Agents of Mycetoma**

Eumycetoma	Actinomycetoma
<i>Acremonium falciforme</i>	<i>Actinomadura madurae</i>
<i>Madurella mycetomi</i>	<i>Actinomadura pelletieri</i>
<i>Madurella grisea</i>	<i>Nocardia brasiliensis</i>
<i>Exophiala jeanselmei</i>	<i>Streptomyces somaliensis</i>
<i>Pseudoallescheria boydii</i>	

Pathogenesis

- The causative organism is believed to enter the body through minor trauma.
- The disease begins as a subcutaneous swelling usually of foot, which enlarges and burrows into the deeper tissues producing characteristic abscess. The abscess bursts with the formation of chronic multiple sinuses discharging viscid,

seropurulent fluid containing granules.

- The colour and consistency of the granules vary with the different causative agents.

Laboratory diagnosis

- Diagnosis is made from examination of granules.
- The granules are composed of very thin filaments in actinomycotic mycetoma while they are broader and often show septae and chlamydo spores in mycotic mycetoma.

Treatment

Actinomycotic mycetoma usually respond well to sulphonamides and antibiotics, but mycotic mycetoma may require amputation.

2. Chromomycosis

(i) It is a chronic, localised infection of skin and subcutaneous tissue.

(ii) The fungus enters the body through a wound.

Aetiological agents

- It is caused by several darkly pigmented fungi of the family *Dematiaceae*.

These include:

Fonsecaea pedrosoi

Fonsecaea compactum

Cladosporium carrionii

Phialophora verrucosa

* The term chromomycosis includes chromoblastomycosis and other infections caused by dematiaceou fungi (Phaeohyphomycosis). Chromoblasto mycosis is usually confined to the subcutaneous tissue of the feet and lower legs. Phaeohyphomycosis may affect cutaneous, subcutaneous, deeper tissues or organs like brain or lung.

Laboratory diagnosis

Chromoblastomycosis

- Histologically, in H and E staining they appear as yeast like bodies with septae, called *sclerotic bodies*.
- These sclerotic bodies can be demonstrated in KOH mounts and by culture on SDA.

Phaeohyphomycosis

- The fungi appear in lesions as distorted hyphal strands. Sclerotic bodies are absent.

Treatment

- Amphotericin B and 5-fluorocytosine have been found useful.

3. Sporotrichosis

- (i) Sporotrichosis is a nodular, ulcerating disease of skin and subcutaneous tissue.
- ii) The fungus gains access through thorn pricks or some injuries.
- ii) The fungus spreads through lymphatics up to regional lymph nodes and rarely beyond that.

Causative agent

- *Sporothrix schenckii* — a dimorphic fungus.

Oral manifestations

There is nonspecific ulceration of the nasal, oral and pharyngeal mucosa, usually associated with local lymphadenopathy. Lesions heal by soft scars but the fungus may still be present in these tissues.

Laboratory diagnosis

- Diagnosis is made by culture as the fungus may not be demonstrable in pus or tissues.
- *S. schenckii* occurs in the yeast phase in the tissues and in cultures at 37°C, and in the mycelial phase in cultures at 22°C-25°C (*dimorphic fungus*).

- *Yeast* phase appears as cigar-shaped cells and mould form contains hyphae carrying flower-like clusters of small conidia borne on delicate sterigmata (Fig. 8).

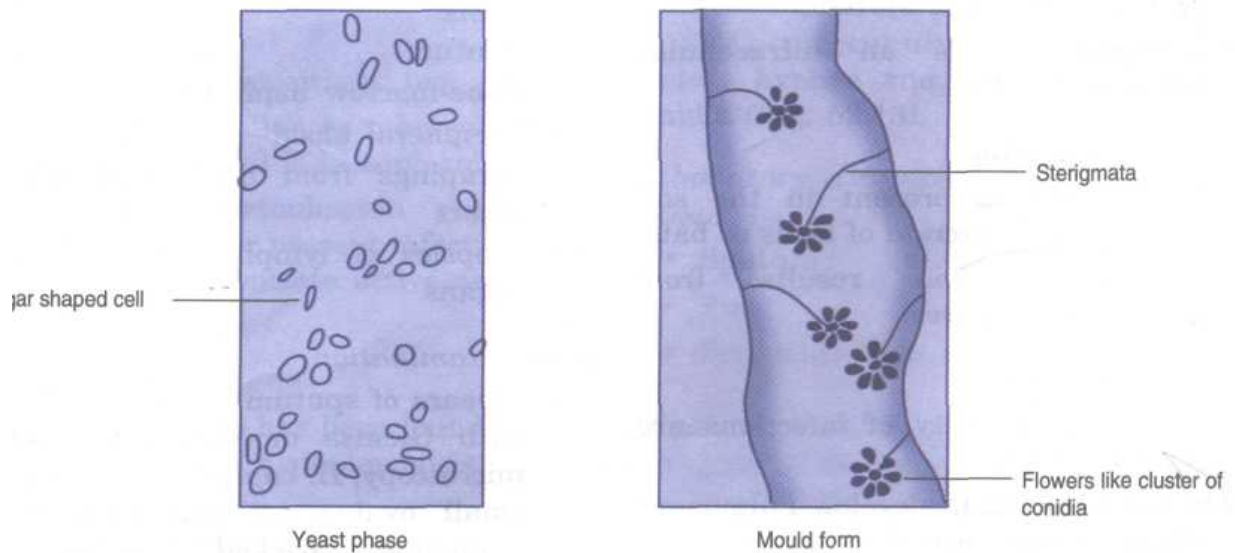


Fig. 8. Sporothrix schenckii

4. Rhinosporidiosis

- Rhinosporidiosis is a chronic granulomatous disease characterised by formation of friable polyps, usually confined to the nose, mouth or eye.
- Causative agent is *Rhinosporidium seeberi*.
- More than 80% cases are reported in India and Sri Lanka.
- The mode of infection is not known but most infections occur in males who have frequent contact with stagnant water or aquatic life.

Oral manifestations

Oronasopharyngeal lesions appear as soft red polypoid growth which spread to pharynx and larynx. These lesions often contain mucoid discharge and are vascular.

Laboratory diagnosis

- The fungus has not been cultivated.

- Diagnosis depends on the demonstration of sporangia.
- Tissue sections stained with H and E stain show large number of endospores tissue and capillaries. The sporangium contains thousands of endospores (Fig. 9).

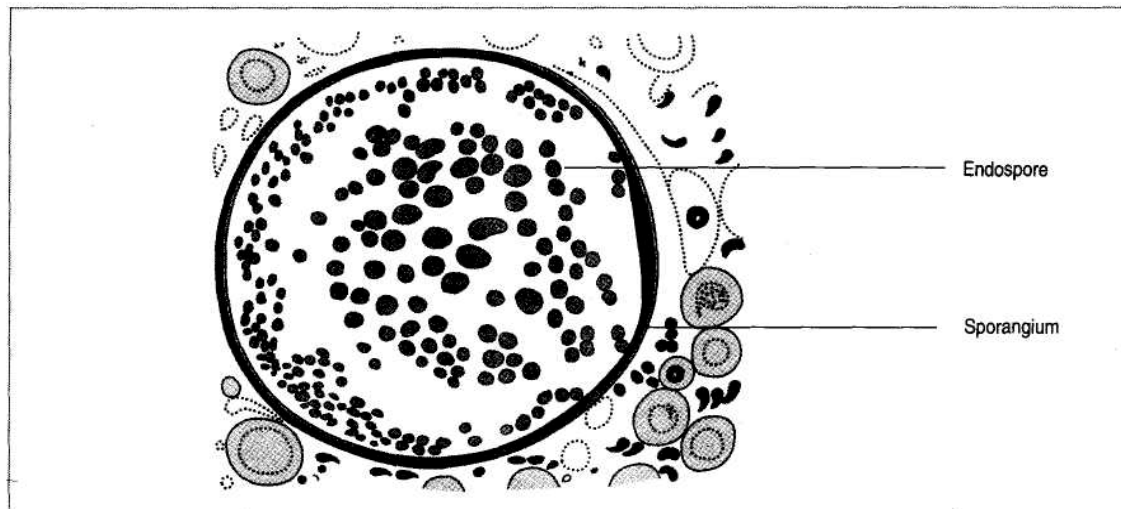


Fig. 9. Rhinosporidiosis: sporangium with numerous endospores

Systemic mycoses

1. Histoplasmosis

- (i) Causative fungus: *Histoplasma capsulatum*, a dimorphic fungus
- (ii) It is primarily a disease of reticuloendothelial system
- (iii) *Histoplasma capsulatum* is an intracellular parasite.

Source of infection

- The fungus is present in the soil enriched with excreta of birds or bats.
- Human infection results from inhalation of spores.

Clinical features

- The large majority of infections are asymptomatic.
- Some individuals develop pulmonary disease which resembles tuberculosis.
- Disseminated histoplasmosis develops only in a minority of infected individuals.

- Involvement of reticuloendothelial system results in lymphadenopathy, fever, hepatosplenomegaly, anaemia and a high rate of fatality.
- Granulomatous and ulcerative lesions may develop on the skin or mucosa.

Oral manifestations

The nodular, ulcerative or vegetative oral lesions may be present on the buccal mucosa, lips, gingiva, tongue to palate. The ulcerative areas are indurated and are usually covered by a grey membrane.

Laboratory diagnosis

Specimens

- * Sputum
- * Bone-marrow aspirate
- * Peripheral blood
- * Scrapings from dermal or mucosal ulcers
- * Biopsies of lymph nodes and other organs.

Direct examination

- Smears of sputum or pus are stained with Giemsa or Wright stains. On microscopy, *H. capsulatum* appears as small oval yeast cell, packed within the cytoplasm of macrophages or monocytes (Fig. 10).

Culture

- SDA or brain heart infusion (BHI) agar with cycloheximide and chloramphenicol are inoculated.
- The yeast phase is formed in cultures at 37°C. White cottony mycelial growth containing large thick walled, spherical spores with tubercles or finger like projections (Fig. 11) appears at 25°C.

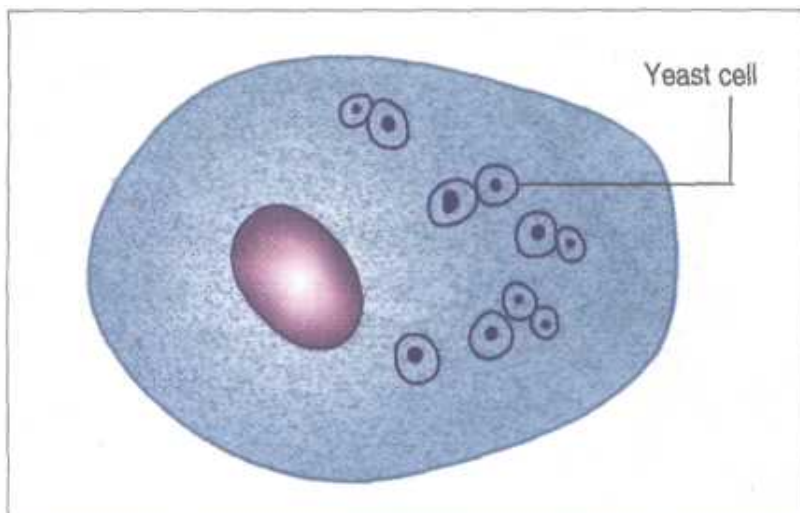


Fig. 10. Histoplasma capsulatum: yeast cells in macrophage

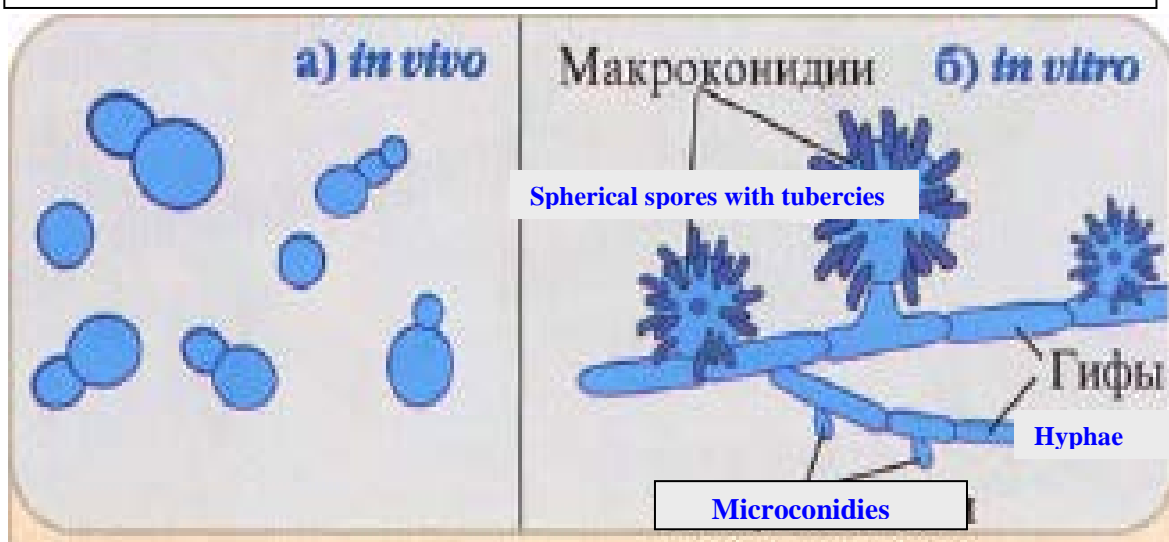


Fig. 11. Histoplasma capsulatum in vivo and in vitro

Histoplasma skin test

- Delayed hypersensitivity test
- The test is similar to tuberculin test but antigen used is histoplasmin.
- A positive histoplasmin skin test indicates past or present infection, but does not differentiate active and past infections.

Treatment

- Amphotericin B has been found useful in treatment of histoplasmosis.

2. Blastomycosis

(i) Causative fungus: *Blastomyces dermatitidis*, a dimorphic fungus.

(ii) It is a chronic infection of the lungs which may spread to other tissues, particularly skin, bone and genitourinary tract.

(iii) As the infection is confined to the North American Continent, it is also known as *North American blastomycosis*.

Source of an infection

- Inhalation of conidia of fungus growing as saprophytes in the soil.

Clinical features

- Asymptomatic.
- Mild primary pulmonary disease.
- Disseminated lesions have been found in immunocompromised patients including AIDS.
- Cutaneous blastomycosis.

Oral manifestation

The oral lesions may be primary in origin or secondary to lesions of some other sites in the body. These lesions may resemble to those of actinomycosis.

However, abscess formation is not as prominent as that in actinomycosis. Tiny ulcers may be the only main clinical feature.

Morphology

B. dermatitidis is a dimorphic fungus. In tissues and in cultures at 37°C, the fungus appears as spherical or oval budding yeast cells with thick, double contoured walls.

Each cell has only a single broad based bud. At 25°C, the culture is filamentous with septate hyphae and many round or oval conidia (Fig. 12).

Laboratory diagnosis

Specimens

- Sputum
- Pus
- Scrapings from skin lesions

Direct examination

- 10% KOH mount shows thick walled yeast cells with a single broad based bud.
- H and E and PAS stains also show yeast cells in sections.

Culture

- SDA or blood agar.
- Mycelial phase occurs slowly incubation at 25°C. Yeast phase seen in cultures incubated at 37°C.

Treatment

Amphotericin B is the drug of choice.

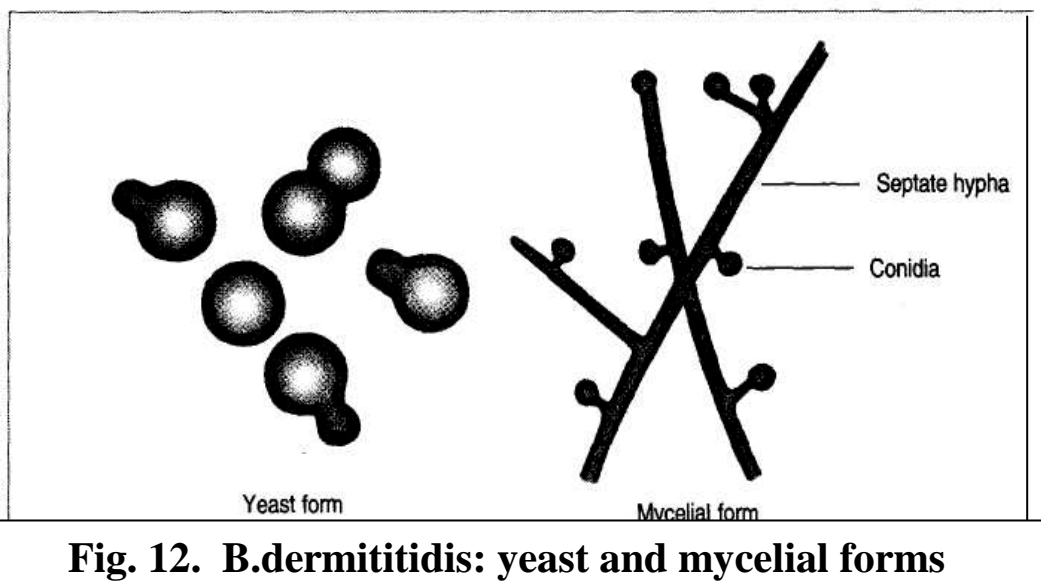


Fig. 12. B. dermatitidis: yeast and mycelial forms

3. Paracoccidioidomycosis

- (i) Causative fungus: *Paracoccidioides brasiliensis*, a dimorphic fungus.
- (ii) It is a chronic granulomatous disease involving lungs, mucosa, skin and lymphatic system.
- (iii) As the disease is confined to South America, it was formerly called *South American blastomycosis*.

Source of an infection

By inhalation of spores from environmental sources.

Clinical features

* Primary pulmonary infection that spreads by haematogenous route to mucous membranes of mouth, nose, lymph node and adjacent skin, producing chronic granulomatous reaction.

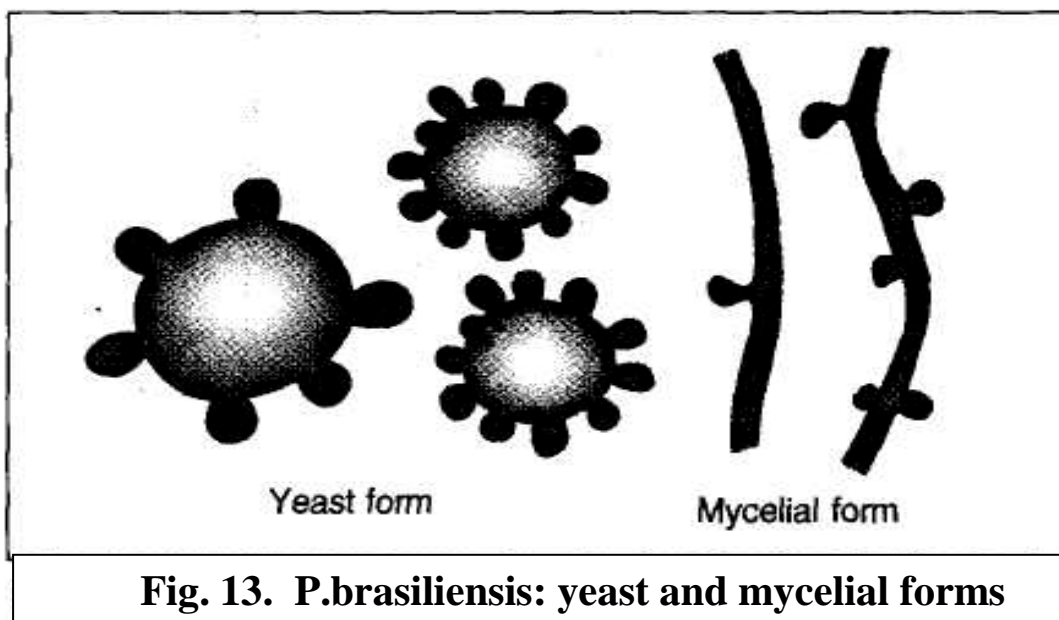
* Ulcerative granulomas of the buccal and nasal mucosa are a prominent feature.

Oral manifestations

The fungus may enter through the periodontal tissues. It produces a severe regional lymphadenopathy. When the fungus penetrates the tissues, it may produce papillary lesions of the oral mucosa. Extensive oral ulceration is also a common feature.

Morphology

• As a dimorphic fungus, it grows as yeast cells with multiple buds in tissues and in cultures at 37°C and in the mycelial phase at 25°C (Fig.13).



Laboratory diagnosis

Specimens

- Sputum
- Pus
- Biopsies from granulomatous lesion

Direct microscopy

- Microscopical examination in KOH mount of the specimen shows numerous yeast cells with multiple buds.
- Tissue sections should be stained with H and E and PAS stains.

Culture

- Mycelial phase of the fungus develops on SDA incubated at 25°C.
- Yeast phase may be obtained by inoculating specimen on enriched media such as BHI agar and incubating it at 37°C.

Treatment

Amphotericin B is the drug of choice.

4. *Coccidioidomycosis*

Causative fungus: *Coccidioides immitis*, a dimorphic fungus.

Source of infection

- By inhalation of dust containing arthrospores of the fungus.

Clinical features

- Asymptomatic.
- Primary pulmonary disease — mild influenza-like fever to severe pneumonia.
- Disseminated disease.

Oral manifestation

Proliferative granulomatous and ulcerative lesions appear on the oral mucosa and skin. These lesions tend to heal by scar formation. Lesions are often chronic in nature. Sometimes it may occur as lytic lesions of the jaws.

Morphology

- Being a dimorphic fungus, it occurs in the tissue as a yeast and in culture (both at 37°C and at 25°C) as the mycelial form.
- The yeast form is a spherule with a thick doubly retractile wall and filled with endospores (Fig. 14). Each endospore gives rise to a new spherule.

- The mycelial phase contains pseudohyphae which fragment into arthrospores (Fig. 14) which are highly infectious.

Specimens

- Sputum
- Pus
- Biopsy material

Direct microscopy

- Microscopic examination of the specimen detects large number of mature spherules.

Culture

- Specimen is inoculated on SDA medium in the test tube.
- Inoculated media are incubated at 25°C for 3 weeks.
- Septate hyphae which fragment into thick walled arthrospores in chains are found.

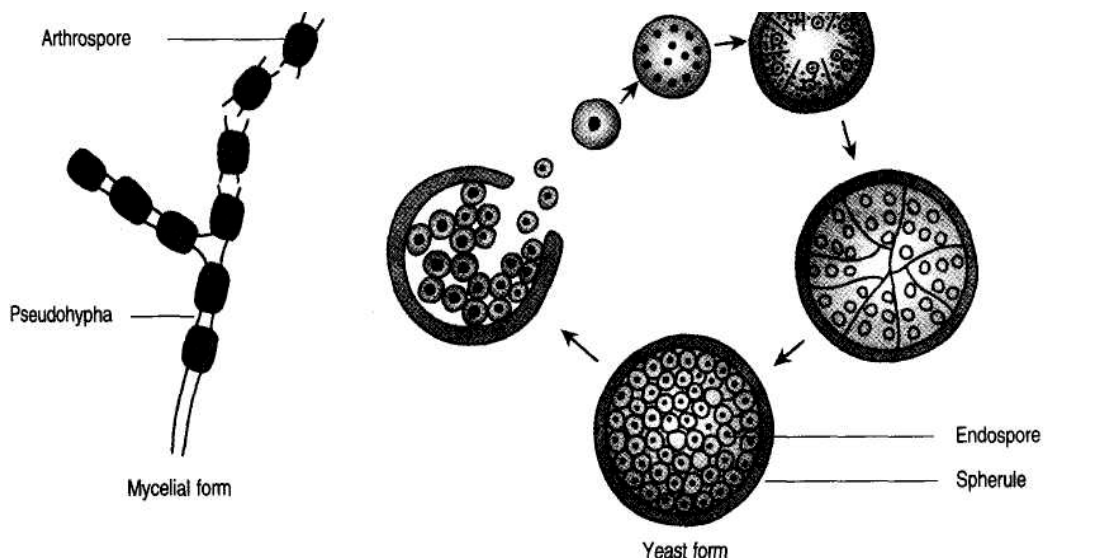


Fig. 14. C.immitis: arthrospore and spherule stages

Skin test

- It is an intradermal skin test using 'coccidioidin', an antigen from the fungus.

5. Cryptococcosis

- (i) Causative fungus: *Cryptococcus neoformans*, a capsulated yeast.

(ii) It is a soil saprophyte and is particularly abundant in the feces of

Morphology

- Spherical budding cell having a prominent polysaccharide capsule (Fig. 15).
- It is a true yeast and is Gram positive.
- Capsule may be demonstrated by India-ink or nigrosin staining.
pigeons.

(iii) Several cases of cryptococcosis have been identified in India.

Antigenicity

- On the basis of cryptococcal polysaccharide antigen, *C. neoformans* has four serotypes (A, B, C and D).

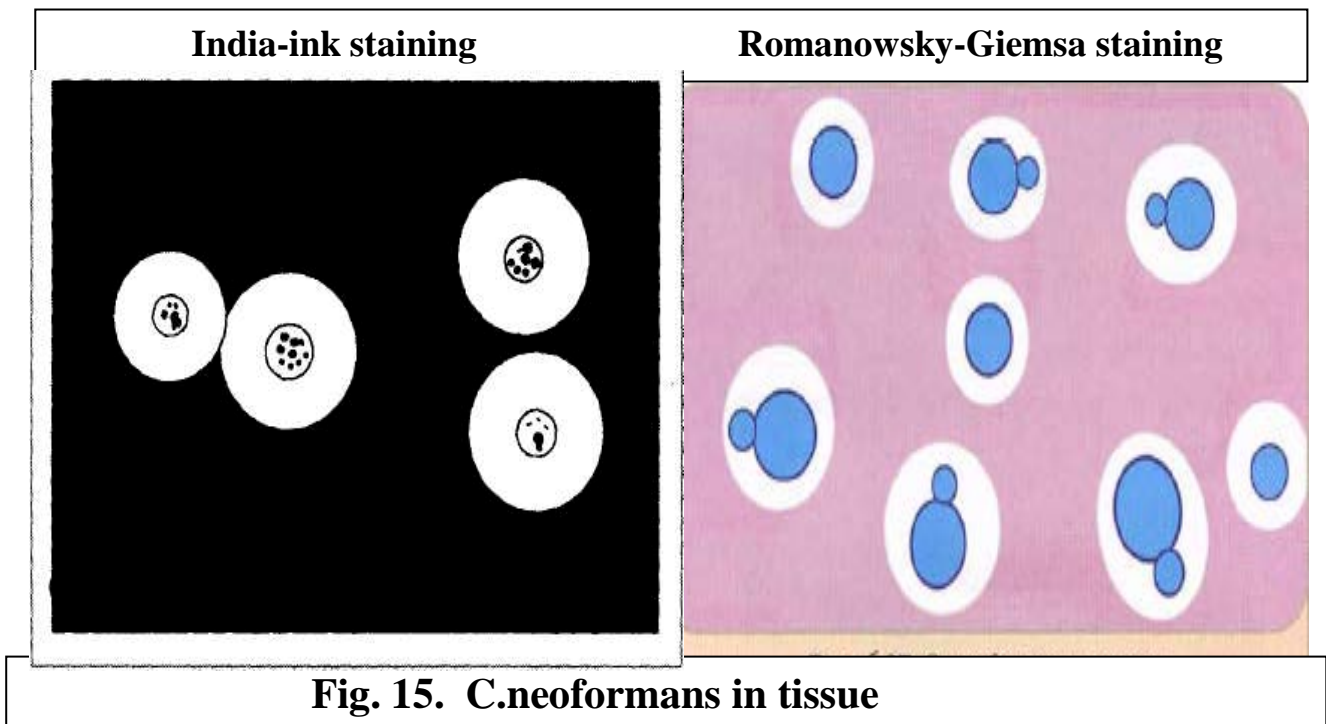


Fig. 15. C. neoformans in tissue

Source of infection

- Infection is usually acquired by inhalation of dust containing yeast cells.

Pathogenesis

- *C. neoformans* is pathogenic in humans and animals.
- The disease is usually seen in immunocompromised host.
- Most infections are asymptomatic.
- Pulmonary cryptococcosis may lead to a mild pneumonitis.
- Cryptococcal meningitis occurs by haematogenous spread. It is often seen in AIDS patients.
- Skin, lymph nodes, bones and other organs may be involved when dissemination of infection occurs. Cutaneous cryptococcosis varies from small ulcers to large granulomas.

Laboratory diagnosis

Specimens

- CSF
- Sputum
- Pus
- Brain tissue

Direct microscopy

- Specimen mixed with a drop of India ink or nigrosin shows round budding yeast cells. In India ink, capsule appears as a clear halo around the yeast cells.
- Gram staining shows Gram positive yeast cells.
- The histopathological examination of tissue can be done by staining with H and E, PAS and mucicarmine stains.

Culture

- Sediment from a centrifuged CSF is inoculated on SDA and incubated at 37°C. Other specimens may also be inoculated on SDA.
- *C. neoformans* grows to form smooth, mucoid, cream coloured colonies.
- Lactophenol cotton blue mount shows budding yeast cells.
- Niger seed (bird seed) agar is a differential medium for presumptive identification of *C. neoformans*. It produces brown colonies on this medium.

Latex agglutination test

Cryptococcal capsular polysaccharide antigen can be detected in CSF, serum or urine by latex agglutination test.

Animal inoculation test

- Intracerebral or intraperitoneal inoculation into mice leads to a fatal infection in case of *C. neoformans*.

Other tests *C. neoformans* hydrolyses urea.

VII. OPPORTUNISTIC MYCOSES

Some saprophytic fungi usually do not infection under special conditions such as immunocompromised individuals and in terminal stages of chronic disease.

The incidence of these fungal infections has increased with widespread use of antibiotics, corticosteroids and immunosuppressive drugs.

These are called *opportunistic fungi*.

- Some of these are common laboratory contaminants in culture media and grow on virtually anything.

Aspergillus and *Penicillium* grow on damp bread and other organic matter.

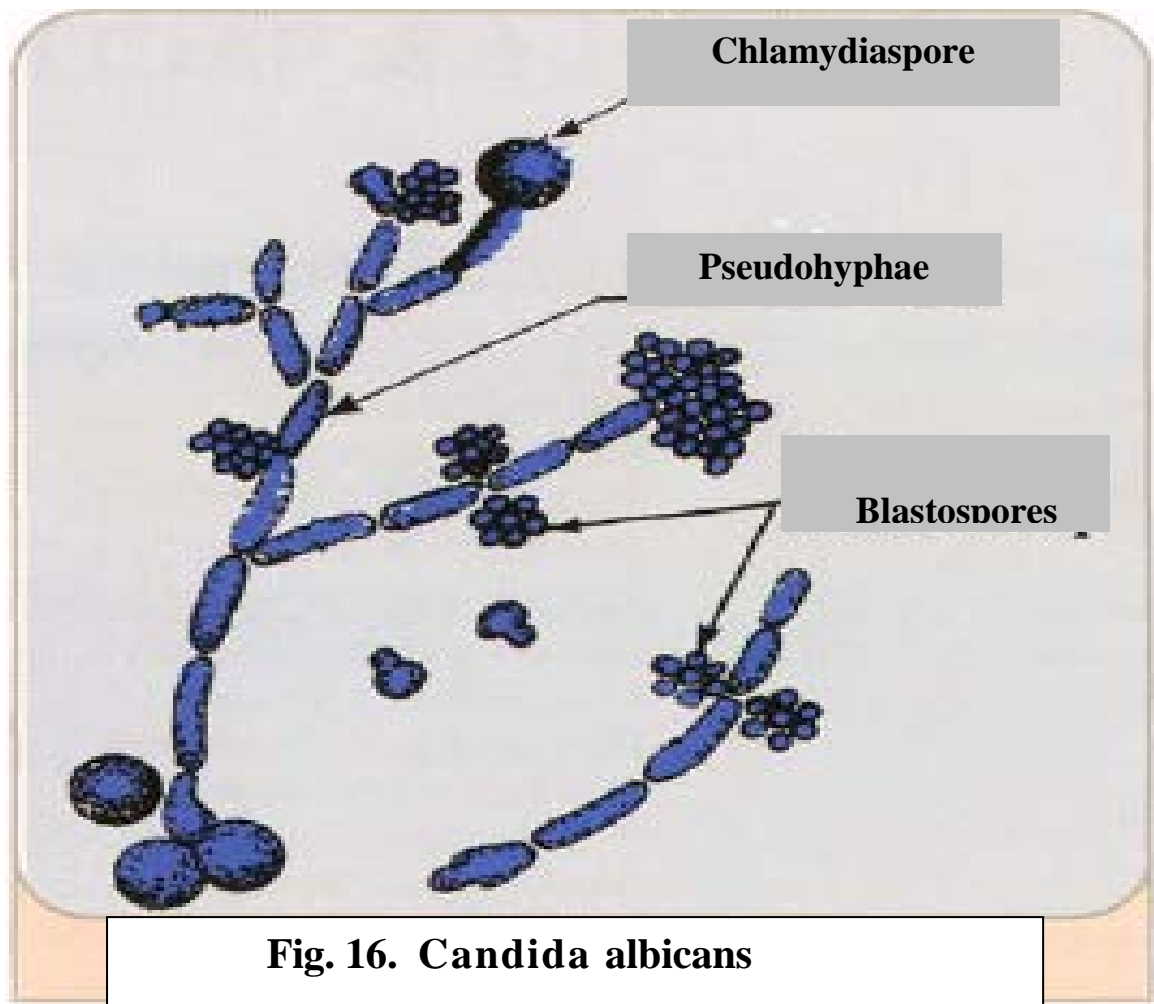
- These fungi can produce serious and even fatal infections in persons who are otherwise debilitated.

Candidiasis

1. Causative fungus: *Candida albicans* (80-90% of cases).
2. Causative fungus: *Candida albicans* (80-90% of cases).
3. Candidiasis is an infection of skin, mucosa and internal organs, caused by yeast like fungus *Candida albicans*, and occasionally by other *Candida* species.
4. *Candida albicans* is the normal inhabitant of skin, gastrointestinal tract, oral and vaginal cavities.

Morphology

C. albicans is an ovoid or spherical budding yeast cell, 3-5 um in diameter. (Fig. 16).



Pathogenesis

- (i) Candidiasis is an opportunistic endogenous infection.
- (ii) Predisposing factors for candidiasis are diabetes, immunodeficiency, malignancy, prolonged administration of antibiotics, patients on immunosuppressive drugs and intravenous catheters.
- (iii) Lesions caused by *Candida* are as follows:

(a) *Mucocutaneous lesions*

- Oral thrush
- Vulvovaginitis
- Balanitis
- Conjunctivitis
- Keratitis

(b) *Skin and nail infections*

- Skin—Infections of axillae, groin, perineum and submammary folds.
- Nails—Infections of finger webs, nail folds and nails may occur. Paronychia and onychia are seen in those persons who frequently immerse their hands in water.
- In infants it may lead to napkin dermatitis

(c) *Systemic candidiasis*

- * Urinary tract infection
- * Intestinal candidiasis: It is a frequent sequel to oral antibiotic therapy and present as diarrhoea.
- * Pulmonary candidiasis.
- * Endocarditis.
- * Meningitis.
- * Septicaemia.

(d) *Oral manifestations*

Various manifestations of oral candidiasis are as follows :

- Thrush
- Chronic oral candidiasis
- Chronic mucocutaneous candidiasis
- Angular stomatitis (angular cheilitis)
- Circumoral candidal dermatitis.

Thrush (Pseudomembranous candidiasis): The lesions are soft, white, slightly elevated plaques frequently occurring on the buccal mucosa of tongue, but may also be seen on the gingiva, palate and floor of the mouth. In severe cases, the entire oral cavity may be affected.

Thrush is very common in patients with HIV infection or in cancer patients undergoing chemotherapy or radiotherapy. Infection may also be seen in neonates and infants due to incompletely developed immune system. It is also common in debilitated and chronically ill persons.

Chronic oral candidiasis: It may be denture-induced stomatitis or chronic hypertrophic candidiasis (candidial leukoplakia). Denture-induced stomatitis is usually seen on the palate. It is due to tight-fitted denture which may

prevent the contact of the underlying mucosa with saliva which have antimicrobial properties. In chronic hypertrophic candidiasis, the plaque is tightly adherent, usually on the cheeks, lips and tongue.

Chronic mucocutaneous candidiasis : Both intraoral and extraoral lesions may be present. The oral lesions are similar to other types of candidiasis. In diffuse type of mucocutaneous candidiasis, severe candidiasis of skin, nails and mucous (such as oral, conjunctival, vaginal) develops.

Angular stomatitis (angular cheilitis) : Lesions are present on the lips and are common in immunocompromised individuals.

Circumoral candidal dermatitis : It involves the lips and area around lips.

Laboratory Diagnosis

(i) Direct microscopy

Gram stained smears and KOH mounts from lesions of skin, nail or mucous membranes show budding Gram positive yeast cells.

(ii) Culture

- (a) Candida species grow well on SDA and ordinary bacteriological culture media e.g. blood agar.
- (b) They grow at 25-37°C within 24 hours.
- (c) Cream coloured, smooth, pasty colonies appear.
- (d) Gram stained smear from colonies shows Gram positive budding yeast cells.

(iii) Identification

To differentiate *C. albicans* from other species, the following tests are done.

- (a) Germ tube test : *C. albicans* has ability to form germ tubes within two hours when incubated in human serum at 37°C (*Reynolds-Braude phenomenon*).

- (b) Chlamydoconidia: Chlamydoconidia develop in a nutritionally deficient medium such as *cornmeal agar* at 20°C. They can be seen at the end of pseudohyphae.

Treatment

- (i) Predisposing factors are to be removed in all cases.
- (ii) Topical application of polyene (nystatin) or imidazole (miconazole, clotrimazole) is effective in superficial infections.
- (iii) Amphotericin B is administered alongwith 5 fluorocytosine in systemic infections.

Aspergillosis

1. Aspergilli are ubiquitous in nature.
2. *Aspergillus fumigatus* is the main opportunistic pathogen.
3. Other important species are *A. niger* and *A. flavus*. (Fig. 17).

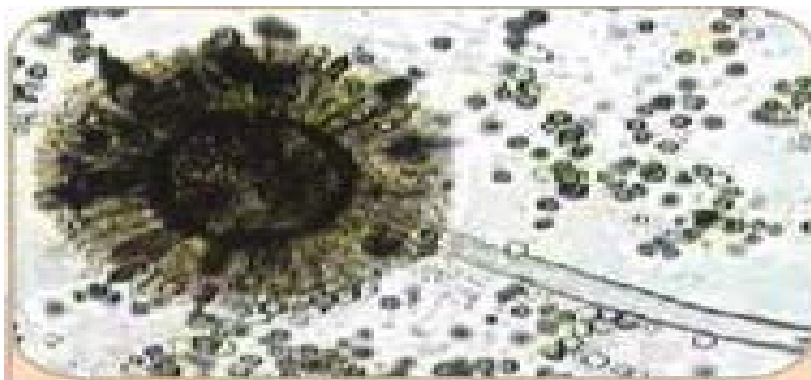


Fig. 17. Aspergillus niger

Pathogenesis

- * Aspergillosis is caused by inhalation of *Aspergillus* conidia or mycelial fragments which are present on the decaying matter, soil or air.
- * When the host defence is compromised, aspergillosis may develop.
- * There are three clinical forms of systemic aspergillosis,

(a) Respiratory disease

- Aspergillus asthma—Hypersensitivity to aspergilli may occur in atopic individuals.

Bronchopulmonary aspergillosis — The fungus grows within the lumen of the bronchioles, which may be occluded by fungus plugs.

- Aspergilloma—The fungus colonises in the preexisting pulmonary cavities such as in tuberculosis or cystic disease. It is often called *fungus ball*.

(b) Invasive aspergillosis

Invasive or disseminated aspergillosis occurs in severely immunocompromised individuals.

(c) Superficial infections

- Sinusitis
- Mycotic keratitis
- Otomycosis

Laboratory Diagnosis

Specimens

- Sputum
- Bronchoalveolar lavage
- Biopsy

Direct microscopy

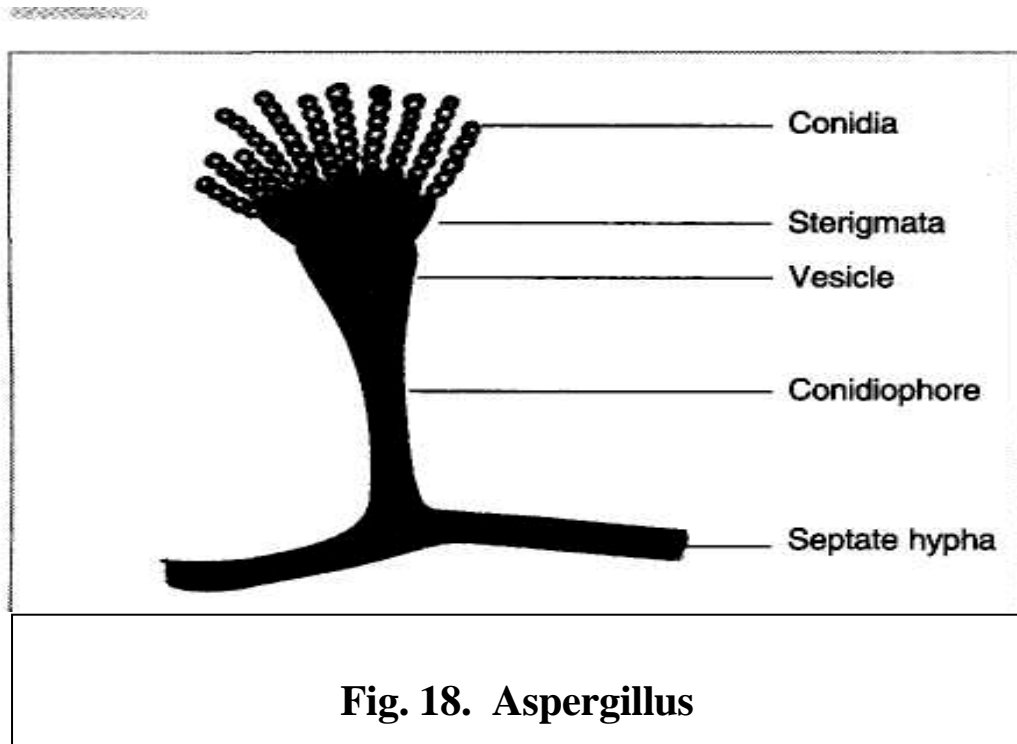
- KOH preparation of the specimen reveals non-pigmented septate hyphae with characteristic dichotomous branching (at an angle of approximately 45°).
- Biopsy sections can be stained with H and E and PAS staining and examined for the characteristic hyphae.

Culture

- The clinical specimen is inoculated on SDA without cycloheximide and incubated at 25°C.
- Colonies appear within 1-2 days and show a velvety to powdery surface. Colonies are coloured. A *fumigatus*—green coloured colonies, A *niger*—black colonies and A *flavus*— golden-yellow coloured colonies.
- Lactophenol cotton blue preparation from colonies shows branching and septate hyphae.

Asexual conidia are arranged in chains, carried on sterigmata, borne on the expanded ends (vesicles) of conidiophores.

(Fig. 18).



Penicillosis

Etiology: Penicillium

Specimen: Skin lesion scrapings

Direct microscopic examination: KOH preparation shows small, round spores and hyphae.

Culture:

On Sabouraud's dextrose agar with chloramphenicol at 25 °C for 1 to 4 days the colonies appear which are white first and later on becomes blue green. Surface of these colonies is usually velvety or powdery.

Microscopic examination of these colonies shows brush like arrangement of conidia, sterigmata and conidiophores.

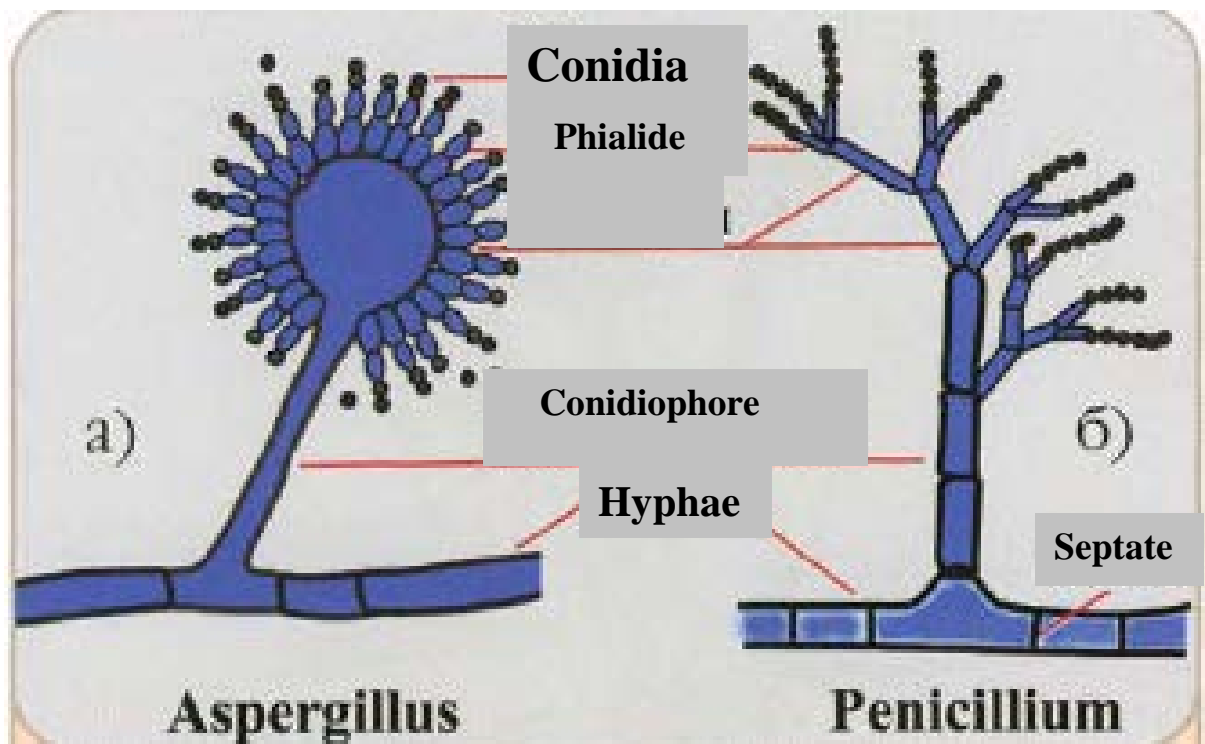


Fig. 19. Fungi: a) genus Aspergillus, b) genus Penicillium

Clinical picture:

Penicillium species are implicated in otomycosis and mycotic keratitis. In otomycosis there is inflammation, puritis, exfoliation of epithelium and often, by deafness when ear canal is occluded by a plug of hyphae. In mycotic keratitis there may be corneal ulcer or hypopyon or both. However corneal trauma, corneal disease glaucoma predispose to mycotic. There may be pulmonary and cerebral penicillosis.

Treatment:

In otomycosis 5% aluminum acetate solution may be used to reduce edema and to remove epithelial debris. Aqueous solution of 0.02 to 0.1% phenyl mercuric acetate, thymol (1%) in meta cresyl acetate and iodochlorohydroxyquin are more effective drugs.

In mycotic keratitis, topical application of amphotericin B is quite useful.

Zygomycosis

1. Zygomycosis includes mucormycosis and entomophthoramycesis. The latter is an infection of the subcutaneous tissue or paranasal sinuses.
2. Three genera of class *Zygomycetes*, *Mucor*, *Rhizopus* and *Absidia* are associated with zygomycosis. (Fig. 20, 21).
3. These fungi are saprophytes of soil, manure and decaying vegetables.
4. These are normally avirulent and are able to cause disease only when general resistance is extremely low.
5. The primary infection is usually in upper respiratory tract or nose.
6. Rhinocerebral form of disease is characterised by spreading lesion from nasal mucosa to turbinate bone, paranasal sinuses, orbit and brain.
7. *Mucor* and *Absidia* are most commonly isolated from mucormycosis.
8. *Rhizopus* causes zygomycosis and otomycosis.
9. *Absidia* may also cause keratitis.
10. Pulmonary mucormycosis is a progressive severe pneumonia. The fungi may spread haematogenously to other areas of the lung and to other organs such as brain.
11. Gastrointestinal mucormycosis may occur in malnutrition, uraemia and diarrhoeal diseases.

Laboratory Diagnosis

Specimens

- * Scrapings from the lesions, skin lesion scraping
- * Pus
- * Tissue biopsy
- * Sputum

Direct microscopy

- KOH wet mounts of specimens show non-septate hyphae.
- Histological sections stained with H and E stain reveal the presence of hyphae.

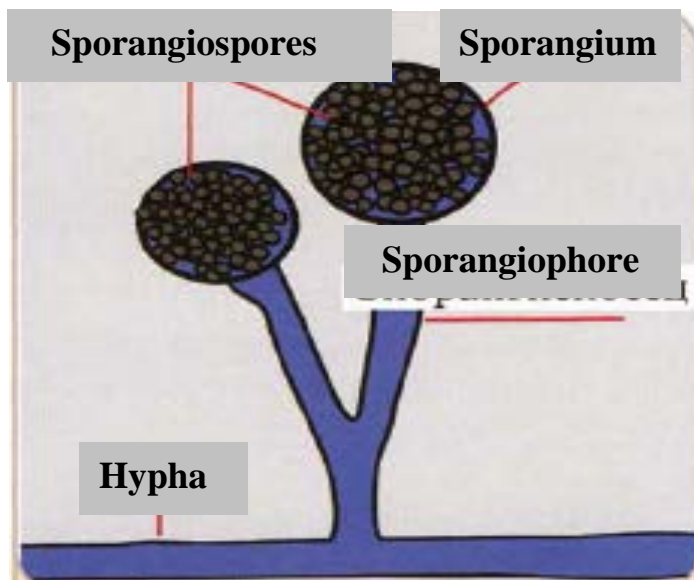


Fig. 20. Fungi: genus Mucor

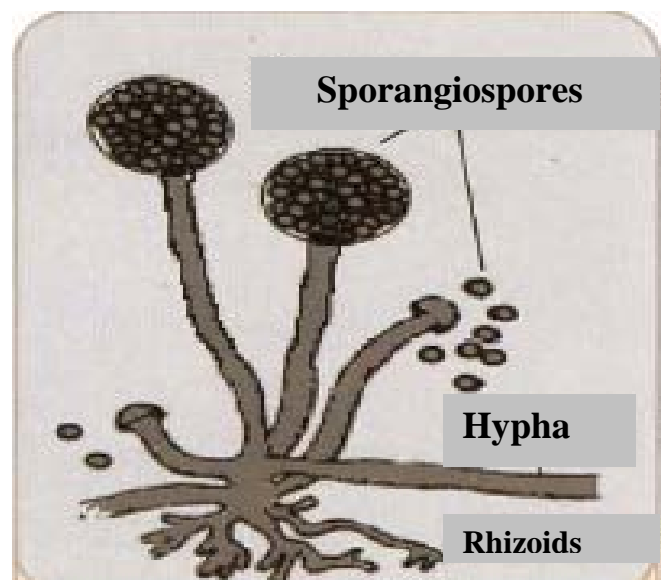


Fig. 21. Fungi: genus Rhizopus

Culture

- Fungi can be readily grown on SDA without cycloheximide at 37°C.

Lactophenol cotton blue preparation of colonies shows branched sporangiophores arising randomly along aerial mycelium. In the case of mucor rhizoids are absent. Rhizopus has rhizoids, and sporangiospore arise in groups directly above the rhizoids. Absidia has also rhizoids but sporangiophores arise from the aerial mycelium in between the rhizoids.

Histological sections:

Microscopic examination shows broad, non septate, irregular hyphae in thrombosed vessels or sinuses surrounded with leukocytes and giant cells.

Clinical picture:

Zygomycosis and phycomycosis (mucormycosis) is a systemic disease which may involve internal organs with predilection for blood vessels. Sometime phycomycosis may be seen as a chronic infection of subcutaneous tissue.

Treatment:

In some cases amphotericin B may be useful.

Pneumocystis carinii

1. *Pneumocystis carinii* causes pneumonia in immunocompromised patients. Until recently, it was thought to be a protozoan, but now it has been included in

fungi. Prior to the introduction of chemoprophylaxis, it was a major cause of death among AIDS patients.

2. *P. carinii* has two stages: thin walled trophozoites and thick walled spherical cysts. Cyst contains 4 to 8 nuclei.

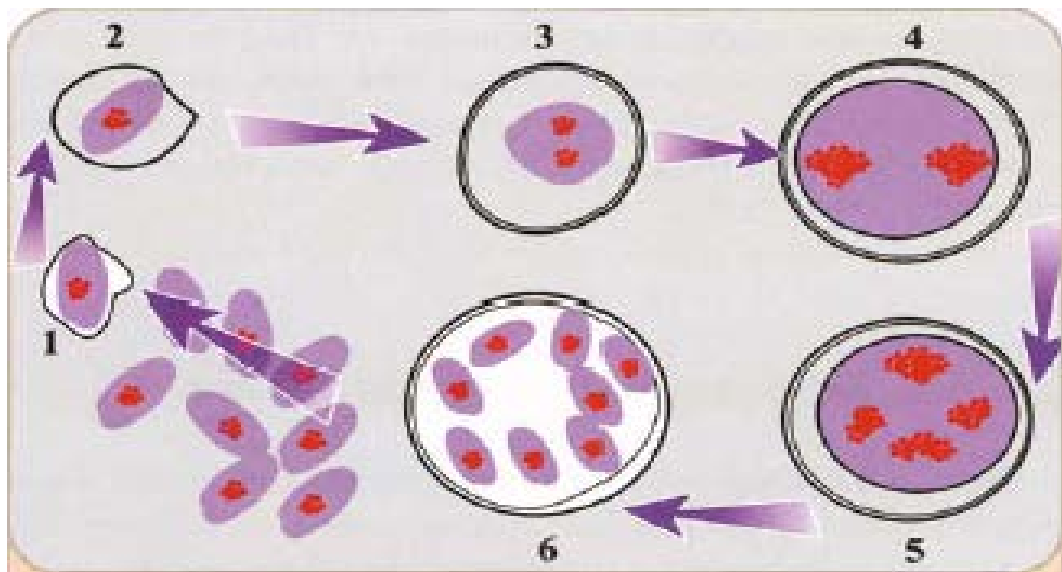


Fig. 22. Scheme of living cycle of pneumocystis: formation of trophozoites, sub-cysts, cysts and 8 nuclei.

3. Bronchoalveolar lavage, lung biopsy and induced sputum are the specimens used for diagnosis.

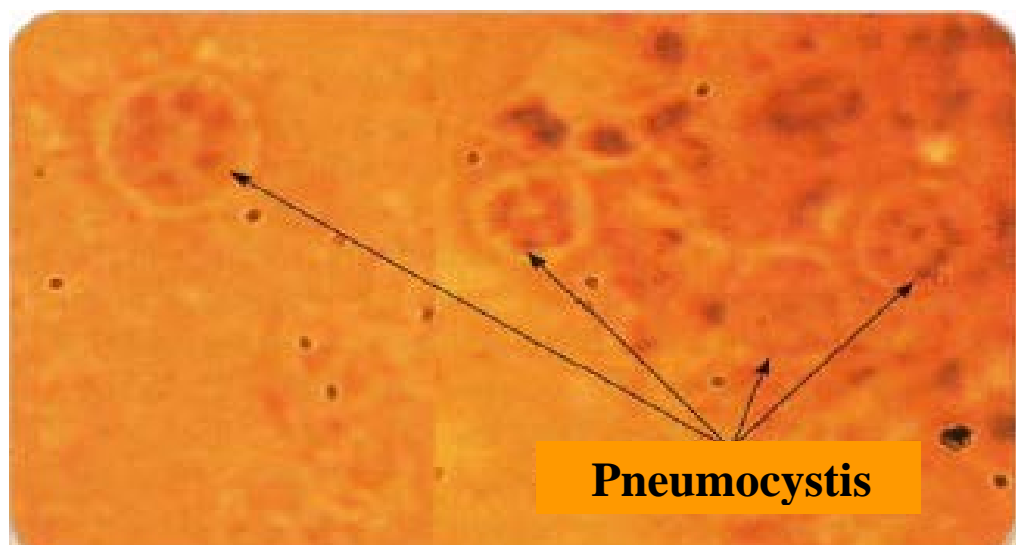
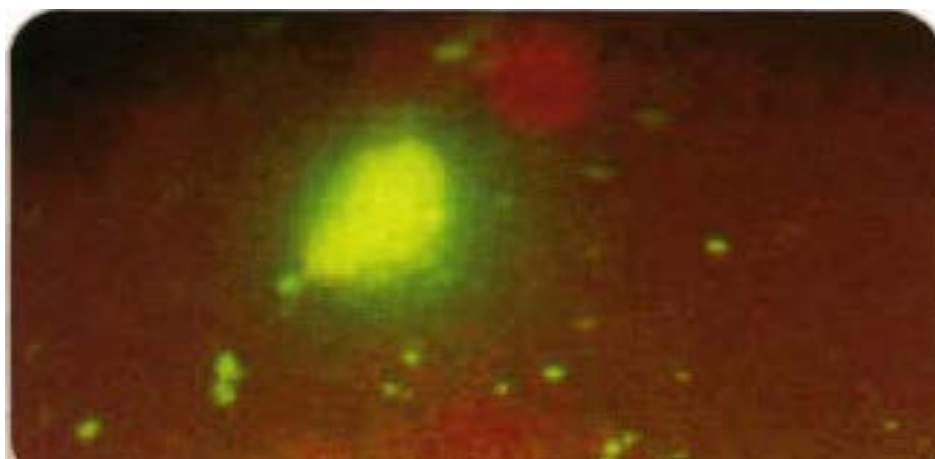


Fig. 23. Pneumocystis carinii in the preparation from lung. Romanowsky-Giemsa staining.

4. Direct demonstration of trophozoites and cysts can be done by Giemsa, toluidine blue, methenamine silver and calcofluor white stains.

Cyst wall stains black with methenamine silver staining. Fluorescent monoclonal antibody staining shows honeycomb appearance of the cyst.



**Fig. 24. Pneumocystis carinii in lung biopsy.
Immunofluorescence.**

5. Trimethoprim - sulphamethoxazole or pentamidine are used in treatment of acute cases of pneumonia.

Antibiotics produced by fungi.

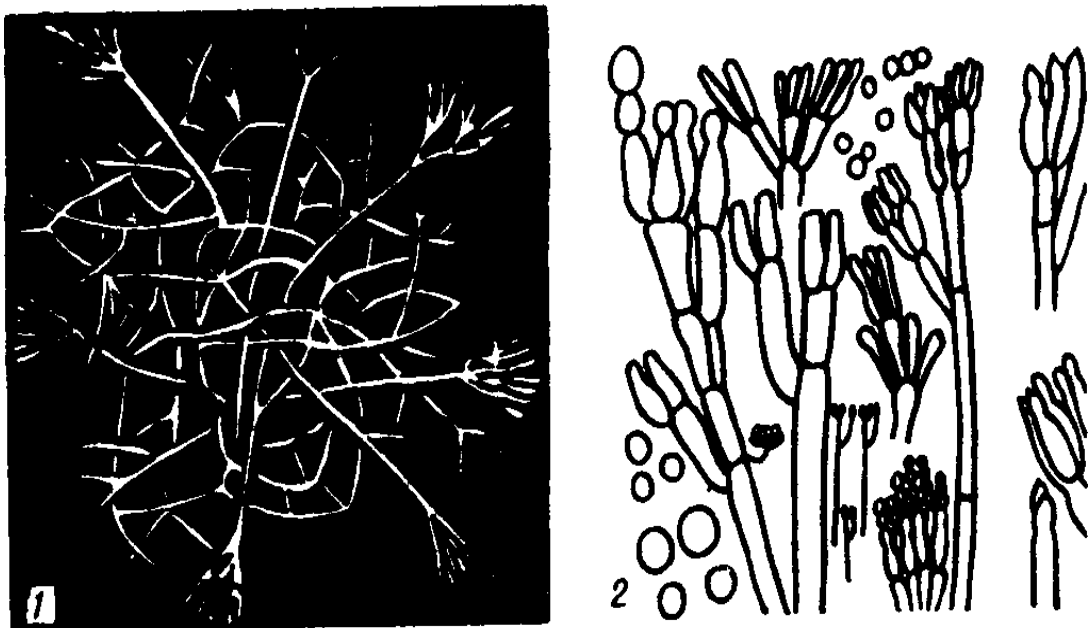
Penicillin is produced by the fungi *Penicillium notatum*, *Penicillium chrysogenum*, etc. Penicillin is produced as sodium and potassium salts.

It dissolves readily in water, but its solutions are not stable. It is a dipeptide consisting of dimethylcysteine and acetylserine.

Penicillin is used in staphylococcal, streptococcal, and meningococcal infections, anaerobic infections, gonorrhoea, syphilis, leptospirosis, anthrax and other diseases.

Penicillin preparations include econovocillin which is a form of long-acting penicillin, maintaining the necessary therapeutic concentration of penicillin in the blood. It is used only for intramuscular injections.

The indications are the same as for the application of penicillin.



Penicillium notatum (1), *Penicillium chrysogenum* (2) - producers of penicillin

New drug forms of penicillin have been obtained, e. g. bicillin-1, bicillin-3, which are retained for long periods in the body.

The former is an N, N'— dibenzylethylenediamine salt of benzylpenicillin, the latter — a mixture of novocain, potassium salt of benzylpenicillin and bicillin-1; and epicillin (hydrogen iodide salt of diethylammonoethyl benzylpenicillin).

Semisynthetic penicillins (methicillin, oxacillin) are used in infection with penicillin-resistant staphylococci; ampicillin is prescribed in mixed infections. Resistance, however, develops faster to semisynthetic preparations than to natural ones.

Novobiocin and ristomycin cause a favourable therapeutic effect.

One product of the life activity of penicillium is griseofulvin obtained in 1939 from mycelia of *Penicillium griseofulvum*.

At present griseofulvin is manufactured by the medical industry.

It is obtained by the deep method of fermentation of *Penicillium putulum*, *Penicillium nigricans*. It is employed for treating trichophytosis, microsporosis, epidermophytosis and favus.

Microcide is produced by *Penicillium vitale*. It is used externally in recently infected wounds and in other acute purulent processes.

It leads to a rapid cleaning of wounds and ulcers from pus, and to a decrease in inflammatory phenomena. It is used for treating patients with burns, frost-bites, infected and unhealable wounds and ulcers.

ANTIFUNGAL ANTIBIOTICS

The number of fungal infections has risen dramatically with the increase in patients who are immunocompromised from AIDS, chemotherapeutic drugs, and organ transplant immunosuppressive drugs.

For this reason you will frequently use the handful of antifungal antibiotics available. Ergosterol is a vital part of the cell membranes of fungi but is not found in the cell membranes of humans, which contain cholesterol.

Most antifungal agents bind more avidly to ergosterol than to cholesterol, thus more selectively damaging fungal cells than human cells.

By binding to or inhibiting ergosterol synthesis, they increase the permeability

of the cell membranes, causing cell lysis.

We can divide antifungal agents into 3 groups:

1) Antifungal agents that are used for **serious systemic infections**:

Amphotericin B, the grandfather of antifungal agents. This drug covers almost all medically important fungi but must be given intravenously (not absorbed orally) and causes many side effects. It may also be given intrathecally (into the cerebrospinal fluid).

Itraconazole, given orally, has now proven useful for many of these infections.

2) Antifungal agents that are used for **less serious systemic infections**:

The oral azole drugs. The prototype is **ketoconazole**. There are now new agents in this class, called **fluconazole** and **itraconazole** (mentioned above).

3) Antifungal agents that are used for **superficial fungal infections**:

Griseofulvin (taken orally) and the many topical antifungal agents such as nystatin and the azole drugs (**clotrimazole** and **miconazole**).

Amphotericin B

The classic antifungal antibiotic is amphotericin B. Most species of fungi are susceptible to it, and although it has many side effects, it remains the drug of choice for most serious systemic fungal infections: Systemic *Candida* infections.

Cryptococcal meningitis: used in combination with flucytosine. Severe pneumonia and extrapulmonary Blastomycosis, Histoplasmosis, and Coccidioidomycosis. Invasive Aspergillosis. Invasive Sporotrichosis. Mucormycosis.

Adverse Effects

On the wards this drug is referred to as amphoterrible and Awfultericin because of its numerous adverse effects:

1) **Renal toxicity:** (Poor Mr. Kidney!) There is a dose-dependent azotemia (increase in BUN and creatinine reflecting kidney damage) in most patients taking this drug. This is **reversible** if the drug is stopped. The creatinine level must be followed closely, and if it becomes too high (creatinine > 3), the dosage may have to be lowered, terminated, or switched to alternate day regimens.

2) **Acute febrile reaction:** A shaking chill (rigors) with fever occurs in some people after IV infusion. This is a **common** side effect.

3) **Anemia.**

4) **Inflammation of the vein** (phlebitis) at the IV site. These side effects are important because they are very common. In fact, when amphotericin B is given in the hospital, it is usually given with aspirin or acetaminophen to prevent the febrile reaction. Daily BUN and creatinine levels are drawn to monitor kidney function. You can see that these side effects are important in day-to-day clinical management!

Properties of **amphotericin B**, the "**amphibian terrorist**": intravenous drug delivery; fungicidal by binding to ergosterol in the fungal cell membrane, causing membrane disruption and osmotic lysis of the cell; nephrotoxicity. To speed amphotericin's travel through the kidneys and decrease renal toxicity, hydration with normal saline is used commonly with traditional amphotericin B. This hydration is generally not required with the newer preparations of amphotericin B. Electrolyte replacement is another important adjunct of amphotericin therapy because amphotericin causes increases in urinary excretion of potassium, magnesium and bicarbonate.

New preparations of amphotericin B are now available that add different lipids (fats!) to the traditional (old fashion) amphotericin B deoxycholate.

The addition of the lipid decreases the nephrotoxicity of the drug, making it less

Amphoterrible.

Amphotericin B colloidal dispersion (ABCD: Amphocil): Ampho B + cholesterol sulfate. Rigors still occur but nephrotoxicity is reduced. **Amphotericin B lipid complex (ABLC):** Ampho B + dimyristoylphosphatidylglycerols and dimyristoylphosphatidylcholines.

Rigors still occur but there is less nephrotoxicity.

Liposomal amphotericin B (Ambisome): A unilamellar liposome containing a mixture of 1 molecule of amphotericin B surrounded by a coating of nine molecules of lipid (soy lecithin, cholesterol, and distearoylphosphatidylglycerol), like a coated jaw breaker. There is little nephrotoxicity or rigors.

Some hospitals make their own concoction by adding amphotericin B deoxycholate to Intralipid (parenteral fat for intravenous feeding) in a mixture of 1-2 mg amphotericin B per ml lipid.

Less nephrotoxicity is seen, but once again we do not yet know enough about antifungal efficacy.

Flucytosine is rarely used alone because of rapid development of resistance. Think of it as the tag team wrestling partner of amphotericin B.

Amphotericin B busts holes in the cell membranes and flucytosine enters and inhibits DNA/RNA synthesis.

Most fungi are resistant to flucytosine, but *Cryptococcus*, and *Candida* are the exceptions. Flucytosine use mostly limited to the treatment of cryptococcal meningitis, in conjunction with Amphotericin B.

Adverse Effects

1) **Bone marrow depression**, resulting in leucopenia and thrombocytopenia. Remember that most antimetabolite type drugs will do this (methotrexate, sulfadruugs, 5-fluorouracil, etc.).

2) **Nausea, vomiting, diarrhea.** This again is common with the antimetabolites, such as the chemotherapeutic drugs.

The reason for these adverse effects is that the drugs damage DNA during its

formation in rapidly dividing cells such as bone marrow and GI epithelial cells.

The Azole Family

The azole family may be classified into 2 groups of drugs: the imidazoles and the triazoles.

IMIDAZOLES	TRIAZOLES
Ketoconazole	Fluconazole
Miconazole	Itraconazole
Clotrimazole	Voriconazole

The azoles inhibit the cytochrome P-450 enzyme system, which is involved in ergosterol synthesis. The depletion of ergosterol disrupts the permeability of the fungal cell membrane.

These drugs are active against a **broad** spectrum of fungi.

Clotrimazole and **miconazole** are too toxic for systemic use and for this reason, are primarily used for **topical fungal infections**, including pityriasis versicolor, cutaneous candidiasis, and the dermatophytosis (tinea pedis, corporis, etc.).

Clotrimazole troches (like candies) are sucked to treat oral *Candida* (thrush), and clotrimazole vaginal suppositories treat *Candida* vaginitis.

Ketoconazole, fluconazole, and itraconazole are tolerated orally and have many important uses for **systemic fungal infections**.

Ketoconazole

Ketoconazole, one of the imidazoles, is the drug of choice for chronic mucocutaneous candidiasis (*Candida* on every surface). It is NOT used for systemic candidiasis (amphotericin B, remember?).

Ketoconazole is currently not used for the treatment of systemic fungal infections. The safer, more efficacious, oral itraconazole and old faithful, amphotericin B, are the first line drugs.

Adverse Effects

- 1) GI: Nausea, vomiting, and anorexia, all common.
- 2) **Hepatotoxicity:** This is usually seen as a temporary rise of hepatic enzymes but on rare occasions can lead to hepatic necrosis. Follow enzymes when on this drug.
- 3) **Inhibition of testosterone synthesis:** Ketoconazole inhibits the cytochrome P-450 system, which is important in testosterone synthesis. The result is gynecomastia, impotence, decreased sex drive (libido), and decreased sperm production.
- 4) Adrenal suppression.

Fluconazole

Fluconazole is one of the triazoles; it is less toxic and has broader antifungal coverage than ketoconazole.

Like ketoconazole it is used for cutaneous *Candida* infections but it is also used as a second-line agent behind amphotericin B for systemic candidiasis and cryptococcal meningitis.

In AIDS patients who have had cryptococcal meningitis, maintenance with fluconazole will prevent relapses.

The big picture with fluconazole is that it kills *Candida albicans* very well:

- 1) Studies comparing it to amphotericin B in the treatment of systemic *Candida albicans* infection (in non-neutropenic patients) demonstrated equivalent efficacy.
- 2) A single dose of fluconazole very effectively clears candida vaginitis.

Itraconazole

This triazole is becoming the next amphotericin B but in an oral formulation without the many amphoterrible side effects!!!

Itraconazole is now used as first-line treatment for chromoblastomycosis, histoplasmosis, coccidioidomycosis, blastomycosis, and possibly for invasive aspergillosis.

The main problem with this drug is poor oral absorption. Taking it with acid drinks such as orange juice or colas enhances absorption (need low pH). A new IV formulation has also been developed to avoid poor absorption.

Voriconazole

Voriconazole has a **Voracious** appetite for fungi!!! Voriconazole is a promising triazole antifungal.

Although more clinical experience is needed, data are sufficient to support its future use in patients with invasive aspergillosis who have failed to respond to agents of choice (i.e. conventional or liposomal amphotericin B, itraconazole).

Other Antifungal Drugs

Nystatin

Nystatin, like amphotericin B, binds to ergosterol, increasing the permeability of the cell membrane and causing cell lysis.

Think "Nasty Nystatin" because this drug is too toxic to take parenterally (intravenously). It is only used topically on the skin and mucous membranes.

Also, since it is not absorbed from the gastrointestinal tract, oral nystatin can be used to treat oral and esophageal infections with yeast or fungi.

You will order nystatin on the wards as **Nystatin, Swish and Swallow** for treatment of oral, esophageal, and gastric candidiasis.

It is also given topically for vaginal candidiasis.

Nasty Nystatin cruises down the esophagus killing fungi on the wall of the esophagus. In one end and out the other!

Griseofulvin

Visualize **griseofulvin** as a greasy fulcrum used to lever the dermatophyte plaques off the skin.

It inhibits fungal growth by disrupting spindle formation, thus preventing **mitosis**.

Note the worker peeling fungus off your **"toe,"sis**. Griseofulvin deposits in

keratin precursor cells in the skin, hair, and nails, where it inhibits the growth of fungi in those cells.

Note that it does not kill the fungi; it just inhibits their growth (static rather than cidal). The uninfected drug-infiltrated keratin precursor cells mature and move outward toward the keratinized layer.

As the older, infected cells fall off with normal cell turnover, this translates into a slow cure of skin fungus.

Adverse effects of griseofulvin are uncommon. They include headache, nausea, vomiting, photosensitivity, and mental confusion, in addition to bone marrow suppression (leukopenia and neutropenia).

Potassium Iodide

Potassium iodide is used to treat sporotrichosis. Remember that you get sporotrichosis from pricking your finger in the garden. "You get Sporotrichosis while Potting plants." If the infection becomes systemic, amphotericin B or itraconazole is better. Summary of the anti-fungal drugs.

Terbinafine

Terbinafine is a new oral fungicidal agent that blocks fungal cell wall synthesis. It blocks ergosterol synthesis by inhibiting the formation of squalene epoxide from squalene.

Terbinafine tends to accumulate in nails, and is therefore useful for tinea unguium (onychomycosis). It also appears useful in the treatment of tinea pedis, tinea capitis, and tinea corporis.

Since it is not metabolized by the cytochrome p450 system (as do the azole antifungals), there is little potential for drug-drug interactions.

Quizzes

1. The fungal nucleus...

- A. Contains true chromosomes
- B. Contains DNA fragments
- C. Differs from the bacterial nucleus
- D. All of the above

2. Asexual spores of fungi are following EXCEPT:

- A. Arthrospores
- B. Chlamyospores
- C. Blastospores
- D. Ascospores
- E. Oidium

3. In smear, that was made from mucous appearances of patient's tonsils, were found big oval cells that bud and form pseudomycelium. What microorganisms are they?

- A. Mucor
- B. Penicillium
- C. Aspergillus
- D. Candida
- E. Rhizopus

4. On what nutrient medium it is necessary to grow fungi?

- A. Sabouraud agar
- B. Endo medium
- C. Ploskirev medium
- D. Buchin medium
- E. Blood agar

5. Which of the following is produced sexually?

- A. Ascospores
- B. Oidium
- C. Conidium
- D. Yeast buds
- E. Blastospores

6. A sporangium contains...

- A. Spherules
- B. Sporangiospores
- C. Chlamydospores
- D. Oidia
- E. Arthrospores

7. Which dermatophyte infects hair, nails and skin?

- A. Trichosporum
- B. Trichophyton
- C. Microsporum
- D. Epidermophyton
- E. Microphyton

8. Candida is the etiological agent in all of the following EXCEPT...

- A. Endocarditis
- B. Meningitis
- C. Mycetoma
- D. Oral thrush
- E. Urinary tract infection

9. Which is a eukaryote?

- A. Mycoplasma
- B. Bacteria
- C. Chlamydia
- D. Fungi
- E. Viruses

10. Which of the following is an endogenous disease in origin?

- A. Aspergillosis
- B. Candidiasis
- C. Phycomycosis
- D. Mycetoma
- E. All of the above

11. Lesions caused by Candida are as follows:

- A. Mucocutaneous lesions
- B. Skin and nail infections
- C. Systemic candidiasis
- D. Oral manifestations
- E. All of the above

12. Candida stain by...

- A. Neisser method
- B. Burry method
- C. Gram method
- D. Loeffler method
- E. Zyehl-Neelsen method

13. Name antibiotic for treatment of fungal infections.

- A. Gentamycin
- B. Penicillin
- C. Amphotericin B
- D. Polymyxin
- E. Tetracyclin

14. Which of the following is true regarding fungal infections?

- A. Dermatophyte infections are exclusively from humans to animals
- B. Rhinosporidium seeberi causes deep infection in humans
- C. Candida albicans is not pathogenic to laboratory animals
- D. Candida infection is usually endogenous

15. Which of the following is false about Candida albicans?

- A. Yeast like fungus
- B. Forms chlamydospores
- C. Blastomere seen in isolates
- D. Gram positive
- E. Causes meningitis in immunocompromised

16. Reynolds-Braude phenomenon is seen in...

- A. Alternaria
- B. Aspergillus
- C. Oorporium
- D. Candida albicans
- E. Actinomycetes

17. Asteroids bodies and cigar-shaped globi may be produced by...

- A. Sporothrix
- B. Sporothrichosis
- C. Phialophora
- D. Aspergillus
- E. All of the above

18. Non-cultivable fungus is...

- A. Rhinosporodium
- B. Sporothrix
- C. Penicillium
- D. Aspergillus
- E. Candida

19. The only deep mycosis common in India is...

- A. Cryptococcosis
- B. Blastomycosis
- C. Coccidioidomycosis
- D. Histoplasmosis

20. Which of the following is false about Cryptococcus neoformans?

- A. Grows at 37° C
- B. Grows on Sabouraud agar
- C. Polysaccharide capsule
- D. Urease negative
- E. All of the above

21. Which of the following stain is used for cryptococcus?

- A. Negative India ink
- B. Giemsa
- C. Gram
- D. Albort
- E. Neisser

22. Coccidioides immitis is identified in tissues on the basis of which of the following?

- A. Budding yeast cells with pseudohyphae
- B. Yeast-like forms with very large capsules
- C. Arthrospores

D. Endosporulating spherules

23. Aspergilloma has...

A. Septate hyphae

B. Pseudo hyphae

C. Metachromatic hyphae

D. No hyphae

E. Chromatic hyphae

24. Name sexual spores of fungi.

A. Oospores

B. Ascospores

C. Zygosporos

D. Basidiospores

E. All of the above

25. Name vegetable spores of yeasts.

A. Blastospores

B. Sporangiospores

C. Oospores

D. Ascospores

E. Conidiospores

26. Blastospores are formed by budding from parent cell, as in...

A. Moulds

B. Yeasts

C. Fungi

D. Dimorphic fungi

E. Yeast like fungi

27. Which one of the following illnesses is not a fungal infection?

- A. Candidiasis
- B. Aspergillosis
- C. Histoplasmosis
- D. Penicillosis
- E. Chlamydiasis

28. Predisposing factor for candidiasis is...

- A. Diabetes
- B. Immunodeficiency
- C. Malignancy
- D. Prolonged administration of antibiotics
- E. All of the above

29. Candidiasis is...

- A. Systemic mycosis
- B. Superficial mycosis
- C. Cutaneous infection
- D. An opportunistic infection
- E. Subcutaneous mycosis

30. Some saprophytic fungi usually do not produce disease but may cause infection under special conditions such as immunocompromised individuals and in terminal stages of chronic disease. Name the species of Candida.

- A. *C. albicans*
- B. *C. septicum*
- C. *C. novyi*
- D. *C. fallax*
- E. *C. carnis*

31. Class Zygomycetes include ...

- A. *Aspergillus*

- B. Mucor
- C. Penicillus
- D. Candida
- E. Histoplasma

32. Smears of sputum or pus are stained with...

- A. Gram method
- B. Neisser method
- C. Burry method
- D. Loeffler method
- E. Zyehl-Neelsen method

33. Which one of the following illnesses is not a systemic mycosis?

- A. Histaplasmosis
- B. Blastomycosis
- C. Aspergillosis
- D. Paracoccidioidomycosis
- E. Coccidioidomycosis

34. Candida species grow well on Sabouraud dextrose agar. What colonies grow on this medium?

- A. Red with metal shine
- B. Colorless
- C. White and cream
- D. Black and grey
- E. Pink

35. Candida albicans is the normal inhabitant of...

- A. Skin
- B. Gastrointestinal tract
- C. Oral cavity

- D. Vaginal cavity
- E. All of the above

36. What is antibiotic used for treatment of candidiasis?

- A. Penicillin
- B. Nystatin
- C. Polimyxin
- D. Streptomycin
- E. Rifampicin

37. Many fungi produce poisonous substances that can cause acute or chronic intoxication. Name this toxin.

- A. Endotoxin
- B. Enterotoxin
- C. Anatoxin
- D. Antitoxin
- E. Cytotoxin

38. Fungi had been recognized as causative agents of human disease earlier than bacteria. What is the name these diseases?

- A. Chlamydiosis
- B. Salmonellosis
- C. Mycoplasmosis
- D. Shigellosis
- E. Mycosis

39. Fungi can be divided into four classes each of which have one human pathogenic species. Which one of the following groups is not a classification of fungi?

- A. Yeast

- B. Moulds
- C. Fungi imperfecti
- D. Yeast-like fungi
- E. Dimorphic fungi

40. A component of the cell membrane of most fungi is...

- A. Cholesterol
- B. Chitin
- C. Ergosterol
- D. Peptidoglycan
- E. Keratin

41. Yeast-like fungi grow as elongated cells resembling hyphae. Name pathogenic yeast-like fungus.

- A. *Candida krusei*
- B. *Penicillium*
- C. *Aspergillus*
- D. *Candida albicans*
- E. *Mucor*

42. The fungal nucleus...

- A. Contains true chromosomes
- B. Contains DNA fragments
- C. Differs from the bacterial nucleus
- D. All of the above

43. On what nutrient medium it is necessary to grow fungi?

- A. Sabouraud agar
- B. Endo medium
- C. Ploskirev medium

- D. Buchin medium
- E. Blood agar

44. Name antibiotic for treatment of fungal infections.

- A. Gentamycin
- B. Penicillin
- C. Amphotericin B
- D. Polymyxin
- E. Tetracyclin

45. The only deep mycosis common in India is...

- A. Cryptococcosis
- B. Blastomycosis
- C. Coccidioidomycosis
- D. Histoplasmosis

46. Which of the following is false about Cryptococcus neoformans?

- A. Grows at 37° C
- B. Grows on Sabouraud agar
- C. Polysaccharide capsule
- D. Urease negative
- E. All of the above

47. Which of the following stain is used for cryptococcus?

- A. Negative India ink
- B. Giemsa
- C. Gram
- D. Albort
- E. Neisser

48. Coccidioides immitis is identified in tissues on the basis of which of the

following?

- A. Budding yeast cells with pseudohyphae
- B. Yeast-like forms with very large capsules
- C. Arthrospores
- D. Endosporulating spherules

49. Which one of the following illnesses is not a systemic mycosis?

- A. Histoplasmosis
- B. Blastomycosis
- C. Aspergillosis
- D. Paracoccidioidomycosis
- E. Coccidioidomycosis

50. Many fungi produce poisonous substances that can cause acute or chronic intoxication. Name this toxin.

- A. Endotoxin
- B. Enterotoxin
- C. Anatoxin
- D. Antitoxin
- E. Cytotoxin

51. A component of the cell membrane of most fungi is...

- A. Cholesterol
- B. Chitin
- C. Ergosterol
- D. Peptidoglycan
- E. Keratin

52. A component of the cell membrane of most fungi is...

- A. Cholesterol
- B. Chitin

- C. Ergosterol
- D. Peptidoglican
- E. Keratin

53 On what nutrient medium it is necessary to grow fungi?

- A. Sabouraud agar
- B. Endo medium
- C. Ploskirev medium
- D. Buchin medium
- E. Blood agar

54. Name antibiotic for treatment of fungal infections.

- A. Gentamycin
- B. Penicillin
- C. Amphotericin B
- D. Polymyxin
- E. Tetracyclin

55. The only deep mycosis common in India is...

- A. Cryptococcosis
- B. Blastomycosis
- C. Coccidioidomycosis
- D. Histoplasmosis

56. Which of the following is false about Cryptococcus neoformans?

- A. Grows at 37° C
- B. Grows on Sabouraud agar
- C. Polysaccharide capsule
- D. Urease negative
- E. All of the above

57. Which of the following stain is used for cryptococcus?

A. Negative India ink

B. Giemsa

C. Gram

D. Albort

E. Neisser

58. Coccidioides immitis is identified in tissues on the basis of which of the following?

A. Budding yeast cells with pseudohyphae

B. Yeast-like forms with very large capsules

C. Arthrospores

D. Endosporulating spherules

59. Which one of the following illnesses is not a systemic mycosis?

A. Histoplasmosis

B. Blastomycosis

C. Aspergillosis

D. Paracoccidioidomycosis

E. Coccidioidomycosis

60. Many fungi produce poisonous substances that can cause acute or chronic intoxication. Name this toxin.

A. Endotoxin

B. Enterotoxin

C. Anatoxin

D. Antitoxin

E. Cytotoxin

61. The fungal nucleous...

A. Contains true chromosomes

B. Contains DNA fragments

C. Differs from the bacterial nucleus

D. All of the above

CORRECT ANSWERS

Test	Answers				
1	A	B	C	*D	E
2	A	B	C	*D	E
3	A	B	C	*D	E
4	A	B	C	*D	E
5	*A	B	C	D	E
6	A	*B	C	D	E
7	A	*B	C	D	E
8	A	B	*C	D	E
9	A	B	C	*D	E
10	A	*B	C	D	E
11	A	B	C	D	*E
12	A	B	*C	D	E
13	A	B	*C	D	E
14	A	B	C	*D	E
15	A	B	*C	D	E
16	A	B	C	*D	E
17	A	*B	C	D	E
18	*A	B	C	D	E
19	*A	B	C	D	E
20	A	B	C	*D	E
21	*A	B	C	D	E
22	A	B	C	*D	E
23	*A	B	C	D	E
24	A	B	C	D	*E
25	*A	B	C	D	E
26	A	*B	C	D	E
27	A	B	C	D	*E
28	A	B	C	D	*E
29	A	B	C	*D	E
30	*A	B	C	D	E
31	A	*B	C	D	E
32	*A	B	C	D	E
33	A	B	*C	D	E
34	A	B	*C	D	E
35	A	B	C	D	*E
36	A	*B	C	D	E
37	*A	B	C	D	E
38	A	B	C	D	*E

39	A	B	*C	D	E
40	A	*B	C	D	E
41	A	B	C	*D	E
42	A	B	C	*D	E
43	*A	B	C	D	E
44	A	B	*C	D	E
45	*A	B	C	D	E
46	A	B	C	*D	E
47	*A	B	C	D	E
48	A	B	C	*D	E
49	A	B	*C	D	E
50	*A	B	C	D	E
51	A	*B	C	D	E
52	A	*B	C	D	E
53	*A	B	C	D	E
54	A	B	*C	D	E
55	*A	B	C	D	E
56	A	B	C	*D	E
57	*A	B	C	D	E
58	*A	B	C	D	E
59	A	B	C	*D	E
60	A	B	*C	D	E
61	A	B	C	*D	E

PRACTICAL LESSON № 1

THEME: Morphology of fungi.

The goal: Study morphology of fungi.

Control of the initial level of knowledge

CONTROL QUESTIONS:

1. Fungi: classification, structure.
2. Reproduction of fungi.
3. The characteristic of filamentous fungi.
4. The characteristic of penicillium, aspergillus and mucor.
5. What are the methods of learning morphology and fungi structure?
6. Morphology and structure of yeast like fungus Candida.

7. Method of staining of yeast like fungus *Candida*.

Independent work of the students.

1. Study the character of growth of penicillium, aspergillus and mucor on Sabouraud's dextrose agar.
2. Draw structure of penicillium, aspergillus and mucor.
3. Microscopy and draw the smear of yeast like fungus *Candida*. Gram's staining.
5. Draw and write main data into the protocol.
5. Learn the demonstration material.

TASK 1. Complete the table about the...

Morphological features of filamentous fungi

Genus	Mycelium		Vegetative spores	
	<i>vegetative</i>	<i>reproductive</i>	<i>type</i>	<i>arrangement</i>
1. Mucor				
2. Aspergillus				
3. Pernicillium				

TASK 2. Make 5 tests for studies of practical lesson № 1. There should be 5 variants of answers in each test. There can be correct only one variant of answer.

PRACTICAL LESSON № 2

THEME: Laboratory diagnostics of mycosis. Yeast – like fungus Candida.

The goal: Study laboratory diagnostics of mycosis.

Control of the initial level of knowledge

CONTROL QUESTIONS:

- 1. Morphological classification of fungi.**
- 2. Systematic classification of fungi.**
- 3. The main ways of fungi's reproduction.**
- 4. Opportunistic mycotic infections (mycosis): aspergillosis, penicillosis and zygomycosis.**
- 5. The nutrient media and main methods of fungi's cultivation.**
- 6. Organs and tissues in human organism which are damaged in mycoses.**
- 7. Yeast-like fungus Candida: main biological characteristic.**
- 8. Diseases, caused by the yeast-like fungus Candida.**
- 9. Biological preparations which are used for treatment of mycosis.**

Independent work of the students.

- 1. Perform the microscopy the ready Gram-stained preparation of Candida.**
- 2. Study the methods of laboratory diagnostics in mycology.**
- 3. Draw and write main data into the protocol.**
- 4. Learn the demonstration material.**

TASK 1. Mark the methods of laboratory diagnostics of diseases caused by fungi.

Methods	Mycosis
Microscopic	
Microbiological	
Serological	
Allergical	

TASK 2. Make 10 tests for studies of practical lesson № 1. There should be 5 variants of answers in each test. There can be correct only one variant of answer.

PRACTICAL LESSON № 3

***THEME:* Causative agents of the systemic mycoses. Basic principles of laboratory diagnostics of the systemic mycoses.**

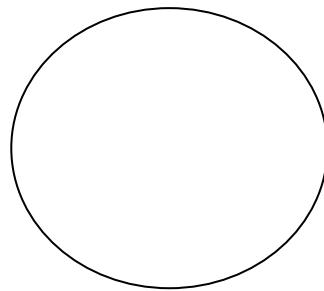
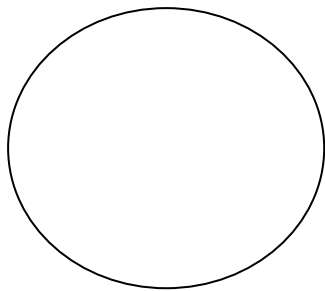
Questions for the learning.

- 1. Morphological and classification of the causative agents of the systemic mycoses.**
- 2. Classification of the systemic mycoses.**
- 3. Laboratory diagnosis of the systemic mycoses.**
- 4. Treatment of the systemic mycoses.**

1. Name the taxonomy of the causative agents of the systemic mycoses.

	Blastomycosis	Histoplasmosis	Coccidioido- mycosis	Cryptococcosis
Genus				
Species				

2. Name the nutrient mediums and main methods of fungi's cultivation.



2. Fill in the table.

Diseases	Source of an infection	Laboratory diagnosis			
		Specimens	Direct examination	Culture	Serological tests
Blastomycosis					
Histoplasmosis					
Coccidioidomycosis					
Cryptococcosis					

4. Name the preparations which are used for treatment of the systemic mycoses.

5. Name the preparations which are used for prophylaxis of the systemic mycoses.

5. Name the causative agent of the systemic mycoses and indicate their tissue and mycelia forms.

