Lesson 20

Introduction to medical mycology.Pathogenesis and principles of microbiological diagnosis of mycoses. antifungal therapy.

Classification of mycoses

Depending on the properties of the etiological agent, localization, form and nature of the pathological process, mycoses are divided into:

- **Superficial mycoses or keratomycosis -** lesions of the stratum corneum of the epidermis and the surface of the hair shaft;
- **Dermatomycosis or epidermomycosis -** lesions of the epidermis, skin and hair;
- **Subcutaneous or subcutaneous mycoses -** pathogens penetrating into the places of microtrauma of the skin involve the deep layers of the dermis, subcutaneous tissues, muscles and fascia;
- **Systemic or deep mycoses -** internal organs and tissues are affected;
- **Opportunistic mycoses -** cause opportunistic fungi;
- *Pathological processes caused by fungi also include mycogenic allergies and mycotoxicoses:*
- **Mycogenic allergies** - caused by fungi or their allergens;
- **Mycotoxicoses** are food poisonings caused by waste products (mycotoxins) of microscopic fungi.

Superficial mycoses

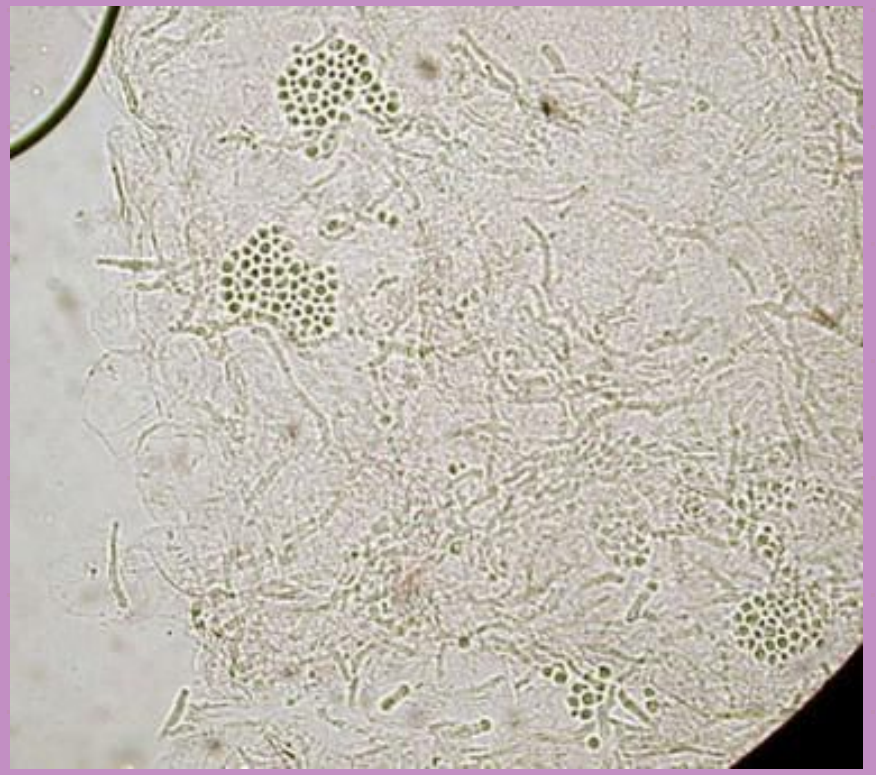
- Superficial mycoses - keratomycosis, are characterized bydamage to the stratum corneum of the epidermis and the surface of the hair shaft.

Keratomycosis is slightly contagious and is characterized by chronic flow.

These include pityriasis versicolor, black lichen, black and white piedra.

The causative agent of pityriasis

- Pityriasis (variegated, multi-colored) is caused by yeast-like fungi of the genus *Malassezia Pityrosporum) - M.furfur, M. globosa, M.restricta*.

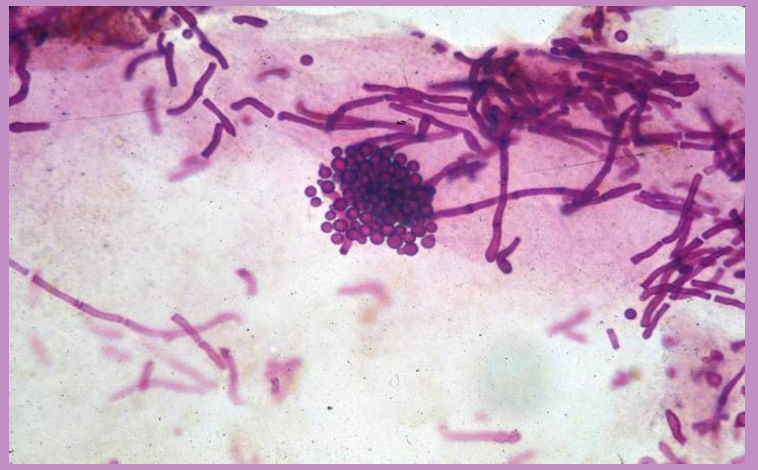


- In scrapings from the affected foci of the stratum corneum of the epidermis, short, slightly curved hyphae and yeast-likecells of a round or oval shape are found. True mycelium is absent.

Malassezia

- They do not grow well on nutrient media. Cultivated on media containing lipid components: after inoculation, a few drops of sterile vegetable oil are added to Sabouraud's medium with an antibiotic. A week later growth is noted in the form of white creamy colonies.

- In smears prepared from isolated colonies, yeast-like bottle-shaped budding cells 2-6 microns in size are found.



Versicolor

Multicolored (spotted) lichen is a chronic disease of the stratum corneum of the epidermis, manifested by the formation of hypo- and hyperpigmented discrete spots on the skin in the chest, neck, back, arms and abdomen.

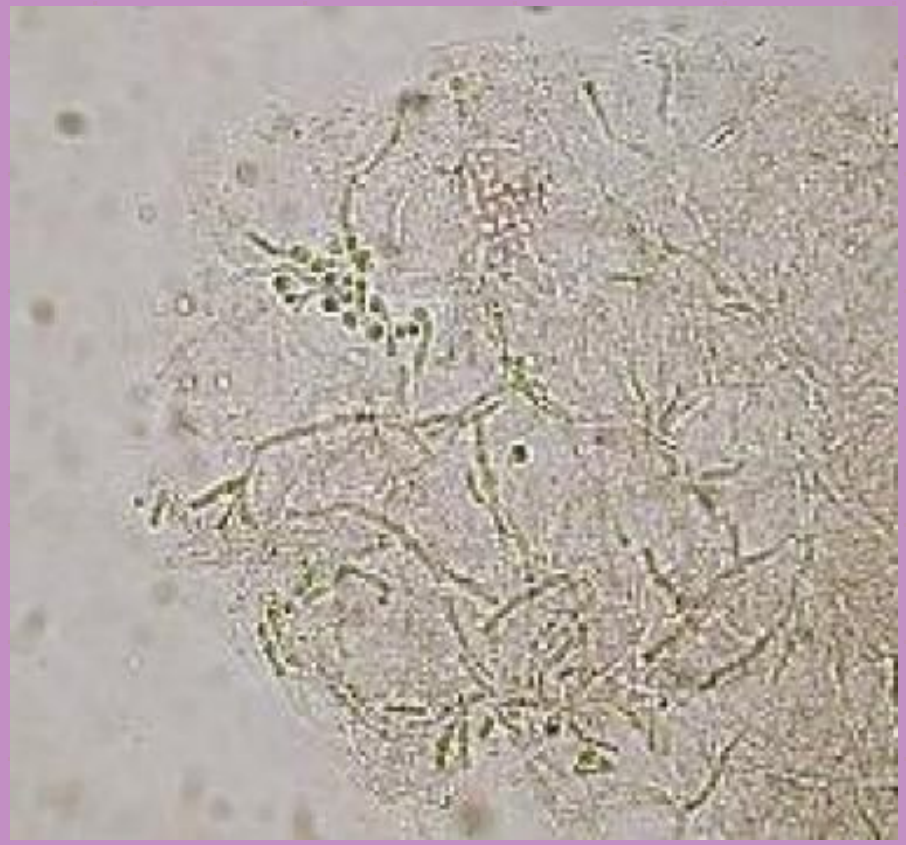
Fungi of the genus Malassezia can sometimes cause fungemia in infants who are parenterally fed, as well as **folliculitis** in some people.

These fungi also cause **seborrheic dermatitis and dandruff** on the scalp.

Microbiological diagnostics

Microbiological diagnosis is based on microscopy of scales from lesions treated with 10-20% KOH.

In preparations prepared from skin scrapings from lesions, short curved hyphae and yeast-like cells (spaghetti with meatballs) of a round or oval shape are found.



Causative agents of skin (dermatomycosis) mycoses

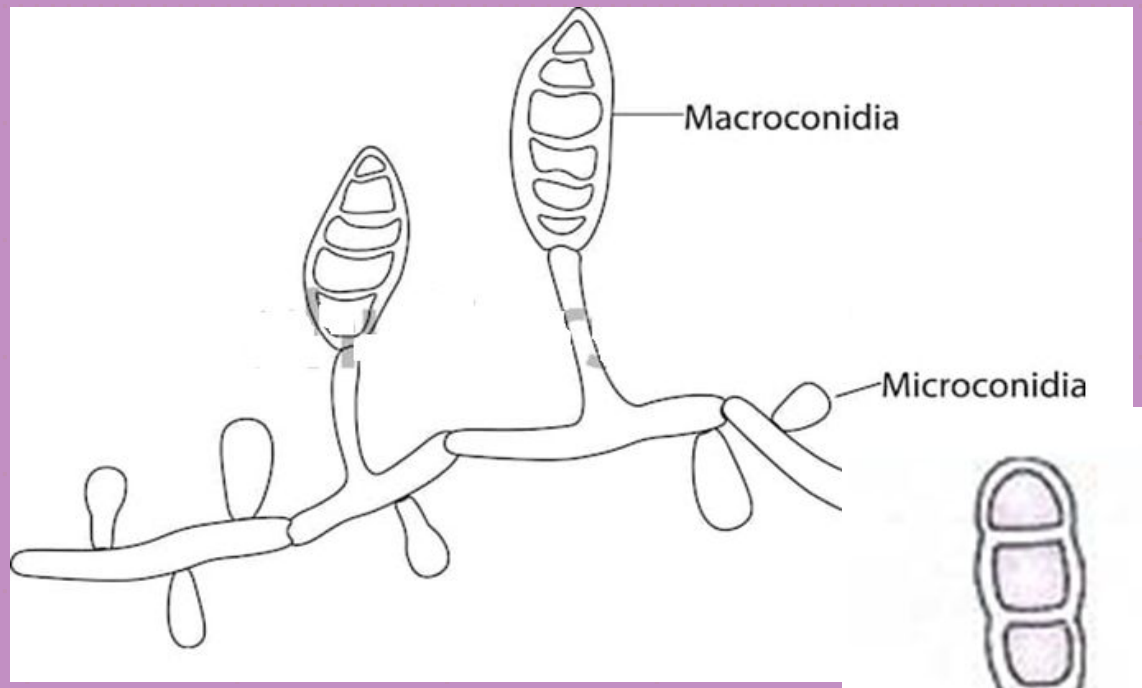
Cutaneous mycoses or dermatomycosis or epidermomycosis affect the epidermis, hair and nails.

The disease is caused by fungi of genera, representatives of which are similar in morphological and biological properties **(fungi of the genus Microsporum,Trichophyton, Epidermophyton).** About 40 species of dermatophytes cause pathological processes in humans.

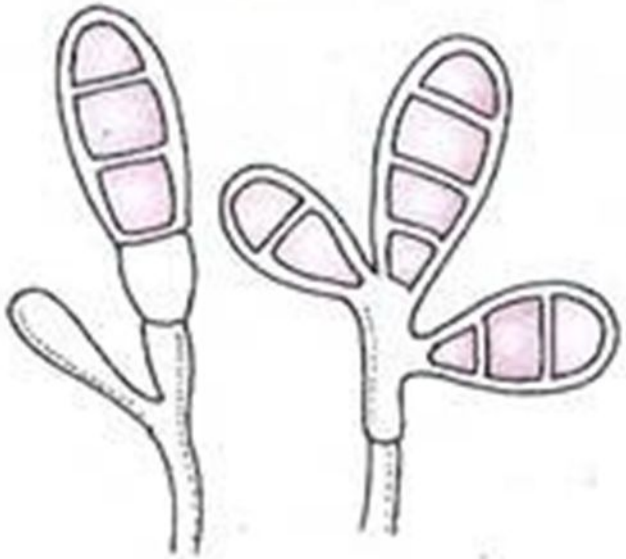
The morphology of dermatophytes observed on the skin, hairs and nails differs sharply from their forms in culture. Therefore, the identification of dermatophytes is carried out on the basis of cultural properties or the study of morphological features.

Dermatophytes grow on Sabouraud's medium at 250C and less often on other media for several weeks. Depending on the species, they form multi-colored, powdery, granular, fluffy colonies.

Morphology of dermatophytes



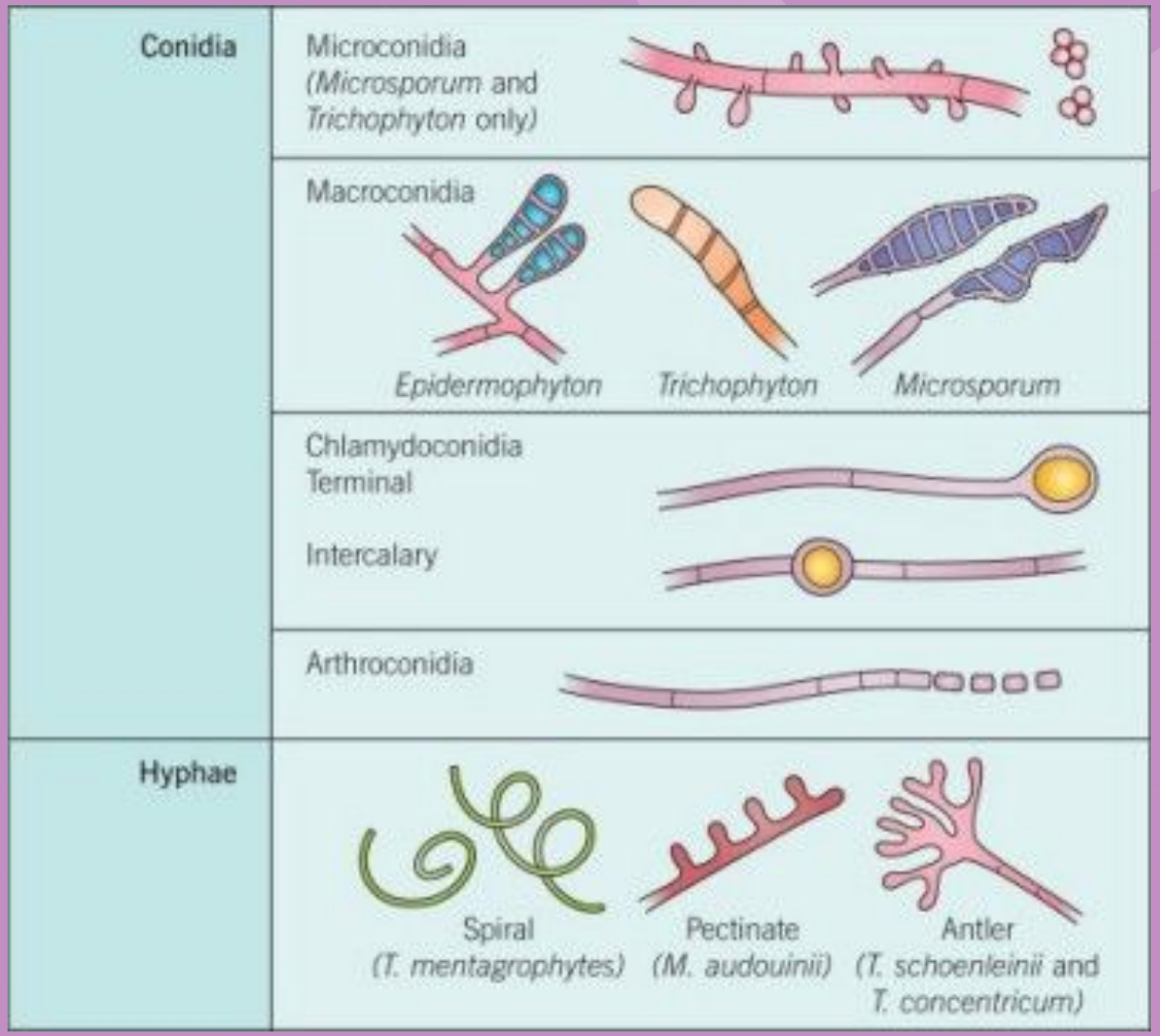
Trichophyton



Epidermophyton

Microsporum

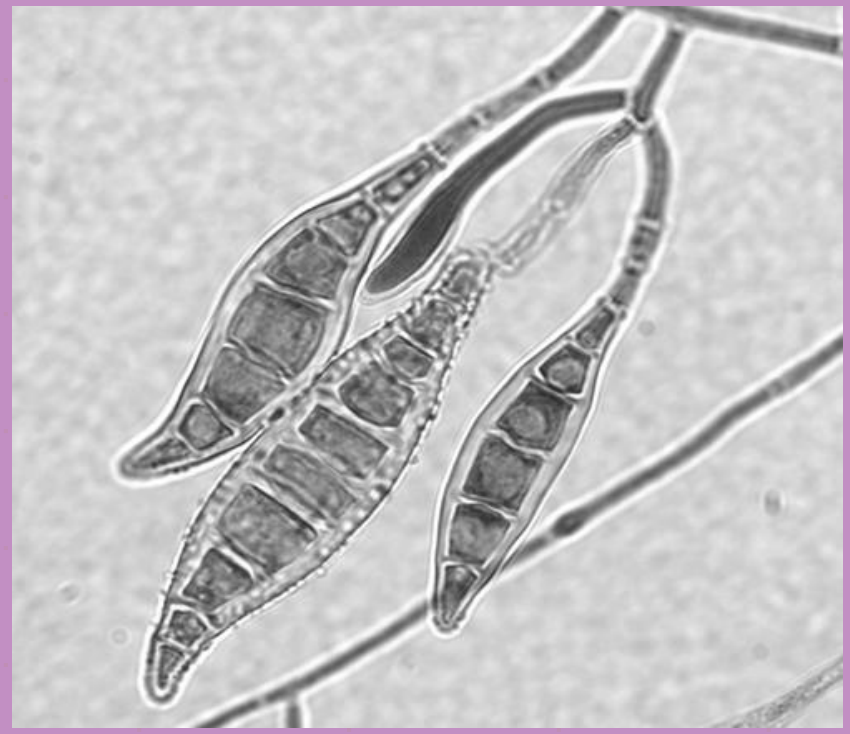
Morphology of dermatophytes



Mushrooms of the genus Trichophyton

Fungi of the genus Trichophyton consist of septate mycelium, large, smooth, multicellular macroconidia and typical microconidia.

Grow on Sabouraud's medium at 250C for two weeks. *Trichophyton mentagrophytes* forms fluffy and granular colonies. On the terminal surface of the branched mycelium there are clusters of rounded microconidia in the form of bunches of grapes.
In isolates, twisted and spiral mycelia can be observed.



Trichophyton rubrum forms mainly red colonies with a white fluffy surface. They produce a water-insoluble pigment that does not diffuse into the medium and is best observed on the back of the colonies. Small pear-shaped microconidia are alternately located on the surface of the mycelium.

T. tonsurans forms flat, reddish-brown colonies with a mealy, velvety surface. Macroconidia are mostly elongated

The septate mycelium of T. schoenleinii, with thickening and branching at the ends, resembles candelabra or deer antlers, which distinguishes them from other species. Fungi of the genus Trichophyton infect hair, skin and nails.

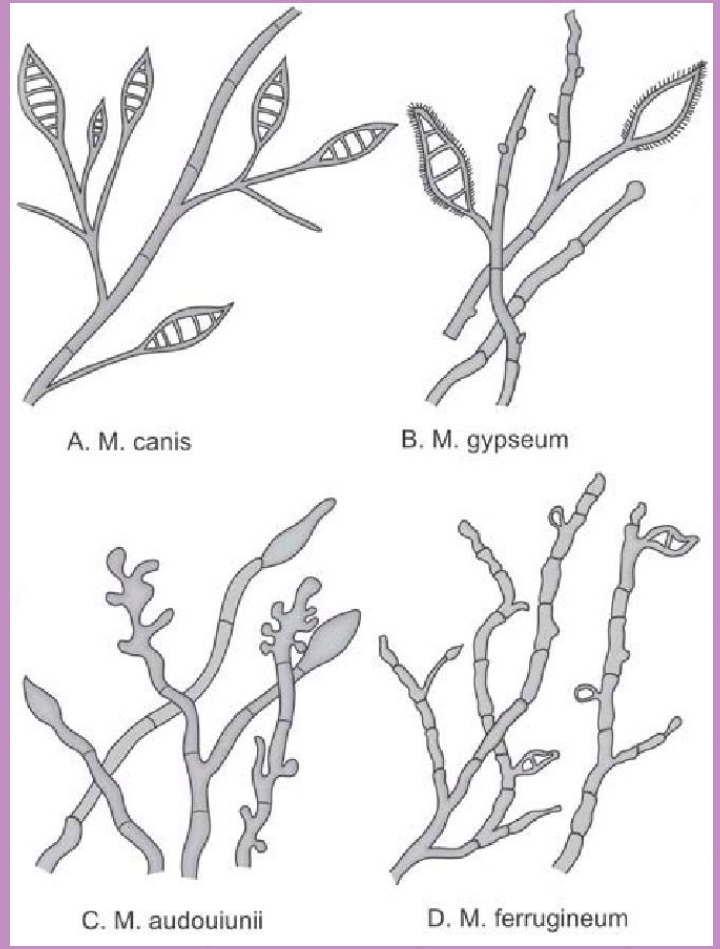
Mushrooms of the genus Microsporum

Mushrooms of the genus Microsporum consist of septate mycelium, thick-walled multicellular spindle-shaped macroconidia with spines.

Macroconidia of Microsporum canis are curved and consist of 8-15 cells.They form mainly yellow colonies with a white fluffy surface.

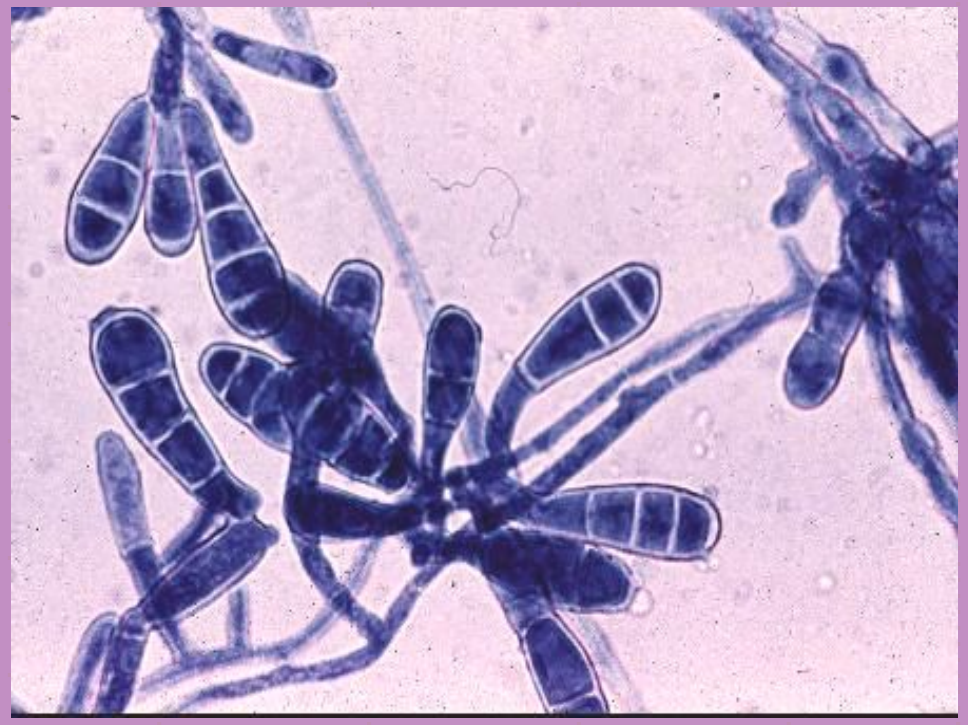
Macroconidia Microsporum gypseum form 4-8 cell macroconidia; superficial leathery colonies

Fungi of the genus Microsporum infect only hair and nails.



Fungi of the genus Epidermophyton

Fungi of the genus *Epidermophyton* consist of septate mycelium, form only macroconidia with a smooth surface, consisting of 2-4 cells. Macroconidia are grouped at the ends of hyphae by 2-3 in the form of bunches of bananas or three-leaf clover.



- Pathogenic representative of this genus for humans is only the species *E. floccosum*.

Fungi of the genus *Epidermophyton* affect only the skin and nails.

Source of infection and routes of transmission

Dermatophyte pathogens are transmitted by contact with a sick person or animal, as well as by contact with contaminated soil. Dermatophytes are divided into three groups according to ecological properties (anthropophilic, zoophilic and geophilic dermatophytes).

- **Anthropophilic dermatophytes are transmitted from person to person.**
- **Zoophilic dermatophytes are transmitted to humans from animals or birds.**
- **Geophilic dermatophytes live in the soil and are transmitted by contact with it.**

Anthropophilic dermatophytes play an important role in human pathology, cause chronic diseases with a moderate course, and are difficult to treat.

Zoophilic and geophilic dermatophytes cause acute inflammatory diseases, their treatment is relatively easier.

Pathogenesis and clinical manifestations of dermatomycosis

Pathogens produce keratinase and elastase, which are the main pathogenicity enzymes that can penetrate the epidermis of the skin, hair and nails and lead to the development of diseases.

- Dermatophytes do not penetrate beyond the basement membrane of the epidermis
- The development of the disease is promoted by increased sweating, weakened immunity, endocrine disorders, etc.
- Depending on the type of fungus, the skin, hair and nails are affected to varying degrees.

Dermatomycosis of the skin

Skin lesions are accompanied by the formation of vesicles, pustules, cracks and foci of peeling and the development of itching. Inflammation may be minor or severe. Skin lesions are observed in all parts of the human body. Depending on the localization, ringworm of the feet **(tinea pedis), hands (tinea manus), body (tinea corporis), pubic region (tinea cruris) are distinguished.**

Nail lesions *(tinea uniquium)* - onychomycosis

- **Fungal lesions of the nails (tinea uniquium) – onychomycosis** is accompanied by a change in color, transparency, thickness, strength and integrity of the nail plate.

- The causative agent of onychomycosis can be any dermatophyte, but more often it is caused by *Trichophyton rubrum and Trichophyton interdigitale.*

Hair lesions

- **Hair affected by fungi break off, baldness, baldness develops.** According to localization, ringworm of the scalp *(tinea capitis*), ringworm of the beard area *(tinea barbae)* are distinguished.

- Basically, the disease is caused by fungi of the genera *Trichophyton and Microsporum*.

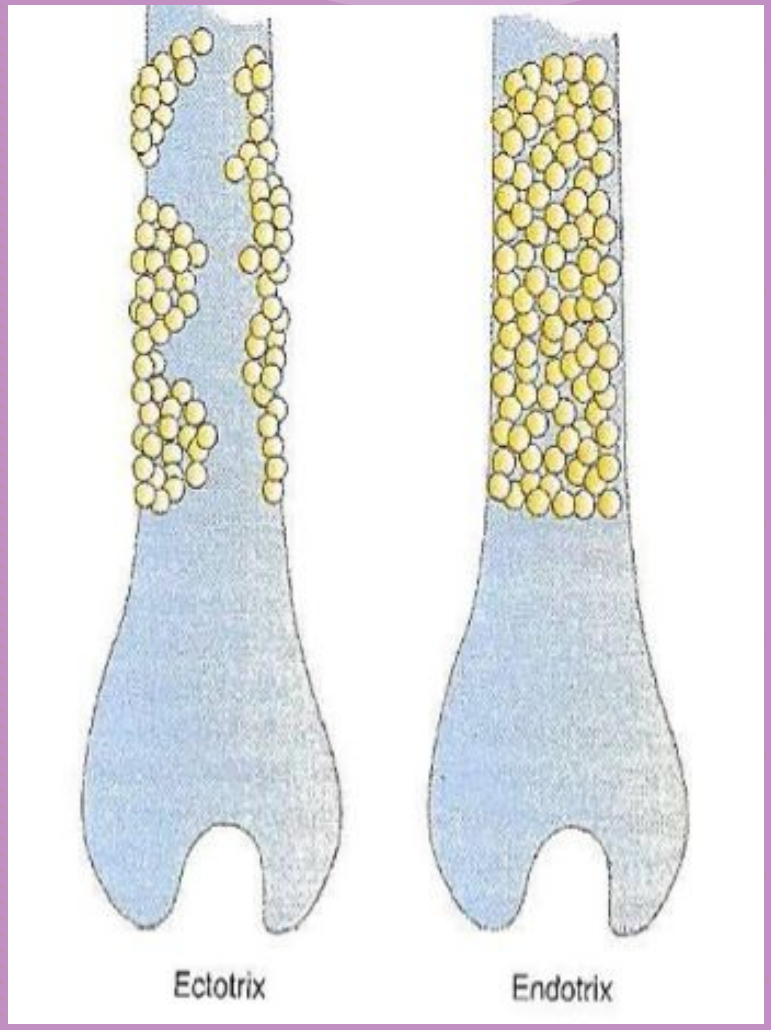
- Arthroconidia of the genus *Microsporum* are located on the surface of the hair shaft (ectothrix), and arthroconidia of the genus *Trichophyton* can be located both outside (ectothrix) and inside the hair (endotrix).

The nature of the damage to the hair

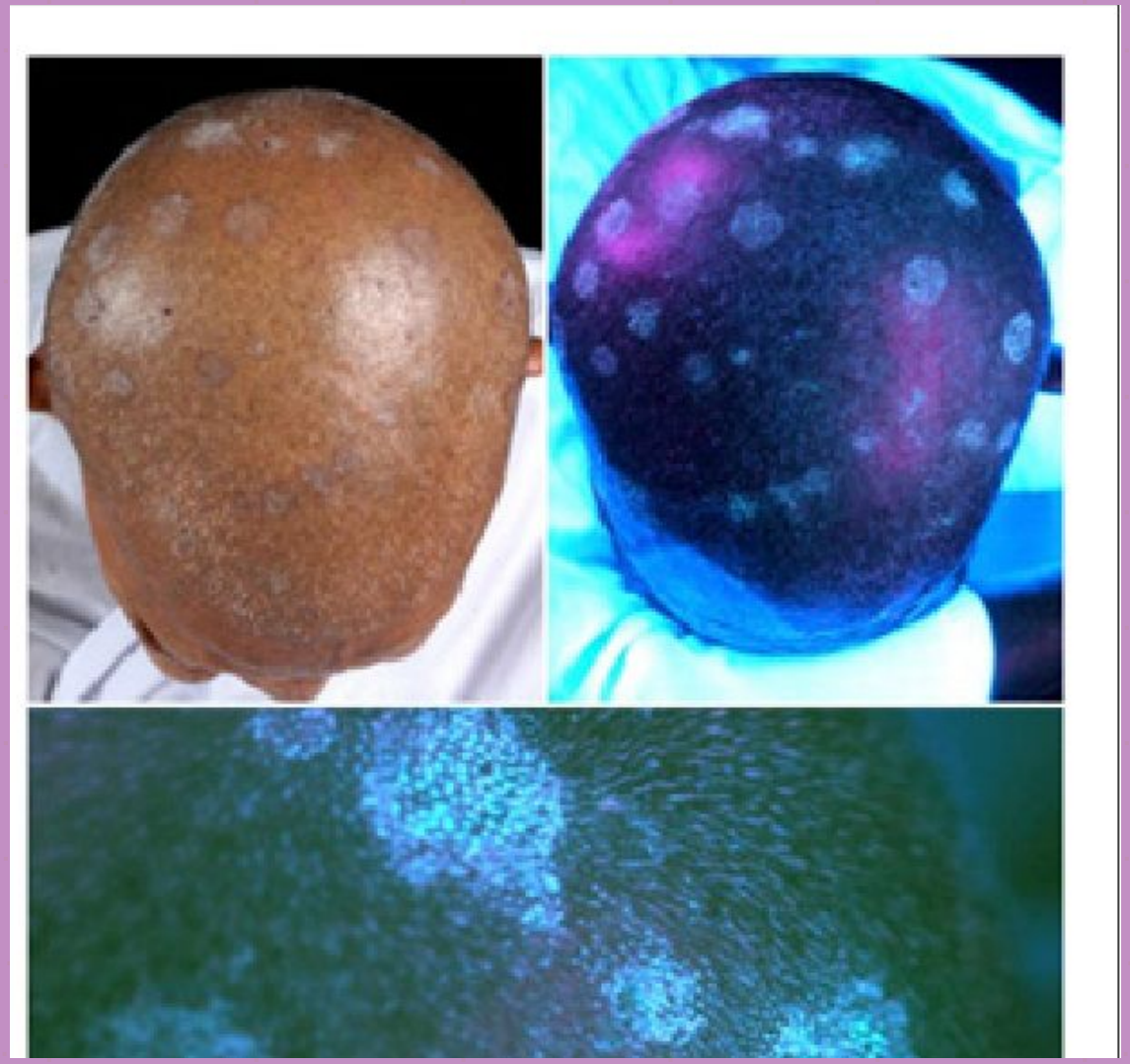
- *In diseases caused by fungi of the genus Microsporum,* spores are located on the surface of the hair - like **ectothrix.** Therefore, when hair is irradiated with ultraviolet rays (using a Wood's lamp), the affected areas glow gree.

- *Spores of fungi of the genus Trichophyton* can be located **both outside like ectothrix**, and inside the hair - like **endothrix.**

- *In some fungi of the genus Trichophyton (T.violaseum and T.tonsurans),* spores are located only inside the hair – like **endotrik**s, no glow is observed when irradiated with ultraviolet rays.



In the case when fungal spores are arranged according to the *ektotriks* type, damaged hair shines greenish when illuminated with ultraviolet rays.



Tinea capitis

- **Dermatomycosis of the scalp (tinea capitis)** is accompanied by damage to the scalp and hair. The infection begins with the invasion of fungal hyphae into the scalp, then spreads to the hair follicles. Accompanied by the formation of crusts, itching, areas of baldness. Hair becomes brittle and breaks easily.

Favus (baldness)

One of the special forms of ringworm of the scalp **(favus, baldness).**

Favus is a chronic disease, mainly in children, caused by T. schoenleinii. Acute inflammation of the hair follicles leads to the formation of crusts around them - **scooters.** After these crusts come off along with the damaged hair, the lesion remains hairless (bald).

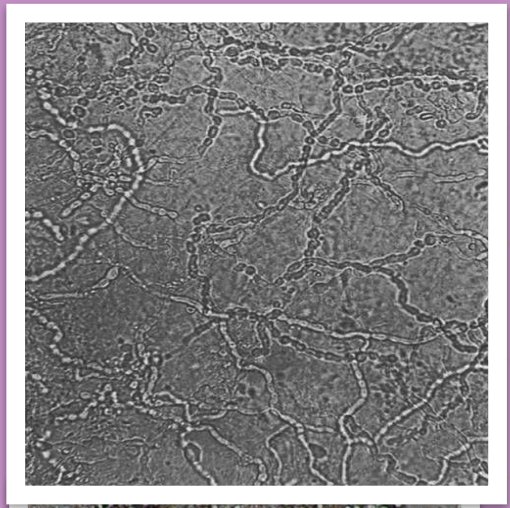
The causative agent does
not form spores inside the affected hair, but septate mycelium and gas bubbles are found.

Microbiological diagnosis of dermatomycosis

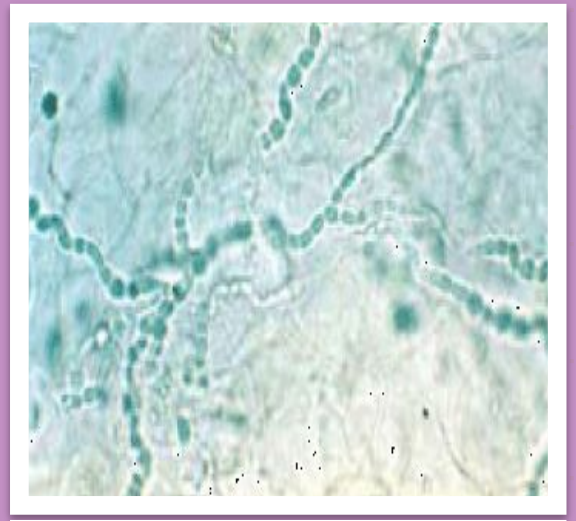
- Apply microscopic, mycological, allergological, serological and biological methods of treatment.
- Microscopic method. Scrapings from the affected skin, scales, nail plates, hair are treated with a 10-20% KOH solution to destroy keratin. Due to the treatment with an alkaline solution, the drug perceives light better, which makes it possible to detect fungal elements in a microscope.

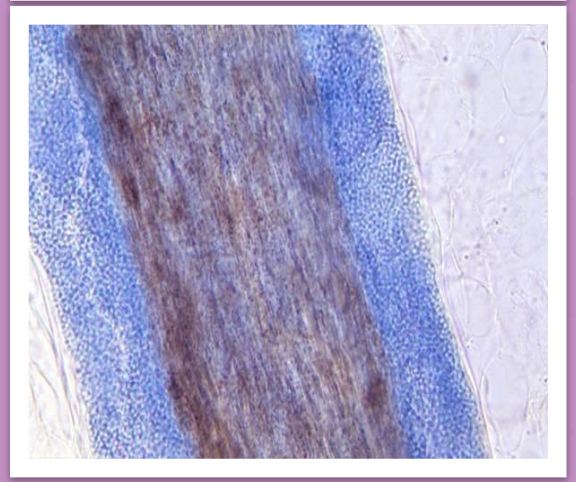
Under microscopy, septate mycelium or chains of arthroconidia (arthrospores). Hair microscopy of arthroconidia fungi of the genus Microsporum located outside the hair – like ectotriks. Spores of fungi of the genus Trichophyton are located on the surface (ectothrix) and inside (endotrix) of the hair. Mushroom spores T.tonsurans and T.violaseum in the affected hair are located only by the type of endotrix.

Dermatophytes in skin scales and affected hair

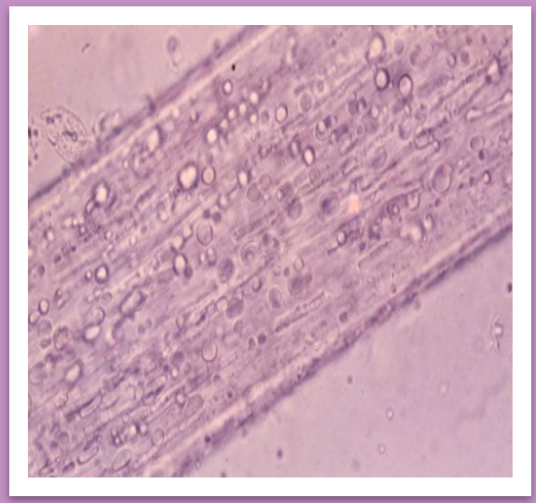


Trichophyton vialoseum **in skin flakes and affected hair**





Microsporum canis: in skin flakes and affected hair



Trichophyton schoenleinii:
pure culture smear, from damaged hair

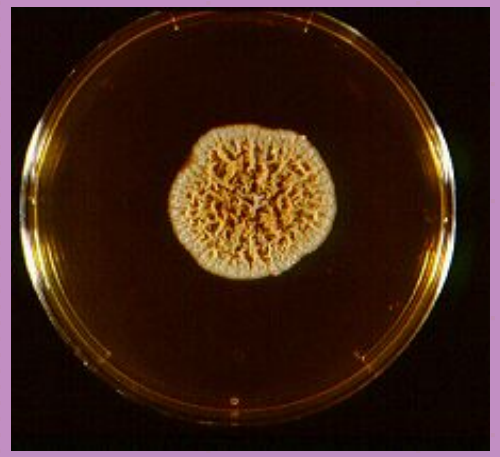
Microbiological diagnosis of dermatomycosis

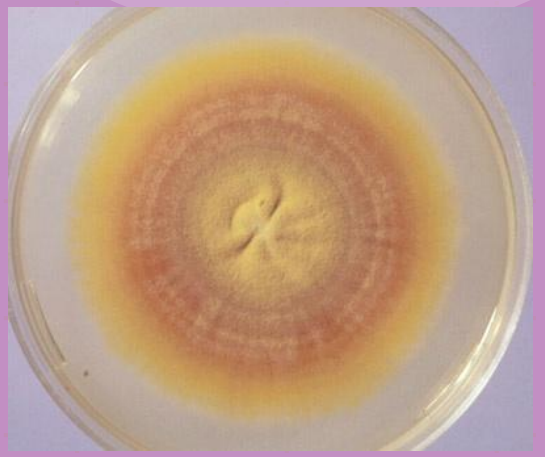
The mycological method is based on the cultivation of samples of skin scales, nail plates and hair (Sabouraud agar, etc.). Samples are incubated at room temperature for 1-3 weeks on Sabouraud's medium with the addition of cyclohexamide and chloramphenicol (to suppress the growth of bacterial flora). Pathogens are identified by the nature of the colonies, as well as morphological features (shapes and features of the location of macro- and microconidia) of preparations from the colonies.

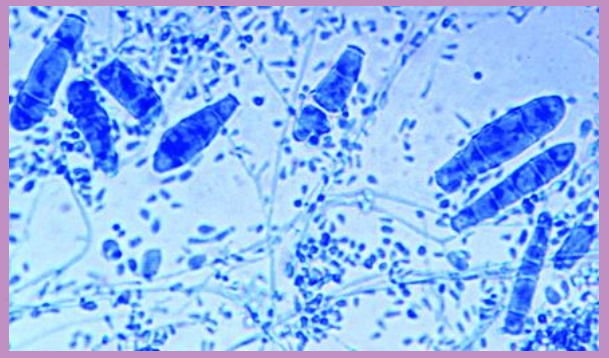
Allergic skin tests are performed with fungal allergens, mainly trichophytin.

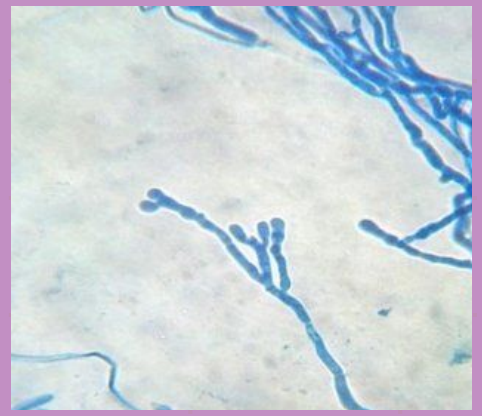
A biological sample is placed on laboratory animals (guinea pigs, white mice, etc.), infecting them in the skin, hair and claws.

Dermatophytes (microscopic picture of culture and pure culture smear)









Microsporium canis

Trichophyton mentagrophytes

Trichophyton schoenleinii

Epidermophyton floccosum

Treatment of dermatomycosis

Etiotropic treatment is carried out with the use of local and systemic antifungal therapy.

Fluconazole, ketoconazole, itraconazole, miconazole, terbinafine, griseofulvin, clotrimazole and other drugs are prescribed.

Modern etiotropic treatment of onychomycosis is carried out mainly in the mode of pulse therapy with itraconazole and is characterized by high efficiency.

Causative agents of subcutaneous (subcutaneous) mycoses

- The causative agents of subcutaneous mycoses live in the soil and on the surface of plants. Penetrating into places of skin microtrauma (damage by a splinter, a thorn, the introduction of other foreign bodies), they cause a chronic granulomatous infection.
- Fungi partially involve the deep layers of the dermis, subcutaneous tissues, muscles and fascia in the infectious process.
- Subcutaneous mycoses include sporotrichosis, chromomycosis, and eumicotic mycetoma.

Subcutaneous mycoses

Sporotrichosis

Sporothrix schenckii

Chromomycosis

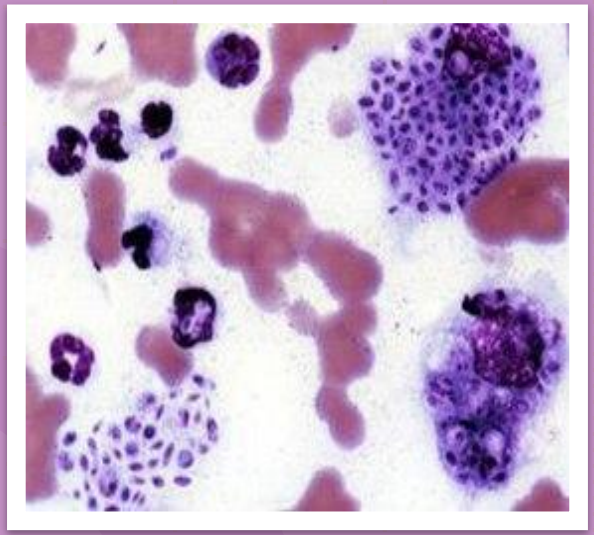
Phialophora verrucosa, Fonsecaea compacta, Fonsecaea pedrosoi, Exophiala jeanselmei and etc

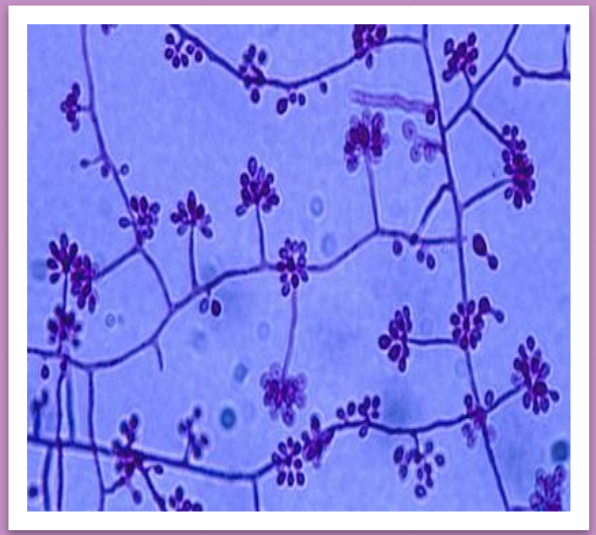
Mycetoma

Madurella mycetomatis, M.grisea, Pseidolles-cheria boydii, Phialophora cryanescens, Exophala jeanselmei and etc

The causative agent of sporotrichosis *(Sporothrix schenckii)*

- Morpho-biological properties. S.schenckii is a dimorphic fungus. In the patient's body it is found in the yeast (tissue) form, in the environment and on nutrient media - in the mycelial form.
- The yeast form forms cigar-shaped, oval cells with a diameter of 3-5 microns
  On nutrient media (simple nutrient media, Sabouraud medium) at a temperature of 18-30 ° C, they first form dark shiny colonies, later the colonies become folded and bumpy.
  The **mycelial form** in preparations prepared from the culture of the fungus is represented by a branching septate mycelium.





Pathogenesis and clinical manifestations of sporotrichosis

S.schenckii in mycelial form lives in the soil of tropical and subtropical zones, as well as on decaying plants. The causative agent enters the areas of microdamages of the skin by contact. The primary lesion is localized on all parts of the body, most often on the limbs.

In most cases, **the skin-lymphatic form** of the disease develops. The granulomatous nodes observed at the site of the introduction of fungi are necrotic with the formation of ulcers.

In some cases,a local form of sporotrichosis develops, which is accompanied by the formation of nodules and the development of lymphangitis.

In weakened individuals, dissemination of the pathogen occurs and visceral **sporotrichosis develops** - the lungs, skeletal system, abdominal organs and brain are affected.

Microbiological diagnostics

In smears from materials taken from the damaged area and treated with KOH, yeast (tissue) forms of the pathogen are detected in rare cases.

In histological preparations prepared from tissue biopaths and stained by the Gomory or Schiff method, fungi can be easily detected. RIF facilitates the detection of fungi in the test material.

The most accessible method for the diagnosis of sporotrichosis is the cultural method. A pure culture of the fungus (mycelial form) is isolated by cultivation on nutrient media at 22-25°C for 7-10 days with the addition of antibacterial drugs.

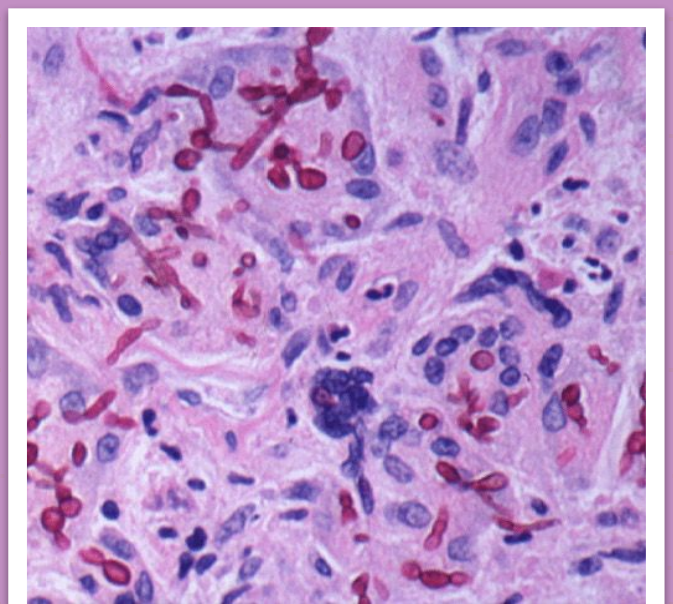
In the blood serum of patients, antibodies are detected in high titers using an agglutination reaction or latex agglutination.

Causative agents of chromomycosis

- The causative agents of chromomycosis - *Phialophora verrucosa, Fonsecaea compacta, Fonsecaea pedrosoi, Cladophialophora carronii, Exophiala jinnelmei,* etc. belong to demacia fungi.
- The brown-black shade of the fungus is due to the presence of melanin in the cell wall.

Causative agents of chromomycosis

The causative agents of chromomycosis are dimorphic fungi.
The **yeast form consists** of identical longitudinally and transversely dividing brown cells (sclerotia) 4-12 microns in size. Due to this division, they form clusters of rounded cells (4-8 pieces).
On nutrient media, they form velvety, wrinkled colonies (mycelial form). **Mycelial forms** are represented by dark brown septate mycelium and various types of conidia. The causative agents of chromomycosis differ in the features of the formation of conidia.



Pathogenesis and clinical manifestations of chromomycosis

The causative agents of chromomycosis live in the soil and on plants. Pathogens are transmitted by contact and enter the body through microtrauma of the skin. Infection occurs when the pathogen enters the microtrauma of the skin, and more often on the feet and legs. Partially progressive warty granulomatous nodules in the form of cauliflower form at the site of penetration due to hyperplasia of the epithelial tissue. In histological preparations, granulomatosis is noted, sclerotia of dark-colored fungi are detected inside leukocytes and giant cells.

Microbiological diagnostics

In scrapings and biopsy materials treated with KOH solution, brown round cells of the pathogen and its septate sclerotia are detected. Identification of sclerotia is a diagnostic sign of chromomycosis.

A pure culture of the fungus (mycelial form) is isolated by cultivation on nutrient media with the addition of antibiotics at a temperature of 22-25 ° C for 5-30 days.

The causative agents of chromomycosis form wrinkled colonies with a velvety surface, consisting of black septate mycelium and various types of conidia. Identification of the pathogen is carried out by their ability to form conidia.

The causative agent of mycetoma

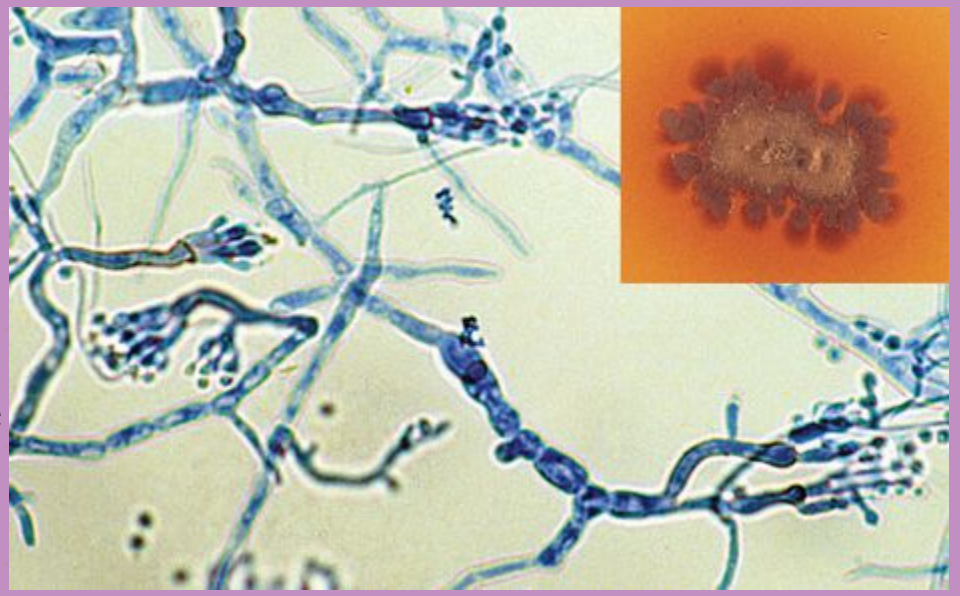
Mycetoma (maduromycosis, “Madura leg”) is a partially progressive chronic local infection of the subcutaneous tissue and connective tissue.

Mycetoma, which is a destructive and painless process, is caused by both actinomycetes **(actinomycetoma)** and fungi **(eumicotic mycetoma).**

Causative agents of eumicotic mycetoma

- The causative agents of eumicotic mycetoma live in the soil. *Madurella mycetomatis, Madurella grisea, Pseidollescheria boydii, Phialophora cryanescens, Exophala jinnelmei, Acremonium falsiforme,* etc. have the greatest etiological significance.
- Mushrooms of the genus *Madurella and E. jeanselmei* are fungi. *P.boydii* is

a homothallic fungus that forms ascospores in culture.

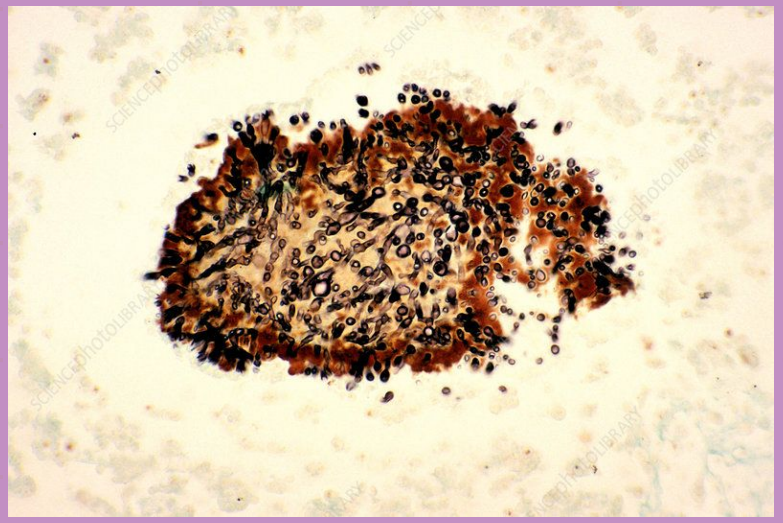


Mushrooms of the genus Madurella

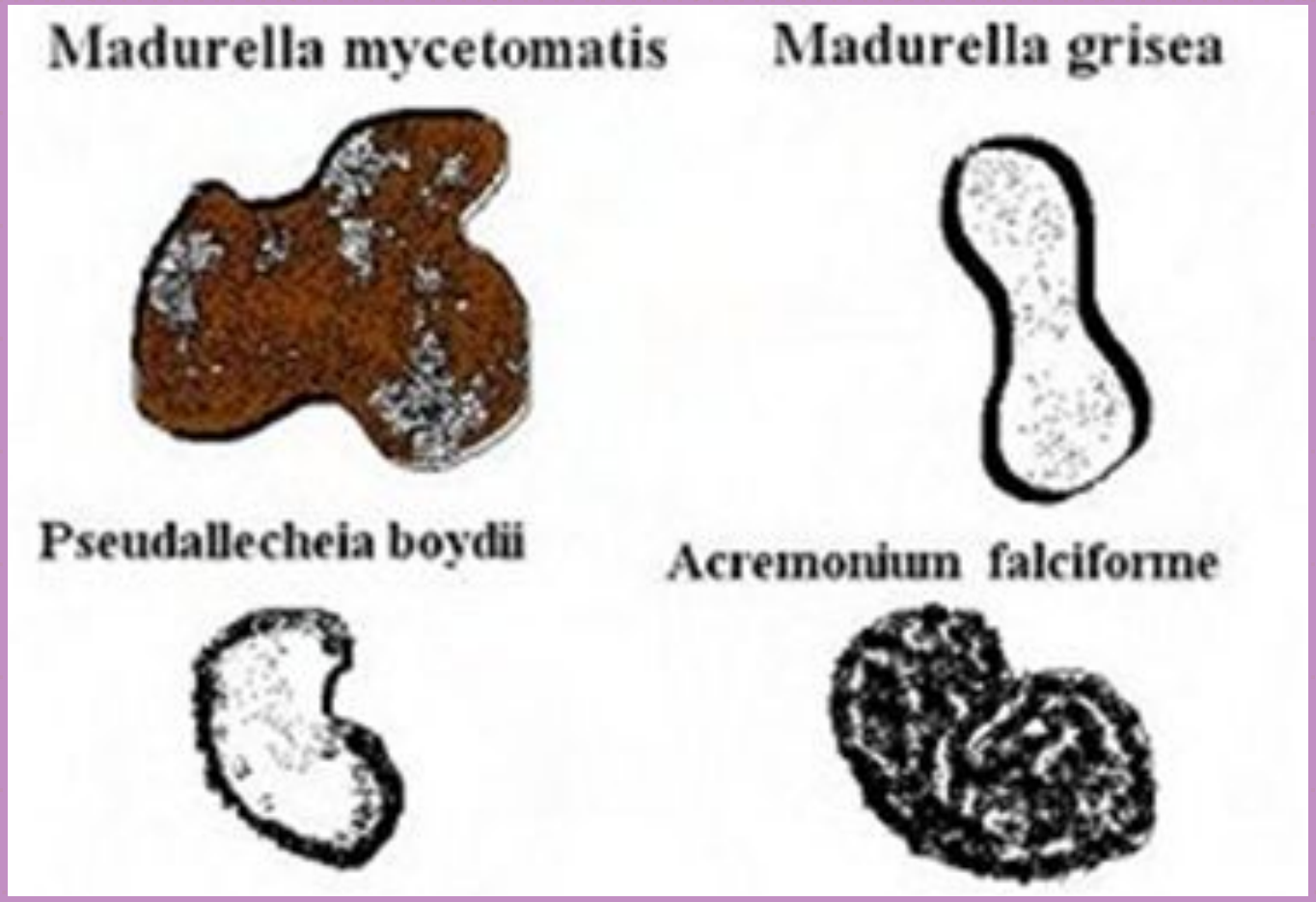
Mycetoma granules

Fungi in tissues form **mycetoma granules** about 2 mm in size. Solid consistency granules do not mix with each other, they consist of septic mycelium with a diameter of 3-5 nm.Along the periphery of the granules, the mycelium changes in shape and expands.

Based on the color of the granules, one can get an ide of the etiological agent. For example, *P.boydii* and *A.falsiforme* granules are white, *M.grisea* and *E.jeanselmei* are black, and M.mycetomatis forms granules of various colors from red to black.



Mycetoma granules



Pathogenesis and clinical manifestations of mycetoma

The causative agents of mycetoma, living in the soil and on the surface of plants, penetrate the body through microtraumas of the skin of the hands and feet.

Mycetoma is more common in people who walk barefoot.

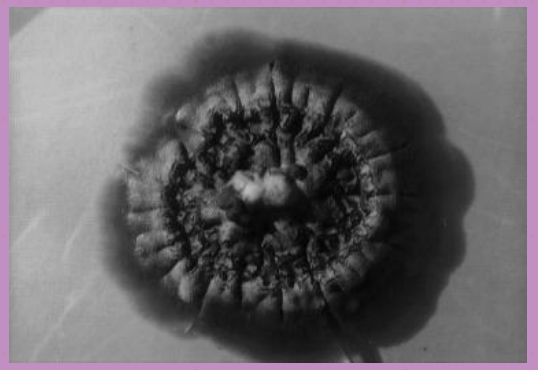
At the site of penetration of the pathogen into the body, granulomas are formed, abscesses with pus, released to the surface through fistulas and containing granules.

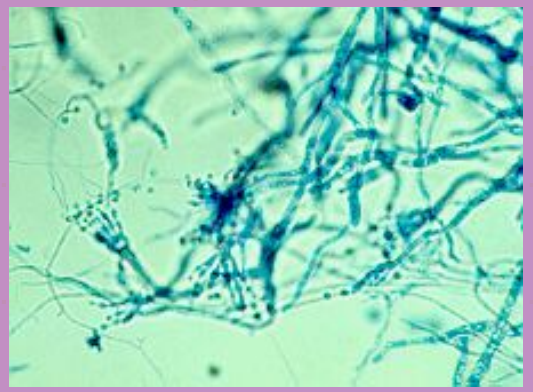
The destructive process affects the fascia, muscles and bones.

In untreated cases, the disease can last for years and spread to deeper layers and the periphery, the affected limbs swell and deform.

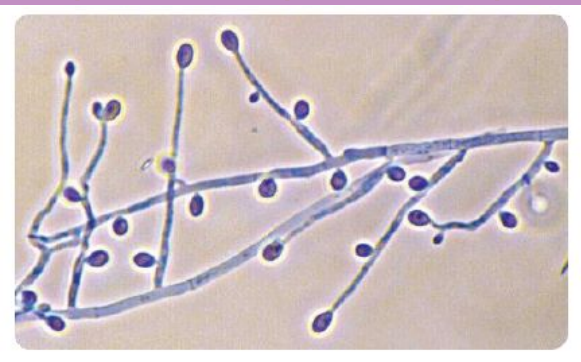
Microbiological diagnostics

The etiological agent can be determined by examining the nature of the mycetoma granules found in the pus.
It is possible to isolate the culture of the pathogen using certain nutrient media.





Madurella mycetomatis



Pseidollescheria boydii

Acremonium falsiforme

Treatment of eumicotic mycetoma

Long-term continuous etiotropic treatment is carried out.

When treating, it is advisable to take into account the characteristics of the pathogen. For example, if fungi of the genus *Madurella* are *detected, itraconazole, ketoconazole, amphotericin* B are prescribed; if fungi of the genus E. jeanselmei are detected, it is recommended to use flucytosine.

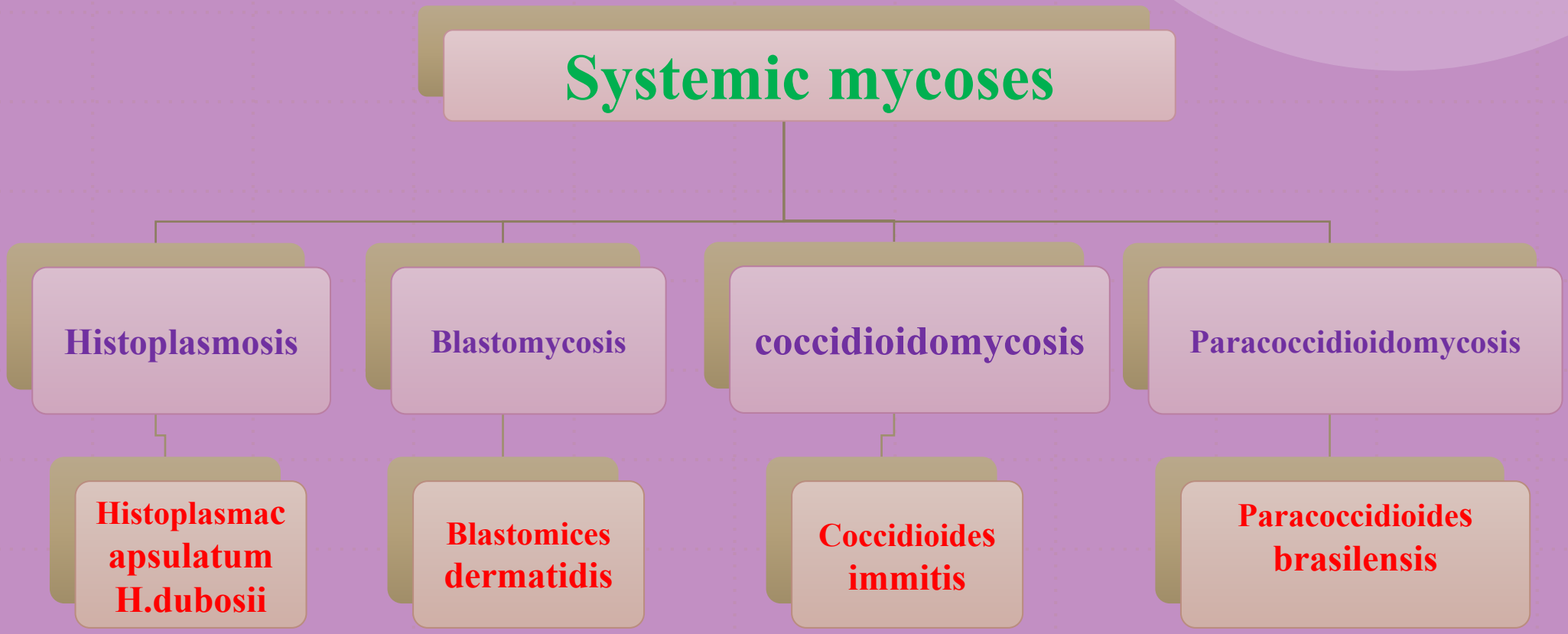
In the case when conservative treatment is late or the treatment is ineffective, surgical intervention (amputation) is performed.

Causative agents of systemic mycoses

Some systemic mycoses - coccidioidomycosis, histoplasmosis, blastomycosis And paracoccidioidomycosis, occurring in certain geographical areas, can be considered as endemic mycoses.

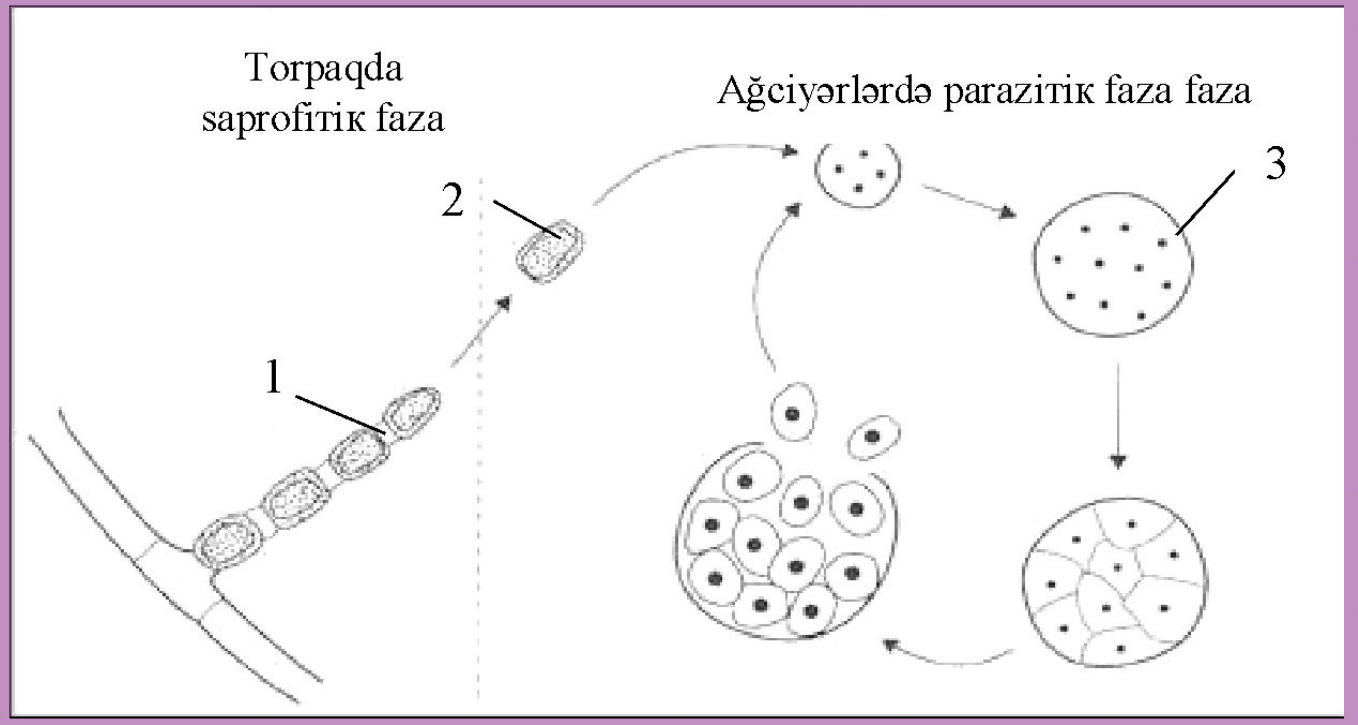
The causative agents of systemic mycoses are **dimorphic fungi** that live in the external environment, mainly in the soil, penetrating the body by inhalation, causing various diseases.

The primary focus mainly develops in the lungs, in some cases the disease is systemic. Diseases are not transmitted from person to person, except in certain cases.


Systemic mycoses
Histoplasmosis
Blastomycosis
coccidioidomycosis
Paracoccidioidomycosis
Histoplasmac apsulatum H.dubosii
Blastomices dermatidis
Coccidioides immitis
Paracoccidioides brasilensis

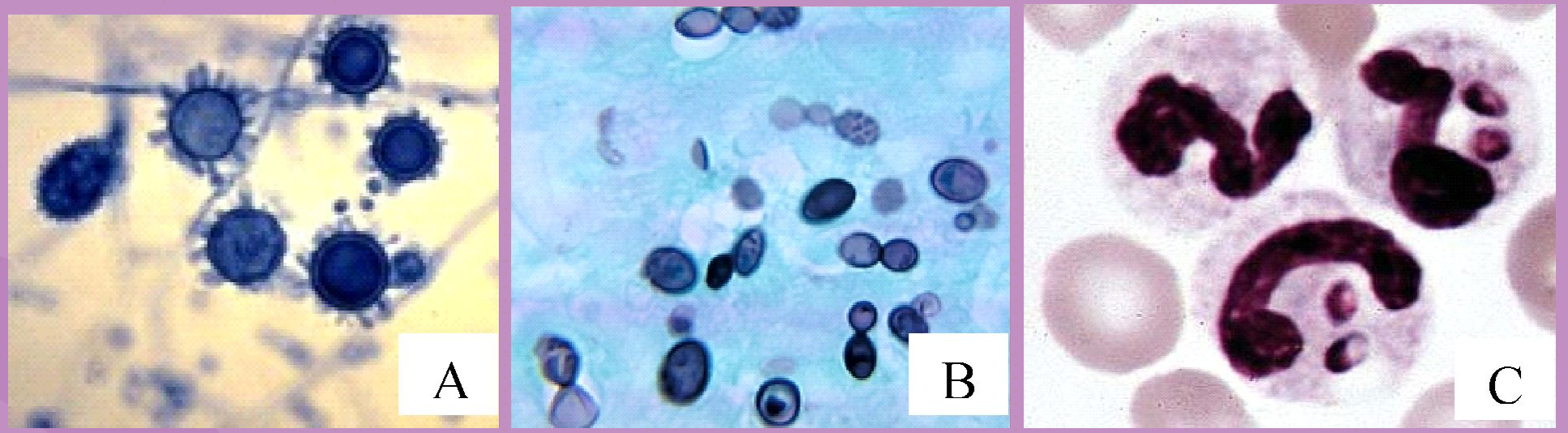
The causative agent of coccidioidomycosis

- Coccidioidosis, or coccidioidomycosis, is caused by *Coccidioides immitis* and *Coccidioides posadasii*.



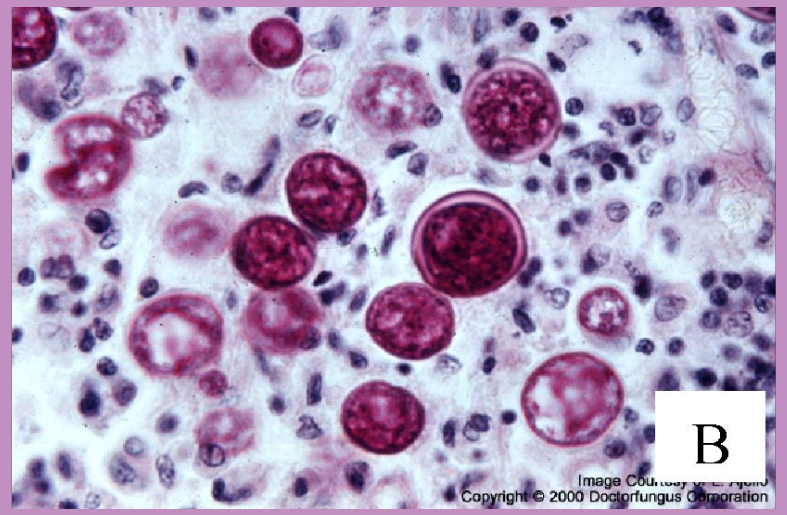
The causative agent of histoplasmosis

- American histoplasmosis is caused by *Histoplasma capsulatum*, which is registered in various countries of America. Biovar *H.capsulatum H.capsulatum var duboisii* is the causative agent of African histoplasmosis, registered only on the African continent.



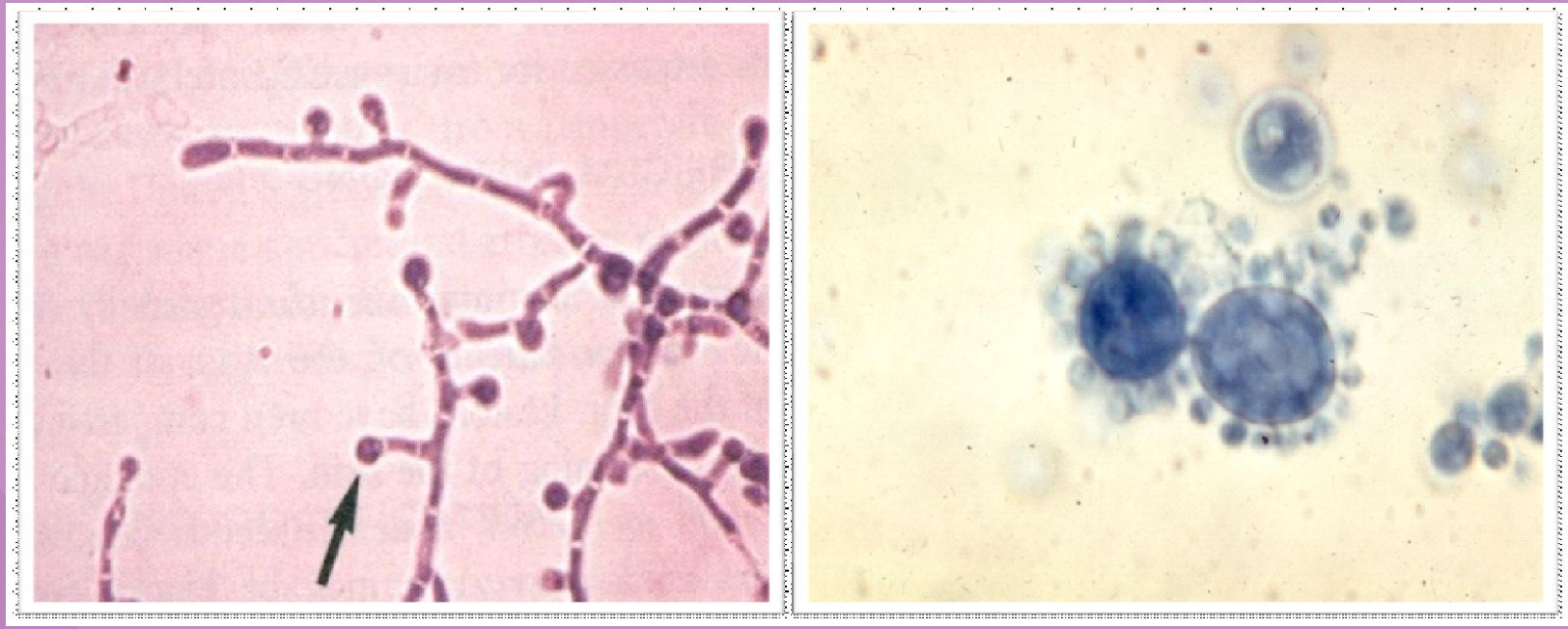
The causative agent of blastomycosis

- Blastomycosis, or North American blastomycosis, is an endemic systemic mycosis that occurs in the United States and Canada. The disease is also recorded in South America, Asia and Africa. The causative agent is *Blastomyces dermatitidis*, a dimorphic fungus that lives in the soil.



The causative agent of paracoccidioidomycosis

- Paracoccidiosis, or South American blastomycosis, is a systemic endemic mycosis that occurs mainly in South and Central America.
  The causative agent, *Paracoccidioides braziliensis*, is a dimorphic fungus that lives in the soil.



Microbiological diagnostics systemic mycoses

Microscopic, mycological, serological and allergic diagnostic methods are used.
Appropriate materials from sputum, blood, skin wounds, pus, urine and internal organs are used for the study.
The microscopic method is based on the detection of tissue forms of pathogens in the studied materials. In materials stained by the Giemsa method, as well as in stained histological samples, small oval-shaped yeast-like cells can be found.
Mycological method. Pathological materials are cultured in Sabouraud's agar.
Serological method. Specific antibodies are determined in the blood serum.

An allergic test for some mycoses is carried out by subcutaneous injection of the corresponding allergen (histoplasmin, coccidioidin).

Opportunistic fungi

Conditionally pathogenic fungi are the causative agents of **opportunistic mycoses**.
Some of these fungi *(genus Candida),* which cause diseases against the background of a weakened immune system, are also found in the normal microflora of the human body.
Activation of fungi in the body causes endogenous diseases.
Other opportunistic fungi *(Cryptococcus, Aspergillus, Mucor, etc.)* are found in the environment - in soil, water, air and in the body and often cause exogenous diseases.

Opportunistic mycoses

Candidiasis	Aspergillosis	Penicillosis	Zygomycosis
C.albicans, C.tropicalis, C.krusei and etc	*A.flavus, A.fumygatus, A.niger and others.*	*P.notatum, P.glaucum, and etc*	*Mucor, Rizopus, Absidia and others*

Mushrooms of the genus Candida

Fungi of the genus Candida are usually found in the microflora of the human body – In the microflora of the skin, mucous membranes and gastrointestinal tract. Some species cause candidiasis in humans.

Clinically significant species C.albicans, C.tropicalis, C.krusei,C.parapsilosis, C.glabrata (Torulopsis glabrata).

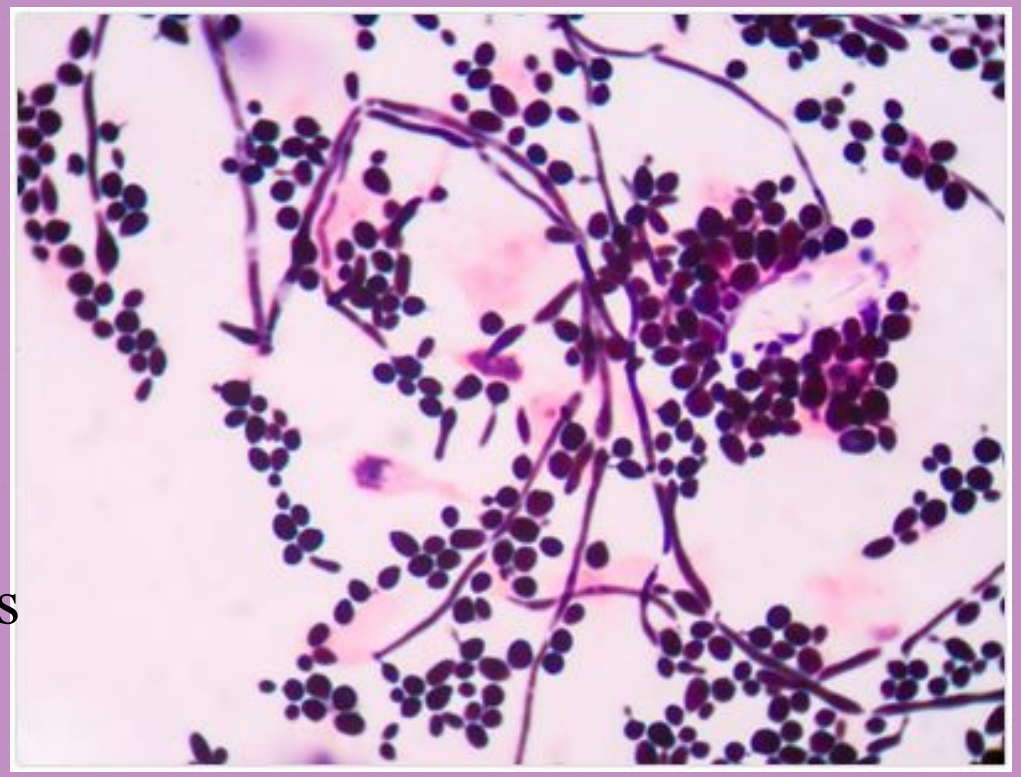
C. albicans and C. tropicalis play an important role in the etiology of candidiasis.

Mushrooms of the genus Candida

Fungi of the genus *Candida* consist of spherical, oval, elongated, budding yeast-like cells 3-6 microns in size.

Budding is represented by chains. Budding cells grow longitudinally, forming pseudomycelium (false mycelium), consisting of cells elongated in a chain.

The exception is *C. glabrata*, which does not form pseudomycelium. Unlike other species, *C. albicans* is dimorphic, since in addition to pseudomycelium, they also form true mycelium.



Mushrooms of the genus Candida

The species C. albicans, which is of particular importance in human pathology, differs from other species in some properties.

They are characterized by the formation of a "*germ tube*" - a real mycelium upon incubation in blood serum for 90 minutes at a temperature of 37°C.

Cultural properties of mushrooms genus *Candida*

Fungi of the genus **Candida**, after 24-hour incubation on conventional nutrient media, as well as on agar with glucose and Sabouraud agar, under aerobic conditions at a temperature of 37 ° C, form convex, shiny, creamy, large cream-colored colonies with a sour smell.

Ecology

Fungi of the genus Candida are found on the skin and mucous membranes of mammals and humans, as well as on environmental objects.

These fungi cause mainly endogenous infection against the background of weakened immune reactivity.

In addition, in very rare cases, these fungi can cause exogenous infections. For example, infection of newborns through the birth canal, as well as during breastfeeding, etc.

Pathogenic factors and clinical features of candidiasis

Candidiasis develops against the background of immunodeficiency, including AIDS,long-term use of broad-spectrum antibiotics, cytostatics, hormonal drugs, metabolic disorders and hormonal disorders (diabetes, etc.).

The main causative agent is **C.albicans.**

According to localization and pathogenetic mechanisms, superficial candidiasis, **systemic candidiasis, as well as chronic candidiasis of the skin and mucous membranes are distinguished.**

Superficial candidiasi

Damage caused by fungi of the genus *Candida* is due to their colonization on the mucous membranes and skin surface and further invasion into epithelial cells.

Factors contributing to the development of superficial candidiasis include AIDS, pregnancy, diabetes, and trauma (burns and superficial maceration).

Candidiasis of the mucous membranes of the oral cavity, or "milk ulcer", is manifested by the formation of a white-gray creamy coating on the surface of the tongue, palate and lips - a pseudomembrane.

Invasion of fungi of the genus **Candida** into the vaginal mucosa causes **vulvovaginitis.**

Superficial candidiasis

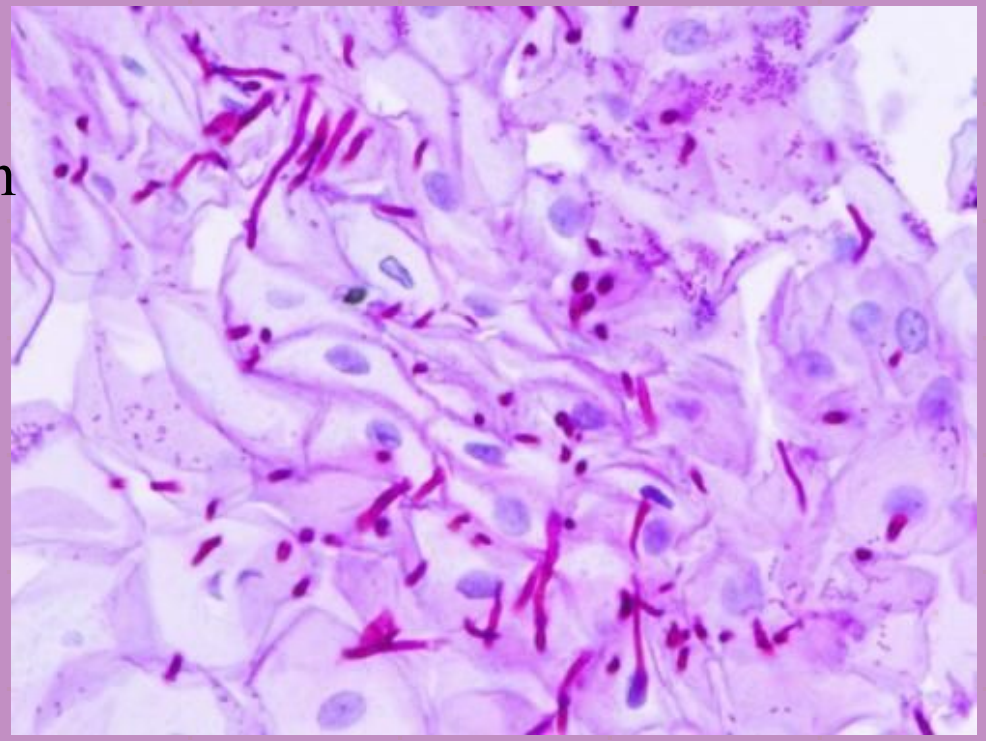
Invasion of fungi of the genus Candida into the skin – **cutaneous candidiasis** - most often develops against the background of injuries, burns, maceration.

The disease is more common in areas of high humidity -in the armpits, groin, perineum, in the folds of the skin, mainly in people who are obese and diabetic.

Invasion of fungi of the genus **Candida** into the nails and periungual tissues leads to **onychomycosis.**

Systemic candidiasis

The penetration of fungi of the genus **Candida** into the blood leads to candidiasis.
Various parenteral manipulations - surgical interventions, intravenous catheters, etc. can contribute to the penetration of fungi into the blood, but in normal healthy people, candidemia is short-term and transient.
In immunocompromised people, *Candida* can colonize and invade internal organs, especially the lungs, kidneys, eyes, endocardium, and meninges.
Systemic candidiasis most often occurs in patients taking corticosteroids and cytostatics,as well as in hematological Patients (with leukemia, lymphoma, aplastic anemia, etc.).



Chronic candidiasis of the skin and mucous membranes

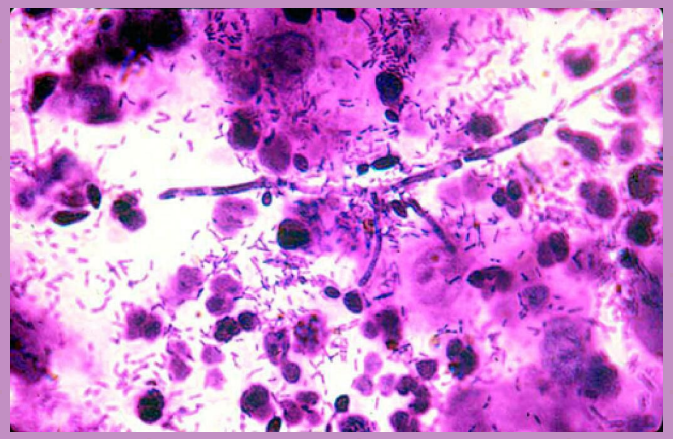
- Mostly occurs in early childhood.
  The disease develops against the background of cellular immunodeficiencies and endocrinopathies, manifests itself as candidiasis of various parts of the skin and mucous membranes.

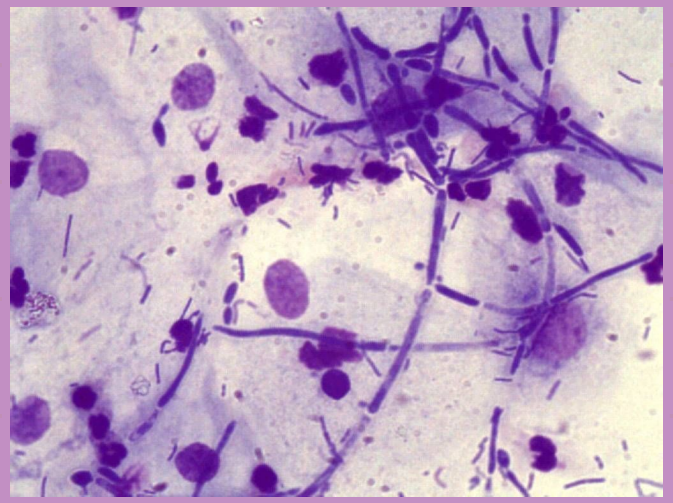
Microbiological diagnostics

Materials taken with a swab from areas of superficial damage, as well as scrapings,blood, cerebrospinal fluid, urine, exudate, etc. are examined.

Microscopic method. A diagnostic sign is the detection of pseudomycelium and budding yeast-like cells in smears prepared from pathological material.

Skin and nail scrapings are examined using the “crushed drop” method with the addition of an alkaline solution.





Cultural (mycological) method

By cultivating pathological materials on Sabouraud's medium and other media, it is possible to isolate and identify the culture of the pathogen.

C. albicans is differentiated from ther species using the germ tube test and their ability to form chlamydospores.

Cultural (mycological) method - interpretation

Fungi of the genus Candida are often found in the normal microflora of the body, so the isolation of the culture does not always confirm the **etiological role of fungi**.
Cultures from sputum and feces, as well as lesions on the surface of the skin, do not always indicate disease and must be confirmed by other methods.
Isolation of culture from the blood indicates systemic candidiasis or transient candidemia.
To confirm the diagnosis of "systemic candidiasis" it is necessary to conduct repeated examinations.

In case the culture is isolated from urine, it is important to estimate the number of cells per 1 ml of the material. Diagnostic value is the presence in 1 ml of 103 or more cells of the fungus (colony-forming unit - CFU) in the urine.

Serological method

The detection of antibodies to fungi of the genus Candida in the blood serum has no diagnostic value, since these antibodies are also found in healthy people.
Although the determination of fungal **mannan polysaccharides** in serum is more pecific, it has low sensitivity.
Recently, new serological methods have been developed based on the search for **beta-glucan polysaccharides** in the blood.

Treatment and prevention of candidiasis

Treatment of candidiasis is carried out, first of all, by determining and correcting the pathogenetic factors of the disease.
In the treatment of superficial candidiasis, nystatin, levorin and azole preparations are mainly used. For systemic candidiasis, amphotericin B is often used in combination with fluconazole or flucytosine. Ketoconazole and other azole drugs are effective in chronic candidiasis of the skin and mucous membranes, but in people with hereditary immunodeficiency, the disease sometimes requires lifelong treatment.

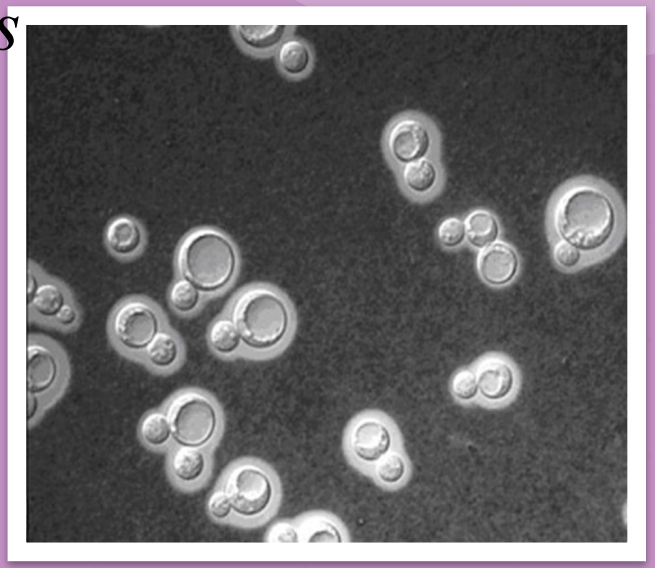
All patients at risk, including those receiving long-term antibiotic therapy, are prescribed **antifungal (antifungal) drugs** to prevent the development of candidiasis.

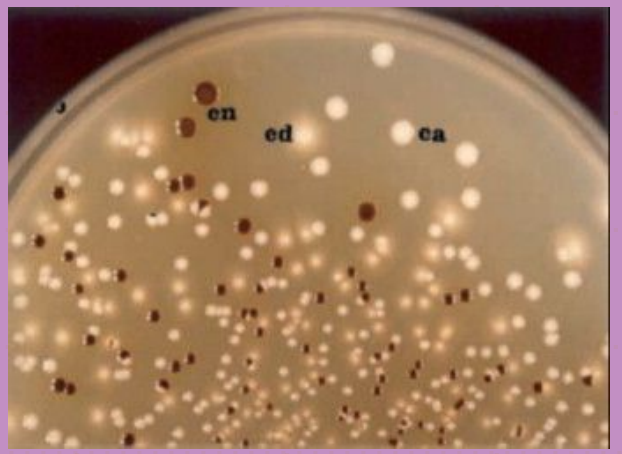
Genus Cryptococcus

Two species of the *genus Cryptococcus, Cryptococcus neoformans* and *Cryptococcus gatti,* cause cryptococcosis in humans.

Cryptococci are spherical yeast cells with a diameter of 5-10 microns surrounded by a thick capsule.

They grow easily in normal nutrient media as well as on Sabouraud's medium.Due to the presence of polysaccharide capsules, they form light and slimy colonies. Glossy, creamy brown colonies may form on Sabouraud agar. While *C.neoformans* and *C.gatti* can grow at both 250C and 370C, saprophytic cryptococci do not grow at 370C.





Genus Cryptococcus (ecology)

C. neoformans is widely distributed in the environment, more commonly found in the dried feces of birds, especially pigeons, but the disease has not been observed in birds.

Infection occurs by aerogenic mechanism, air-dust way. Cases of collective infection are described by inhalation of contaminated dust while working in old buildings contaminated with pigeon droppings.

The disease is not transmitted from person to person.

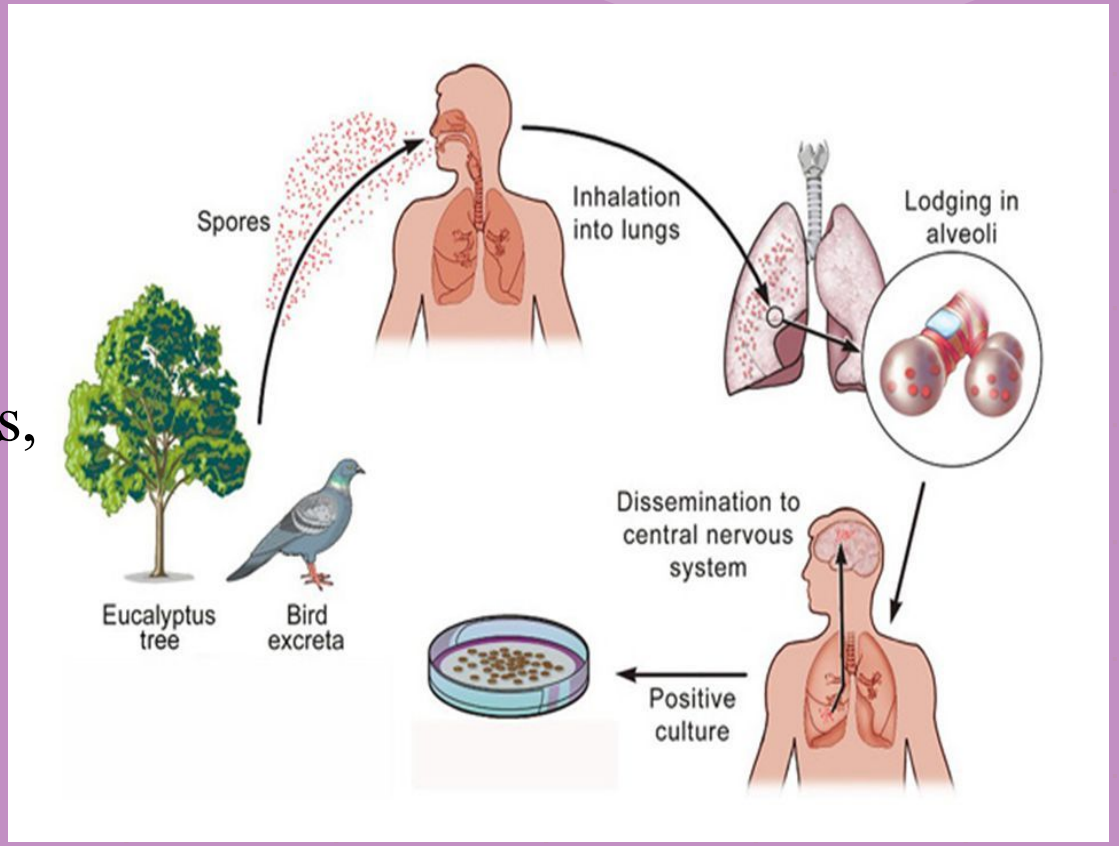
Pathogenesis and clinical manifestations of cryptococcosis

Inhaled mushrooms form the primary focus of inflammation in the lungs.

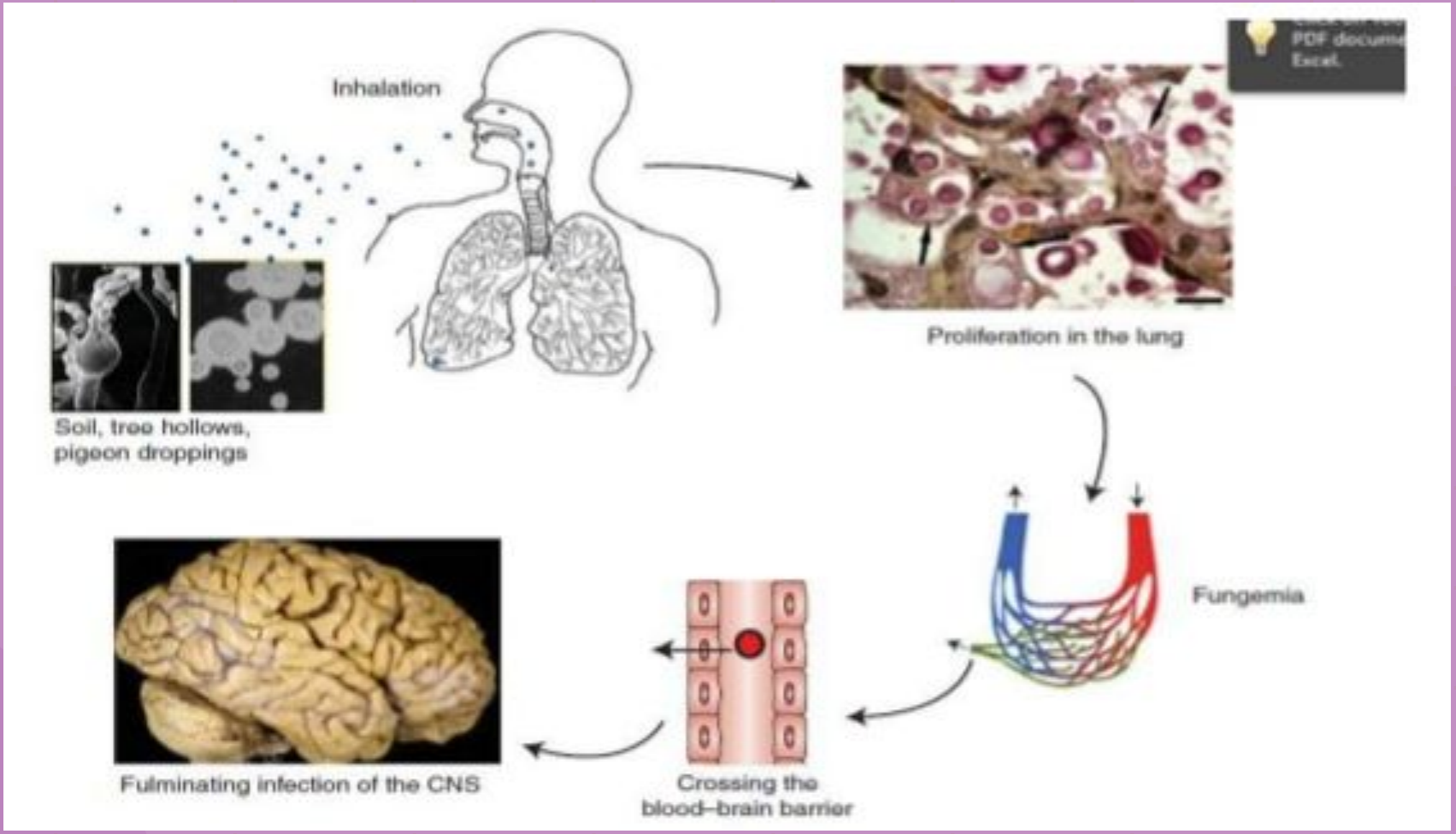
- Primary cryptococcosis is often asymptomatic or flu-like. In most cases, the process ends with a spontaneous cure.

In immunocompromised people, the spread of the fungus from primary foci in the lungs to other organs, especially the central nervous system, causes **cryptococcal meningoencephalitis**.Cryptococcal meningitis occurs in approximately 5-8% of AIDS patients.

As a result of dissemination, other organs may also be involved in the process - skin, adrenal glands, bones, eyes, prostate gland



The pathogenesis of cryptococcal meningoencephalitis



Microbiological diagnostics

The material for the study is sputum, pus, urine, cerebrospinal fluid, tissue biopsy specimens.

- **Microscopic method.** In native and ink-stained preparations, the pathogen surrounded by a mucous capsule looks like rounded yeast cells.
- **Mycological method.** To isolate a pure culture, the test material is inoculated on the medium of Sabouraud et al. at a temperature of 370C. Identification is based on growth at 370C and urease production. On differential diagnostic media containing diphenol, melanin-synthesizing *C. neophorms* and *C. gatti* form creamy brown colonies.

- **Serological method**. It is based on the detection of capsular antigen in blood serum and cerebrospinal fluid by latex agglutination and ELISA (antibodies to the capsular antigen are adsorbed on latex particles). In 90% of patients with cryptococcal meningitis, this reaction is positive. Effective treatment reduces the antigen titer, but the antigen titer remains high in AIDS patients for a long time.

Treatment of cryptococosis

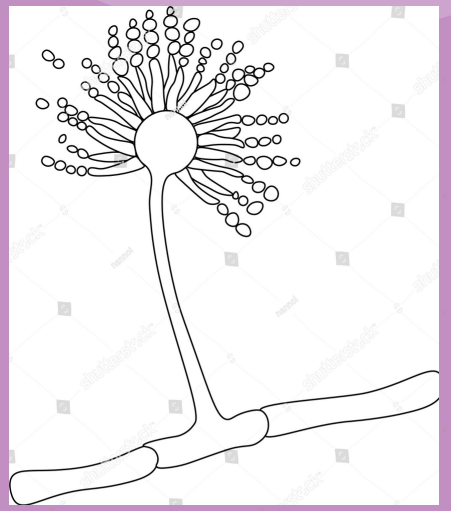
For cryptococcal meningitis, combined treatment with amphotericin B and flusitocin is used.
If this treatment is ineffective, fluconazole is prescribed, which can penetrate the blood-brain barrier.
Patients with AIDS should be treated in combination with effective antiretroviral therapy.

Genus Aspergillus

Fungi of the genus *Aspergillus belong to ascomycetes (type Ascomycota).*
Aspergillus live everywhere: in soil, water, air, on the surface of decaying plants.
Some species, in particular *A.fumigatus* in *humans cause opportunistic mycosis*
- aspergillosis. The disease can be caused by other types of fungi *Aspergillus*
- A.flavus, A.niger, A.terreus.

Genus Aspergillus

Aspergillus are molds with septate, branching mycelium. At the ends of the mycelium there is a characteristic spore apparatus. Chains of exospores (conidia) in the form of a trickle of water poured from a watering can are located on the surface of sterigmata (metula, phialides) - cells arranged in one or two rows at the expanded terminal end of the conidiophore.



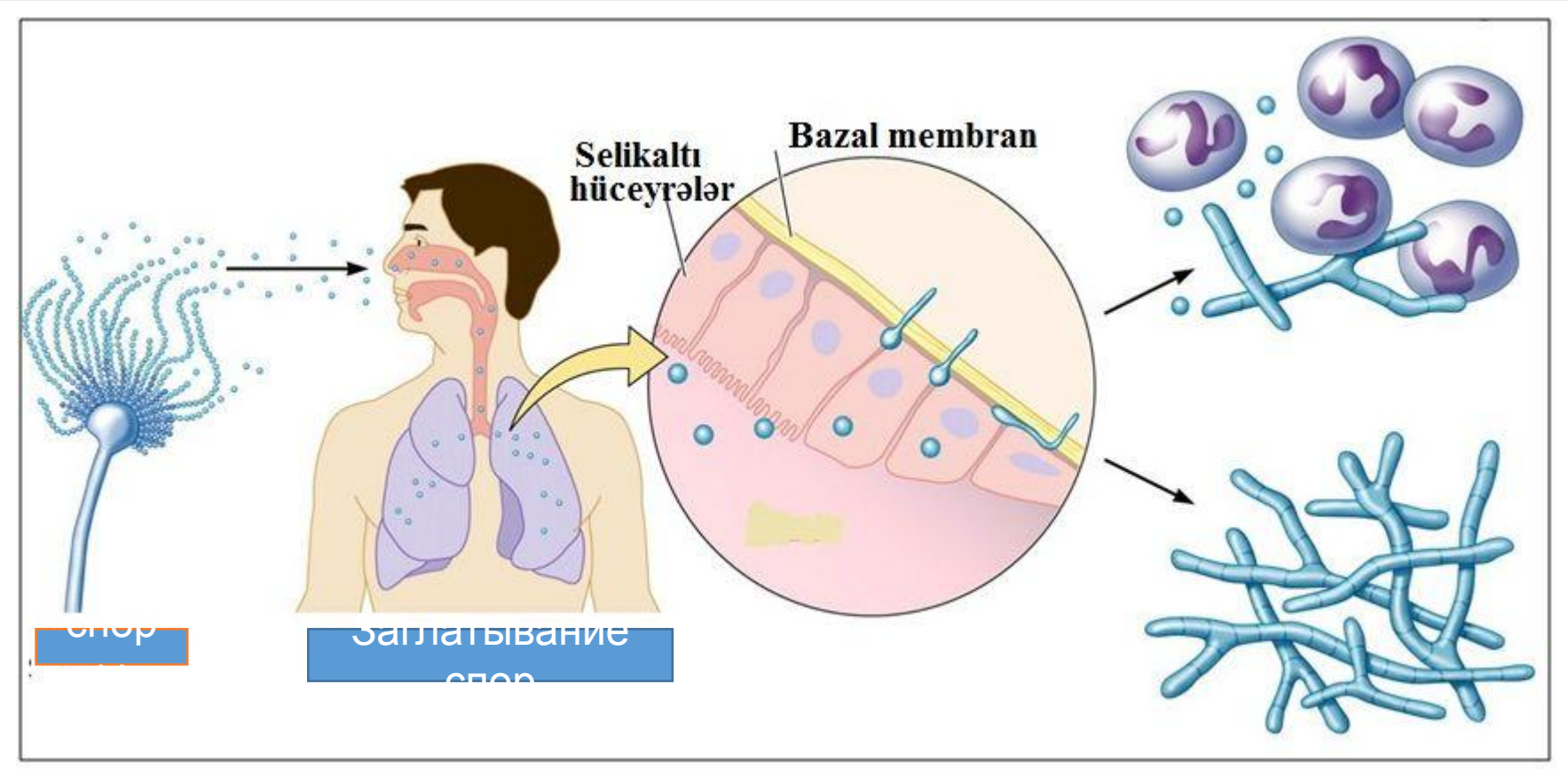
Conidia of different sizes and colors (black, green, yellow, white, etc.), which is used to identify species.

Aspergillus, being aerobes, grow both on simple nutrient media and on Sabouraud's medium at a temperature of 24-37 ° C. After 2-4 days, white fluffy colonies grow on dense media, which are subsequently stained additionally.

Pathogenesis and clinical manifestations of aspergillosis

Spores that enter the lungs through the respiratory tract when inhaled can cause allergic reactions in people with atopy.
In immunocompetent individuals, the spores are taken up by alveolar macrophages and destroyed. However, in immunocompromised people, especially those with leukemia and bone marrow transplantation, as well as in patients receiving corticosteroids and cytostatics, spores develop by forming hyphae and penetrating into the lung tissue, causing a mycotic process.

Aspergillosis pathogenesis

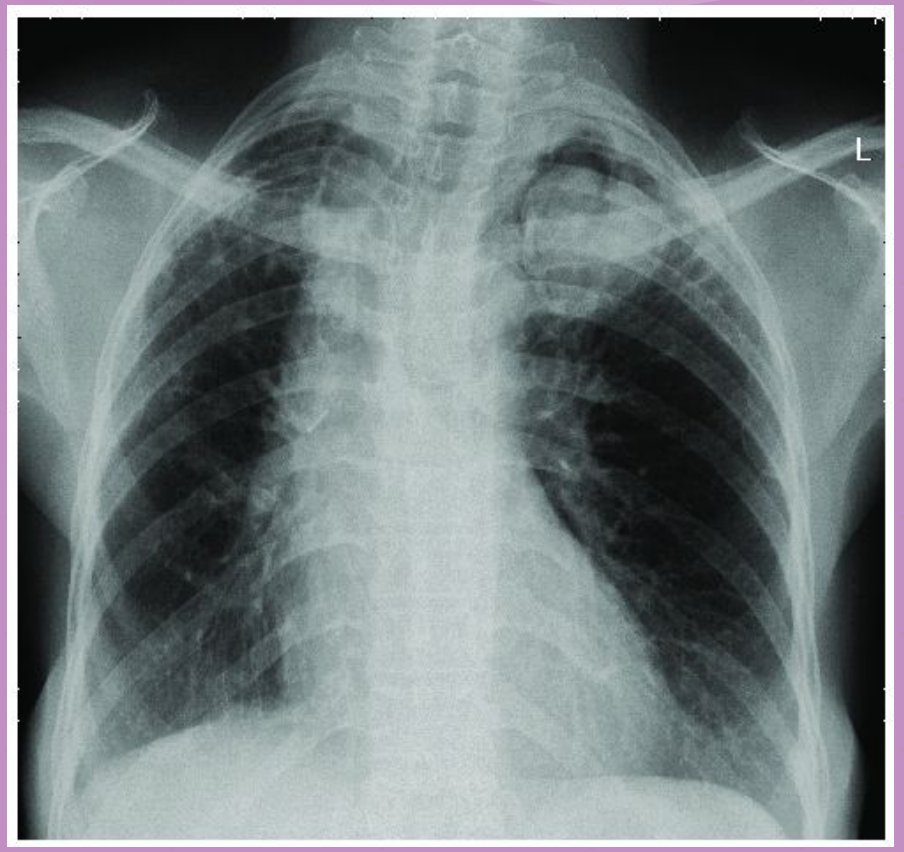


Common clinical manifestations of aspergillosis

Allergic aspergillosis. The formation of IgE to surface antigens of spores penetrating the lungs can lead to **allergic processes** - atopic bronchial asthma in individuals with atopy.
Allergic bronchopulmonary aspergillosis. Sometimes in people with atopy, hyphae formed from spores colonize the bronchi without penetrating the lung tissue. Such cases are characterized by the occurrence of bronchial asthma, eosinophilia, the formation of transient infiltrates in the lungs.
As a result of repeated inhalation of large doses of conidia, **exogenous allergic alveolitis** develops in normal healthy
individuals.

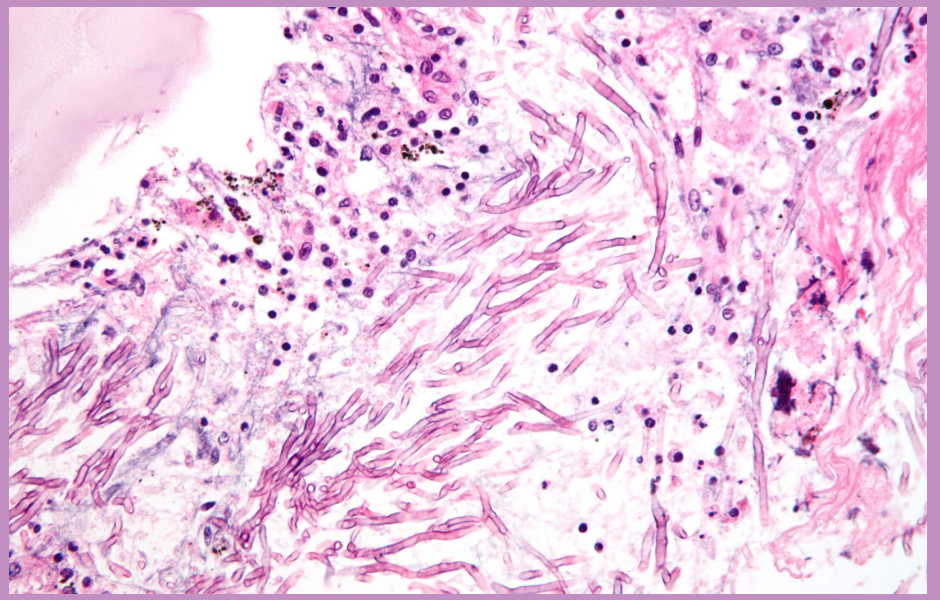
The most common clinical manifestations of aspergillosis

Aspergilloma develops in patients with chronic cavernous processes in the lungs (tuberculosis, sarcoidosis, emphysema). Spores penetrating the lungs multiply, forming a large number of hyphae inside the caverns and lead to the appearance of a globular tumor-like formation - aspergilloma.



The most common clinical manifestations of aspergillosis

İnvasive aspergillosis. Invasive infection caused by the formation of hyphae from spores entering the lungs can lead to the development of acute pneumonia.



Invasive aspergillosis occurs in immunocompromised people, including AIDS patients, as well as in patients with leukemia and bone marrow transplantation, and in people taking corticosteroids.

In some cases, **dissemination** of the disease from the lungs to other organs is possible - the gastrointestinal tract, kidneys, liver, brain.

The growth of some fungiof the genus *Aspergillus*, especially *A.flavus* in food sometimes leads to the development of toxins in them - aflatoxins. Consumption of such food causes food poisoning in humans - aflatoxicosis.

Microbiological diagnostics

Sputum, bronchial lavage, lung tissue biopsy, etc. are mainly used for research.

Microscopic method. In preparations prepared from sputum and treated with KOH, one can detect septate, thick (up to 4 microns) mycelium of fungi of the genus *Aspergillus*. Mycelia are also found in histologically stained specimens.

The mycological method is based on the cultivation of pathological materials on nutrient media. Culture isolation is not always sufficient to confirm the diagnosis. Contamination of test materials with fungi of the genus *Aspergillus* should be excluded.

Serological method. In most patients with allergic forms of aspergilloma and aspergillosis, antibodies to A. fumigatus are detected in the blood serum by means of a precipitation reaction. However, serological tests for the diagnosis of invasive aspergillosis are not informative due to immunodeficiency. Despite this, in invasive aspergillosis, the detection of galactomannan polysaccharides of the Aspergillus cell wall in the blood serum is of diagnostic importance.

The causative agents of mucormycosis

Mucormycosis (zygomycosis) is an opportunistic disease caused by fungi of the Zygomycota type (Mucor, Rhizorous, Absidia, Rhizomucor, etc.).

Zygomycetes are fungi with non-septate branched mycelia. They reproduce asexually (sporangia) and sexually (zygospores).

Mycelium is attached to the substrate with the help of special branches – rhizoids. The tips of the reproductive micelles of these fungi - conidia – expand to form sporangia, in which sporangiospores are located. Zygomycetes develop as molds on ordinary nutrient media, as well as on Sabouraud's medium under aerobic conditions at 22-37°C for several days.

Pathogenetic and clinical features of mucormycosis

Mucormycosis is an opportunistic mycosis, **the risk group** mainly includes diseases accompanied by acidosis –in particular, patients with diabetes mellitus, as well as leukemia, lymphoma, people taking corticosteroid treatment, as well as with extensive burns, immunodeficiency.
An important clinical form of the disease is **rhinocerebral mucormycosis**. The transformation into hyphae of sporangiospores, penetrating into the nasal cavity and further into the blood vessels, leads to thrombosis, heart attacks and necrosis.

Inhalation of sporangiospores in high-risk individuals may develop invasive **pulmonary zygomycosis**. There are also gastrointestinal and cutaneous forms of the disease.

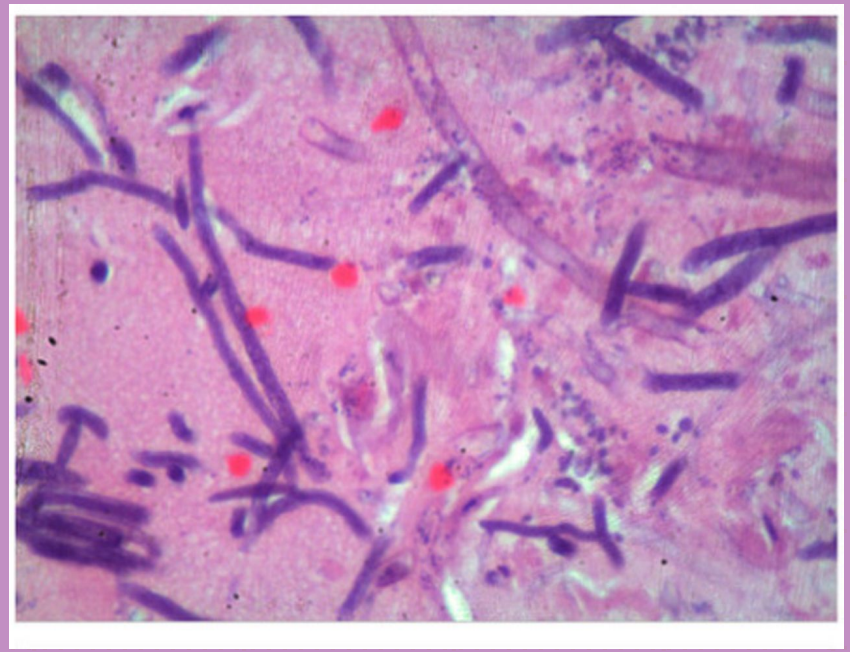
Microbiological diagnostics

The sampling of materials for research is carried out depending on the localization of the disease.

Microscopy of smears from pathological materials reveals unsepted thick hyphae.

Fungal culture isolation is not always sufficient to confirm the diagnosis.

It is necessary to exclude contamination of the studied materials with zygomycetes.



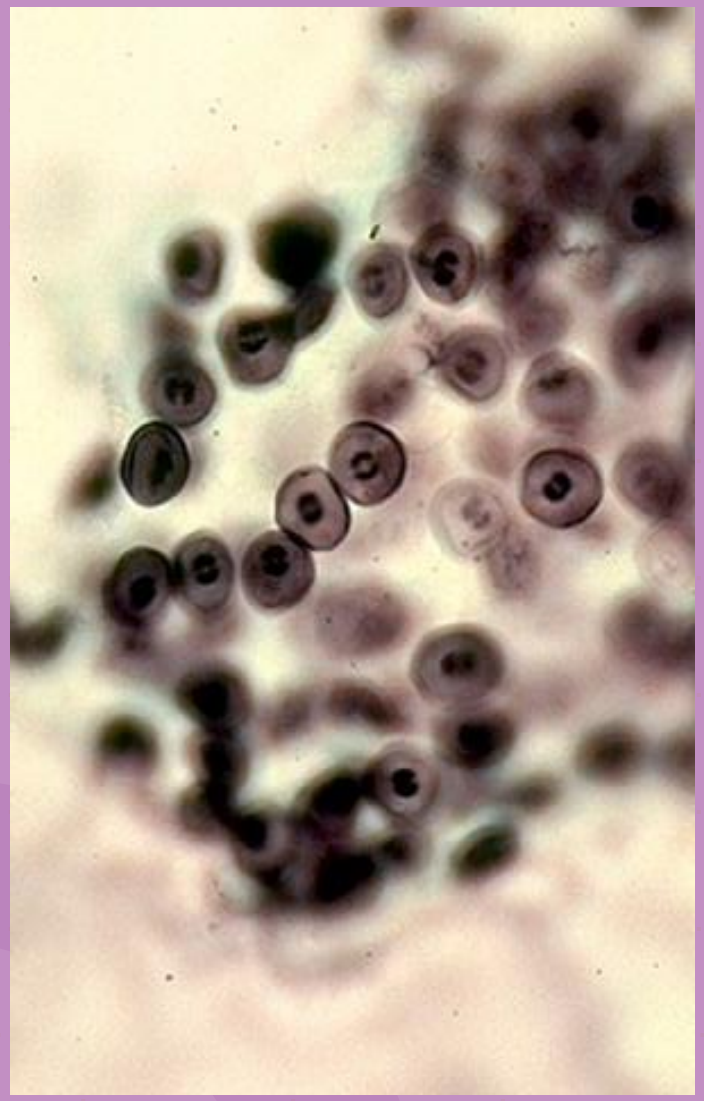
Pneumocystis jiroveci

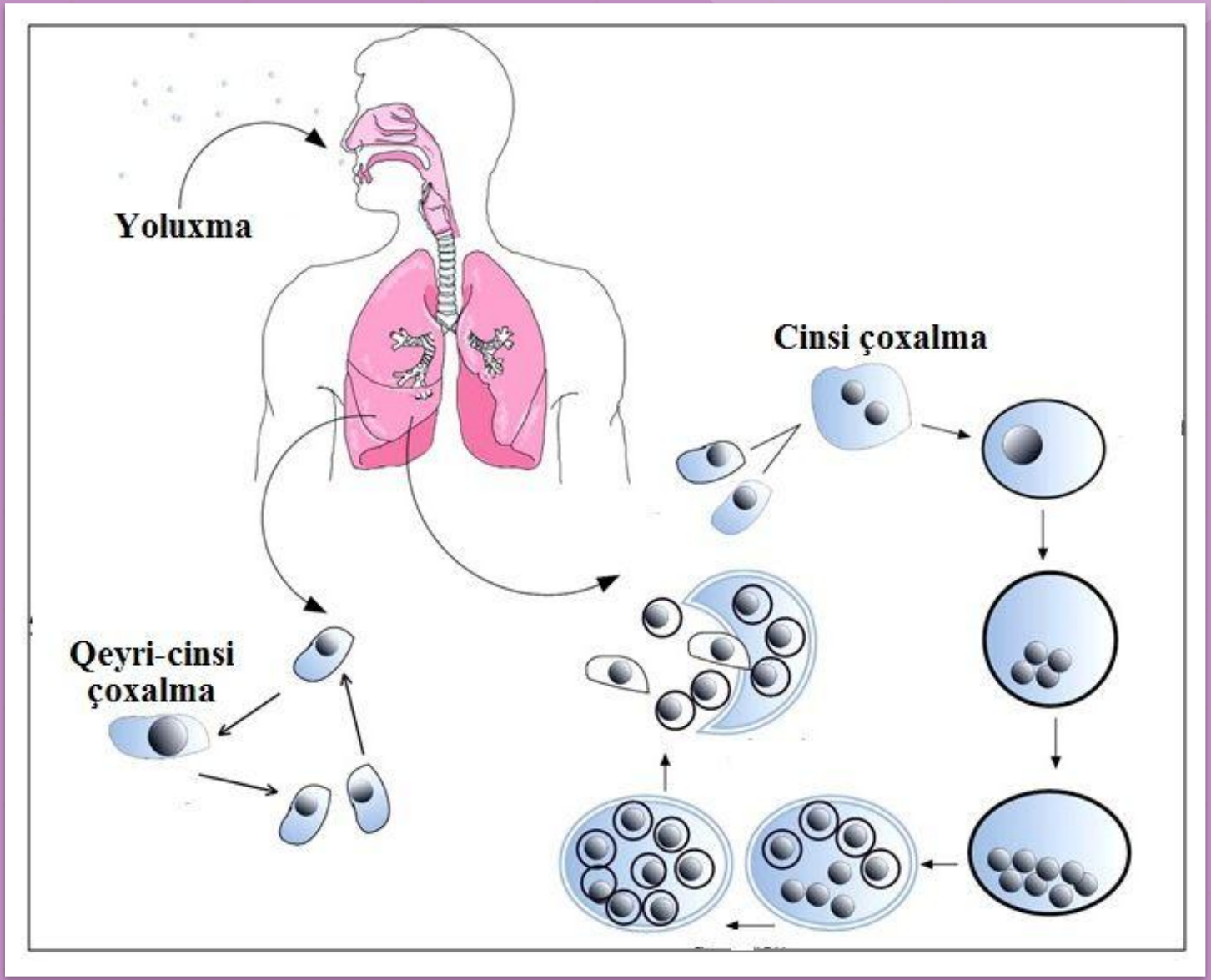
Pneumocystis jiroveci causes pneumonia in immunocompromised people.
Microorganisms of the genus *Pneumocystis* were previously classified as protozoa.
However, molecular biological and genetic studies have shown the similarity of this fungus with ascomycetes. Despite this, in terms of morphological and other properties, sensitivity to antimicrobial drugs, they are similar to protozoa, therefore they are considered a transitional form between protozoa and fungi.

Pneumocystis jiroveci

P.jiroveci form two different morphological forms - trophozoite and cyst.
Trophozoites are oval or amoeboid cells 1-2 microns in size with thin walls. They reproduce sexually and asexually. In the process of sexual reproduction, haploid trophozoites combine to form cysts. Cysts have thick walls, spherical or elliptical shape, 4-8 nuclei (or sporozoites).
The cyst size varies within 4-6 microns.
Sporozoites have a small nucleus and are surrounded by a double membrane and are 1-2 microns in diameter. After leaving the cyst, they turn into trophozoites. In clinical materials, trophozoites and cysts are found in the form of dense masses.

Pneumocystis jiroveci





Pathogenetic features of pneumocystis pneumonia

In cases of immunodeficiency, with the exception of AIDS, interstitial plasma cell pneumonitis develops as a result of infiltration of the intercellular regions of the alveoli by plasma cells.
With pneumocystis pneumonia observed in AIDS, plasma cells are not detected, blockade of gas exchange in the alveoli leads to respiratory failure.
P.jiroveci is an extracellular (extracellular) parasite.
In the lung tissue, it forms parasitic accumulations outside the cells tightly attached to the alveolar epithelium; their development is usually limited to the surfactant layer on the surface of the alveolar epithelium.)
P.jiroveci does not cause disease in people with normal immunity

Microbiological diagnostics

Trophozoites and cysts can be found in smears prepared from bronchial lavage and sputum stained with Giemsa, silver, and toluidine blue.

In Giemsa-stained smears, the fungal cytoplasm is blue and the nucleus is reddish purple.

Pathogens can also be detected in smears using **RIF.**

P.jiroveci is an uncultivated microorganism, therefore the **culture diagnostic method is not used.**

P.jiroveci is part of the obligate microflora of the human body, so the detection of antibodies in the blood serum has no **diagnostic value**.

Treatment is with trimethoprim/sulfamethoxazole (biseptol) and pentamidine.