Color Atlas of medical mycology

Jean Delacrétaz
Dodé Grigoriu
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The soil, air, water, household or farm dust, plants, and animals are all reservoirs of microscopic fungi, dermatophytes, yeasts and mouds in unsuspected abundance. While most of these fungi are saprophytes, a few may cause disease in plants, animals or man. Their classification under saprophytes is, however, not necessarily final, and the adaptation to parasitic life of some hitherto harmless strains should be watched for. To become pathogenic, such 'opportunistic' species require particular local or general conditions and present complex pathological entities in which their role is often difficult to assess.
In general the frequency of mycotic diseases tends to increase under the twofold influence of treatment with antibiotics, corticoids, and antimitotics which promote fungal growth, and of population movements which facilitate their dissemination. The latter also extends the geographic distribution of the species: *Tr. soudanense* and *M. Langeroni* mycoses have for example appeared in Switzerland following the presence of young African refugees in the area. This is why all physicians, whether dermatologists or not, should be fully aware of this pathology. This Atlas has been designed for their benefit. Following three preliminary sections that briefly review the biology, classification and investigation of fungi, the Atlas deals with the superficial and systemic mycoses.
As regards therapy, our survey is intentionally brief. Current research promises significant developments that will drastically alter present-day therapy; we have therefore intentionally refrained from details of treatment that may soon become obsolete.

Biology of Fungi

Fungi rank among the lower plants (thallophyta), they lack photosynthetic pigment, are heterotrophic, and, as living organisms, contain cytoplasm and a nucleus (which distinguishes them from microbes whose nucleoplasmic mass is not differentiated). The high fermentative activity and varied modes of reproduction give the fungi a special morphologic plasticity that definitely sets them apart from the higher plants and animals. Furthermore, if we consider that fungi build colonies composed of intertwined filaments (true tissue or prosenchyma), while the parenchyma (false tissue) of plants and animals is made of juxtaposed cells, we can understand why some workers consider fungi as a separate kingdom, the *regnum fungorum*. A fungus comprises a thallus which contains a vegetative and a reproductive system.

The vegetative system is made up of germ tubes (hyphae or filaments) that contain the cytoplasm and nuclei and together constitute the mycelium. The system may be septate or uninterrupted.

In septate mycelia (Fig.1 & 2), transverse septa divide the tubes into more or less uniform segments which contain cytoplasm and one or more nuclei; contiguous segments communicate with each other through minute orifices situated at the center of the septa (septal pores) (Fig. 7 & 8).

Hyphae grow centrifugally and the protoplasmic mass travels through the septal pores always toward the distal areas to be built, the older sections becoming emptied of their contents. This behavior is typical of the eumycetes, which include most of the pathogenic fungi. The mycelium may branch laterally by dichotomy in an umbellated, cross, or other pattern. Continuous non-septate (coenocytic) mycelium has no septum (Fig. 3 & 4); the cytoplasm and nuclei circulate freely in the branches of filament; this pattern is characteristic of the phycomycetes.

Some fungi called yeasts only show a pseudomycelium derived from a budding process (Fig. 5 & 6). The various components adhere to each other while retaining their individuality; they can be easily detached and may also form branching chains.

The reproductive system may be of several types:

a) *Sexual reproduction*

The fusion of a male and a female gamete results in the formation of an egg which, through successive divisions, generates spores. If the spores remain in the egg, which is then called an ascus, they are referred to as ascospores. If, on the other hand, they fuse outside the egg through tiny tubes called sterigmata, the egg, to which they remain attached by these sterigmata, is referred to as a basidium and the spores as basidiospores.

b) *Asexual reproduction*

Asexual reproduction follows a variety of patterns:

– Segments between two septa start bulging, take the appearance of 'pillow-shaped' spores and may remain in a chain-like arrangement. They are the arthrospores.

– Asexual spores may also appear on the sporiferous apparatus of varying form and size and may be of several types. The main types are the following:

– Chlamydospores, the so-called preservation or resting spores, result from the swelling of a mycelial segment. They contain a condensed and rich protoplasm and are surrounded by a thick or double envelope. They may be intercalated or terminal (Fig. 9a & 9b).

– Aleuria are external spores for preservation and propagation. They are formed by budding of the mycelial filament to which they remain attached by a pedicle (Fig. 10a & 10b).

– Conidia are external spores for propagation. They are born by budding from the mycelial filament from which they are easily separated.

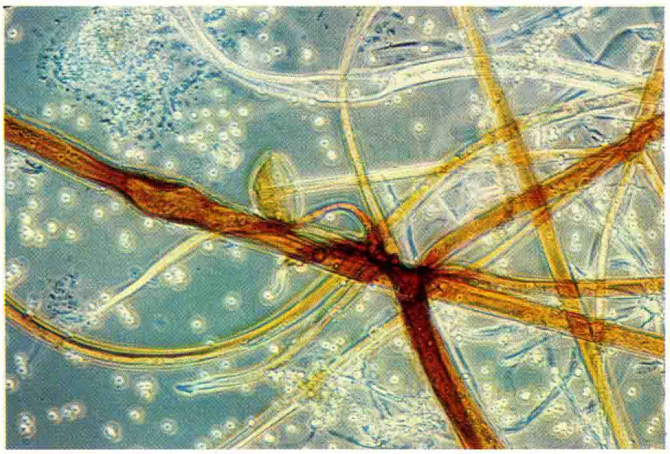
– Blastospores are born by budding from a parent cell from which they are easily detached when grown to become able to bud themselves (Fig. 11).

Morphologically, fungi differ in their appearance depending on whether they are in a parasitic state, e.g., in pathologic material, or in a saprophytic state, e.g., in cultures. Thus in pathologic material budding organs are generally absent. Furthermore, some fungi are yeastlike in the parasitic state and filamentous in the saprophytic state. This feature is known as dimorphism and can be seen in Paracoccidioides brasiliensis, Blastomyces dermatitidis, Coccidioides immitis, Histoplasma capsulatum, Histoplasma Duboisii, etc. In addition different species, mostly among the dermatophytes, may assume confusing similarity

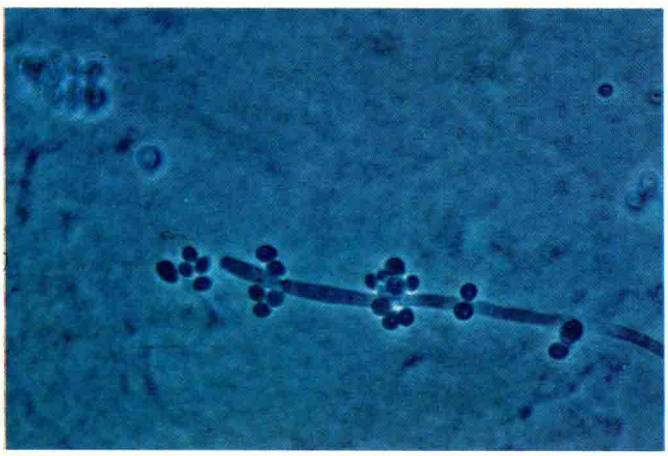
1 Septate mycelial filaments.

2 Septate mycelial filaments.

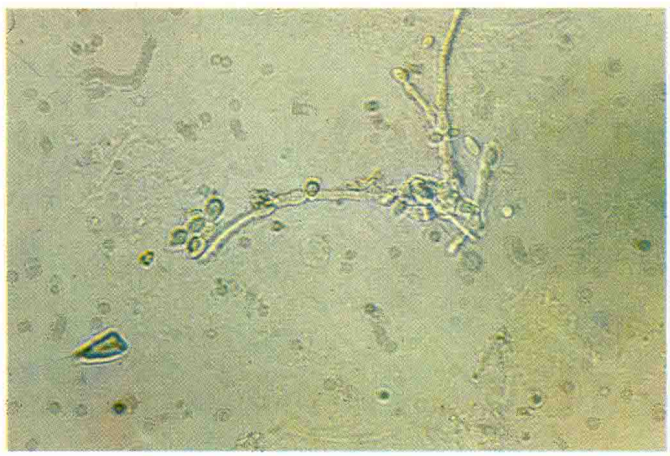
3 Coenocytic mycelial filaments without septa.



4 Coenocytic mycelial filaments without septa.



5 Mycelial pseudo-filaments. Culture fragment.



6 Yeast cells and mycelial pseudo-filaments. Direct microscopic examination.

in their parasitic state. The microscopic and macroscopic morphology of the colonies of the same given species varies considerably according to the culture media: the shape, aspect, dimensions, color and pigment of the colonies; the shape, dimensions and pigment of filaments; the septa, multiplication, etc., will vary according to the composition of culture medium and will depend, among other things, on the nature of sugar or peptones, the pH, the relative humidity and temperature. The simultaneous growth of other fungi or bacteria may also alter the morphology of the cultured fungus, such alterations being reversible as soon as standard conditions are restored. It is therefore essential to use consistently the same components in culture media, to incubate them under steady conditions of humidity and temperature and to avoid any secondary infection by microbes or contaminant fungi.

Pleomorphism (Fig.12) indicates degeneration of the fungi, especially of dermatophytes, which lose their macroscopic and microscopic characteristics and change into white, downy or cottony colonies; the mycelium thins out, septa diminish and fructification organs gradually disappear. This process develops fairly rapidly and may be partial or total. Fungi undergoing such change become less pathogenic, with experimental lesions that are more superficial than those caused by the primary form, and retrocultures always grow the pleomorphic form. This irreversibility, long recognized as being absolute, is now questioned; some methods (switch to earth base media, Borelli's recovery medium, etc.) are reported to restore the culture's original form.

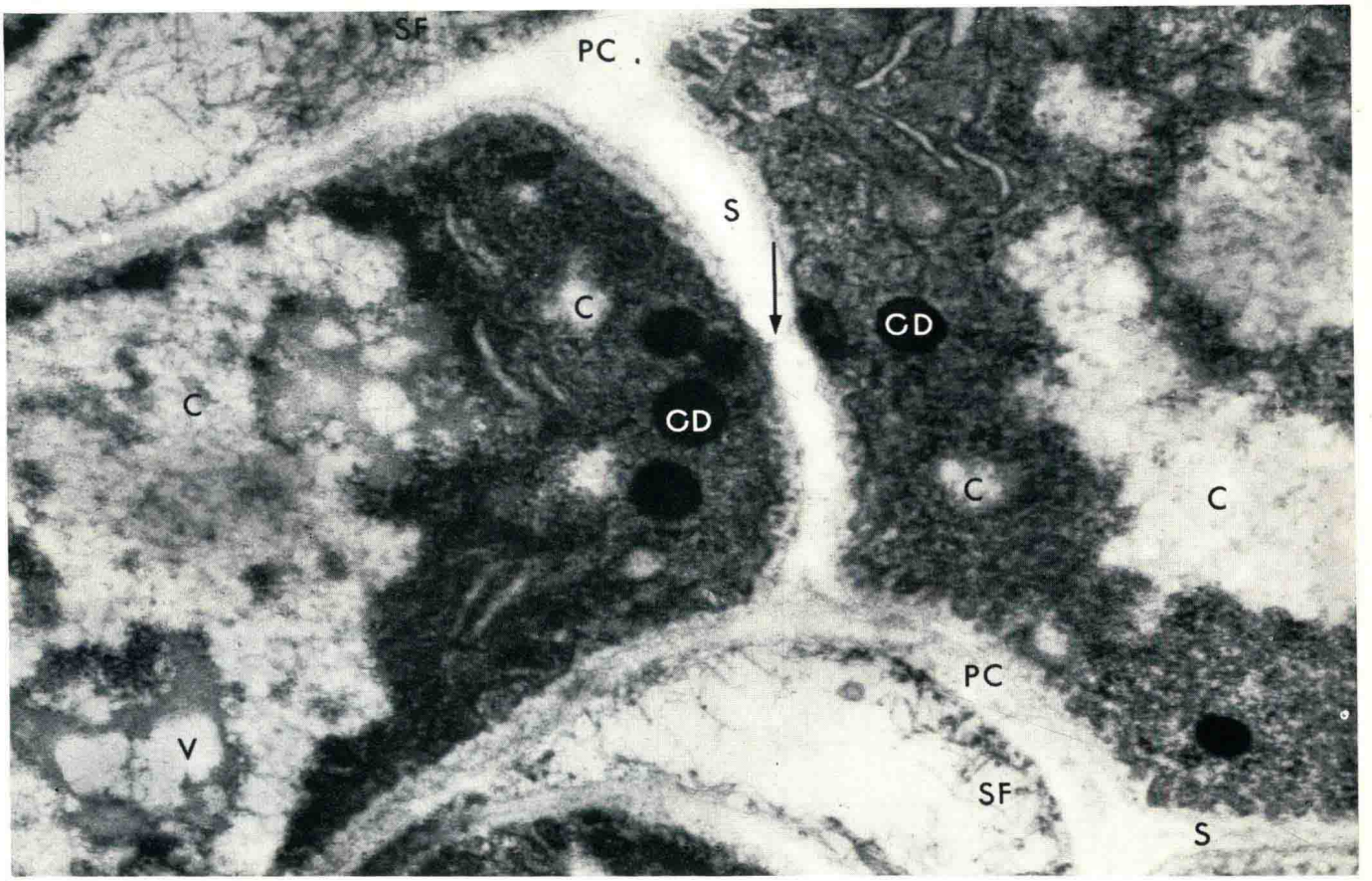
Fungi have a broad range of enzyme action; in particular they possess hydrolases, oxidases and reductases. These enzymes enable them to use for their growth the many organic and inorganic compounds found in their environment; in addition their enzymatic apparatus enables the fungi to carry out many syntheses, especially of antibiotics and toxins.

Generally aerobic, fungi can grow in temperatures that vary greatly according to the species and range from 0° C to 50° C, the optimum range being between 20° C and 37° C. Light favors growth of some species. Humidity stimulates fungi while dryness is poorly tolerated and causes preservation forms of which chlamydospores are an example. The optimum pH level for fungus growth is generally between 5 and 7.

7 Hypha of Microsporum canis. 5 day culture on Sabouraud's medium. Glutaraldehyde-Osmium fixation with lead-uranyl-acetate contrast. Arrow points to the nearby septal pore. x 45,000.

8 Hypha of Madurella mycetomi. Culture on Sabouraud's medium. Osmium fixation with lead-uranyl-acetate contrast. x 54,000.
The open septal pore allows the passage of cytoplasm, dense corpuscles and some mitochondrial membranes.

Legend:
C = Chitin
CD = Dense corpuscle
M = Mitochondrium
PC = Cell wall
S = Septum
SF = Fibrillary surface of the cell wall
V = Vesicle
small arrows indicate cytoplasmic membrane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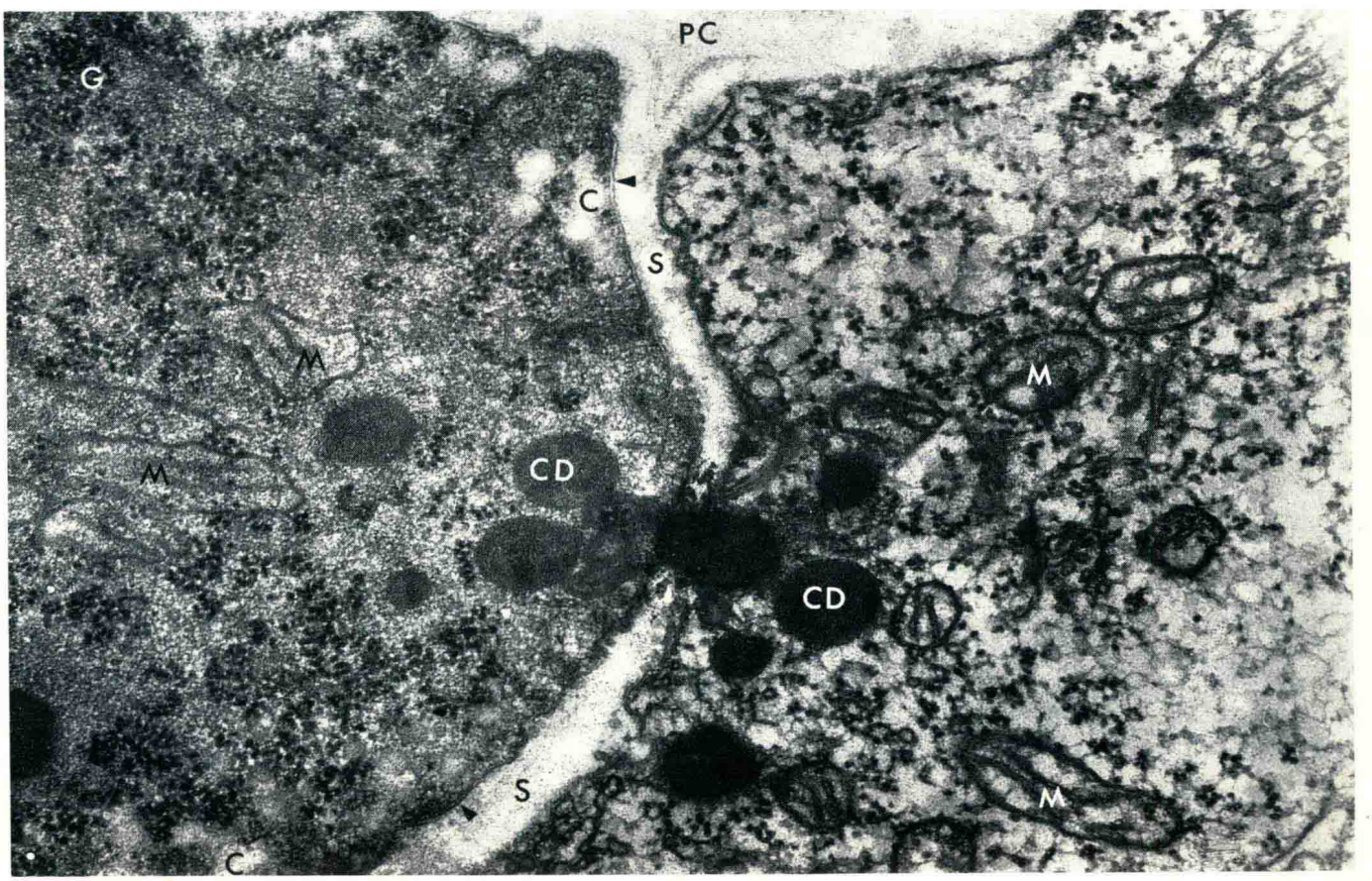
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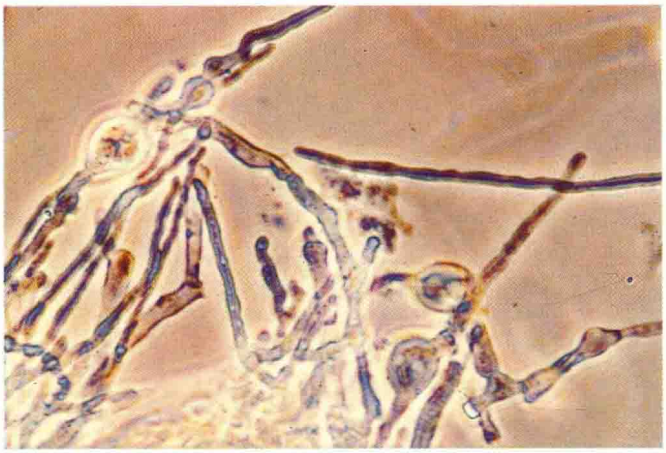
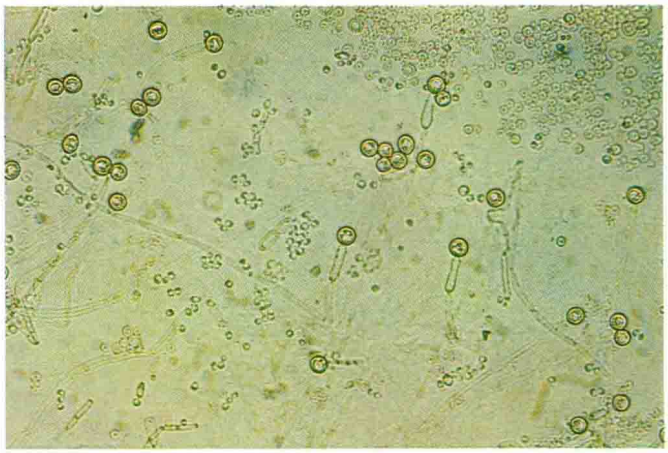
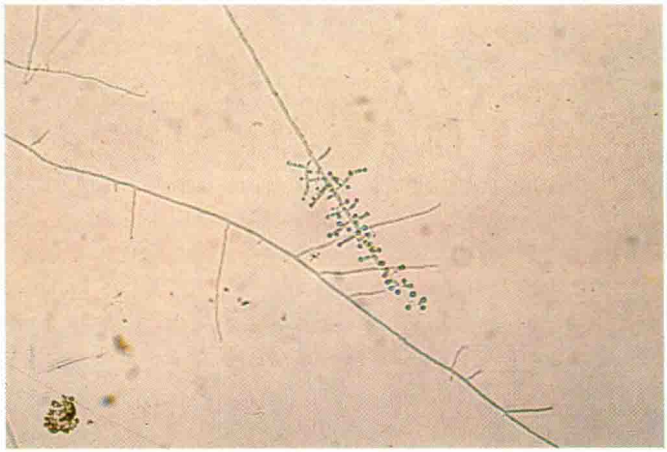


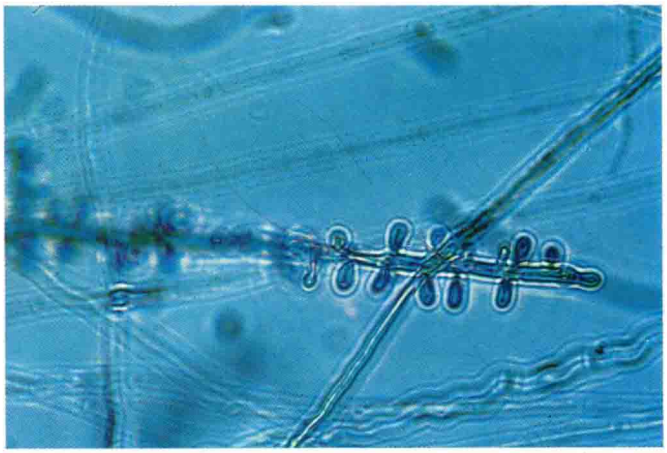
9a Chlamydospores: culture fragment of a dermatophyte (Tr. violaceum).



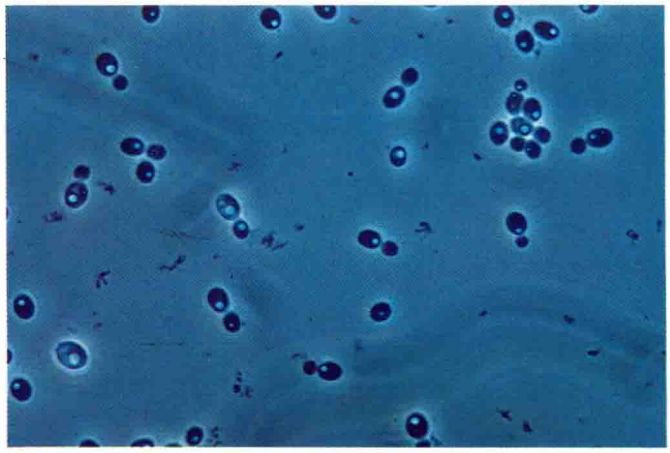
9b Chlamydospores: culture fragment of a yeast (Candida albicans).



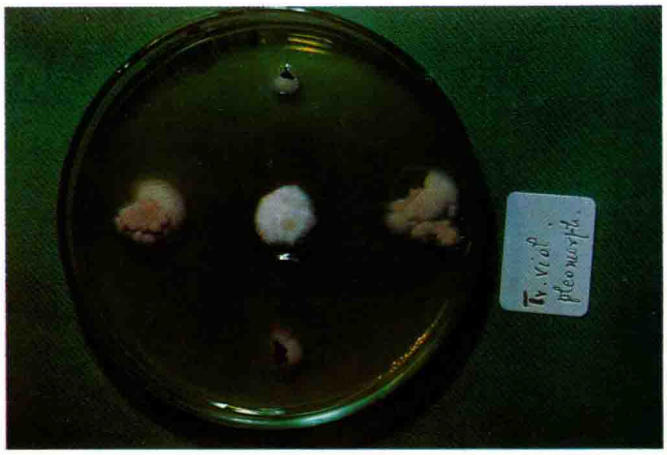
10a Bunched aleurias remaining attached to carrying filament.



10b Aleurias in acladial distribution.



11 Blastospores.



12 Partial pleomorphism.

Classification of Fungi

There is as yet no generally accepted classification of fungi. The one used here has the merit of being simple. Fungi belong to the family of thallophyta, characterized by the presence of a thallus, and include
- algae
- lichens
- bacteria (schyzomycetes)
- fungi (mycetes)

According to the structure of their mycelium, fungi are divided into two major groups:

1. *Phycomycetes* are lower, primitive fungi composed of a thallus made of branching, non-septate filaments. Some are apt to cause systemic mycoses (mucormycoses, etc.).

2. *Eumycetes* are phylogenically more developed, and grow a filamentous branching thallus whose filaments, however, are septate.
According to their mode of reproduction, eumycetes are subdivided into:
a) *Ascomycetes,* characterized by asci (sing. ascus) containing ascospores; they include the dermatophytes and some yeasts pathogenic to man.
b) *Basidiomycetes,* characterized by basidia and basidiospores nonpathogenic to man or animals.
c) *Fungi imperfecti* or *adelomycetes,* a provisional order grouping fungi with a strictly asexual mode of reproduction.

For all intents and purposes, medically one has to distinguish between the following:
- dermatophytes
- yeastlike fungi and yeasts
- dimorphous fungi
- certain 'opportunistic' saprophytes that become pathogenic only under special circumstances
- actinomycetes (filamentous bacteria).

Identification of Fungi

To avoid errors the clinical diagnosis of a mycotic disease should always be confirmed by the demonstration and identification of its causative agent.

Techniques for collecting pathological material

Skin scrapings
Scrapings should be collected by means of a blunt curette, vaccination scarifier or scalpel from margins of the most recent lesions in the area of spread. The material should be collected from several sites and in sufficient quantity to permit, if needed, further tests and cultures.
If the lesions have been recently treated, the collection of specimens should be postponed for three days during which time saline washes or compresses should by applied. Preliminary cleansing of the lesions with ether, though often advocated, is not essential.

Body and scalp hair
Hairs of abnormal appearance in color, shape or length are plucked with forceps and should pull out easily and painlessly. Scaly areas should be examined for the presence of hairs broken off at scalp level, and which may look like mere ostiofollicular blackheads. In cases of suppurative tinea (ringworm) specimens should be collected from the margin of lesions, being careful to avoid hairs surrounded by a purulent sheath.

Nail scrapings
A nail file and a pair of scissors should be used to obtain specimens from affected nails; subungual scale debris should be collected with a blunt scoop. In paronychia scales at the root of the nail as well as any pus in the nail-bed should also be collected.

Exudates
These should be collected from the discharging lesions with a sterile swab slightly moistened with saline. The top of intact vesicles, blisters or pustules should be removed with forceps or small scissors, and their contents extracted on a sterile swab.

Pus
Boils and abscesses as well as mucosal lesions should be aspirated with a syringe or pipette and pus collected with a wire loop or sterile swab.

Body fluids (blood, spinal fluid, urine, etc.)
Samples are collected in the same way as for bacteriological tests, care being taken to avoid contamination.

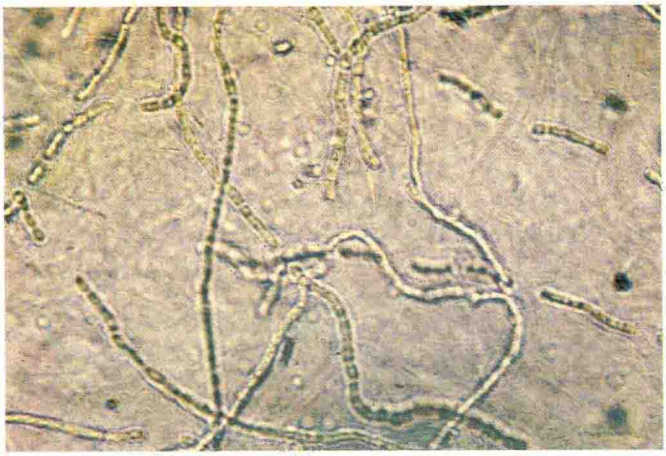
Sputum
In order to avoid contamination of the oral cavity and pharynx by saprophytes, sputum should as a rule be collected by bronchoscopy. If such a procedure is not feasible, it should only be collected after careful disinfection of the mouth for two or three days with a solution of Lugol, gentian violet or tincture of phenylmercury borate.

Stools
Fresh warm stools only must be used for specimens; a swab can also be obtained by a rectal syringe.

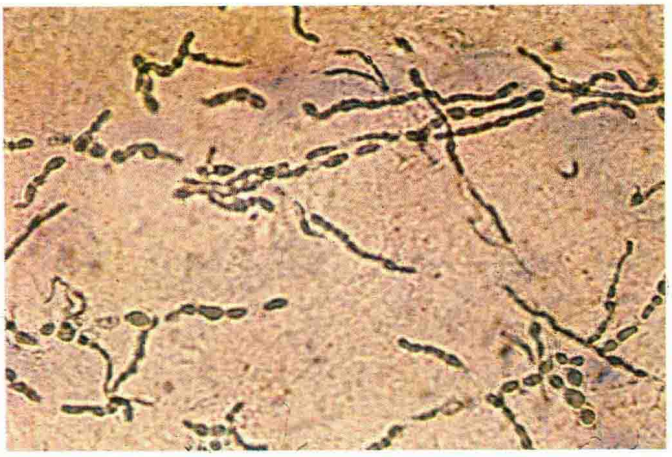
Examination of specimens

Direct examination can be performed with or without staining. Examination without staining (fresh preparation) (Fig. 13–18). The initial step is to clear or to dissociate pathological products in an alcohol-saline solution containing 20–30% caustic potash, chlorallactophenol or 10% sodium sulphide.
We prefer sodium sulphide which has a faster clearing action at room temperature and gives a clearer microscopic image with less artefacts. The specimen is placed on a slide, the clearing solution is added and a cover slide applied. According to the thickness of the material to be examined, a satisfactory dissociation will take 5 to 20 minutes. A negative test should be repeated.

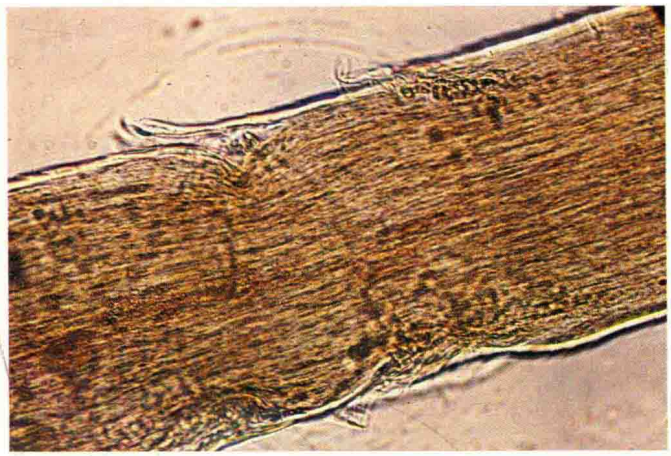
Errors to be avoided
The mycelial filaments – tubes of uniform diameter that run through the keratin cells – should not be confused with the following:
a) the fungus mosaic (Fig. 19), a kind of network composed of components of irregularly shaped, light-refractive elements usually located at the fringe of cells and generally considered as artefacts;



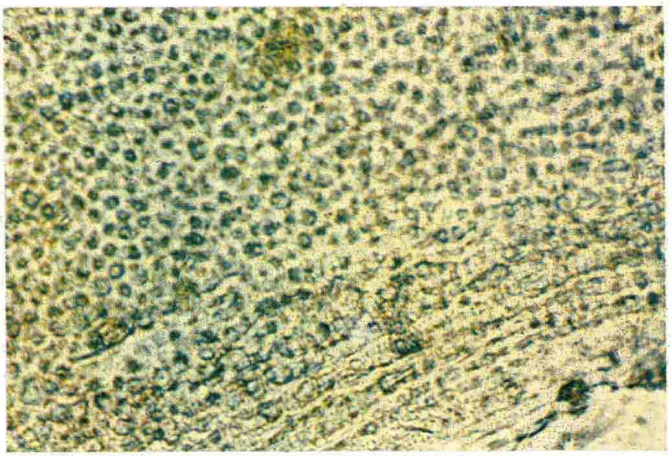
13 Direct microscopic examination of a scale showing mycelial filaments and rectangular arthrospores.



14 Direct microscopic examination of a scale showing fragmenting mycelial filaments and rounded arthrospores.

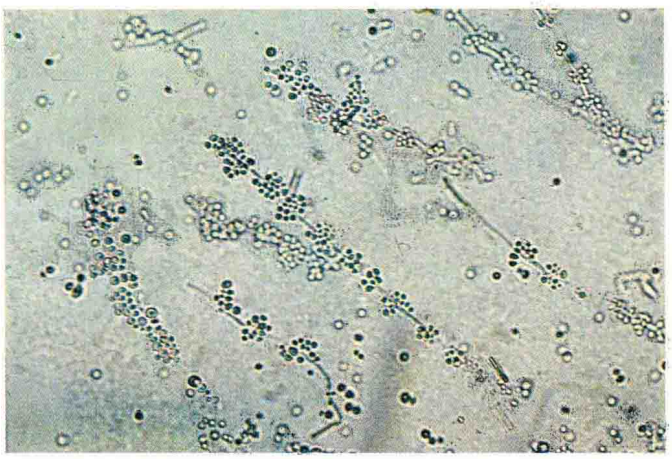


15 Direct microscopic examination of a hair: early parasitism (penetration of spores).



16 Direct microscopic examination of a hair: numerous spores inside the hair.

17 Direct microscopic examination of a hair: rectangularly fragmented filaments inside the hair.



18 Direct microscopic examination of a culture fragment of Candida albicans.

b) the cotton threads (Fig. 20) always of irregular calibre spreading out at the ends in a brushlike manner;
c) some elongated crystals (Fig. 21), irregular or polyhedral in shape, which readily disappear when a drop of distilled water is added to the preparation.
Spores and especially yeast cells should not be confused with fat droplets (Fig. 22) that sometimes appear as rounded, refractive bodies with a double membrane, irregularly shaped; nor with polyhedral crystals (Fig. 23).

Examination after staining and fixing
This examination applies mainly to fluid and semi-fluid pathological material. Fixing is over the flame. The commonest staining is with methylene blue, May-Grünwald-Giemsa and especially the Gram (the majority of fungi being Gram-positive) (Fig. 24). The Hotchkiss-McManus stain and Gömöry's silver method are mainly used to demonstrate the membranes.

Cultures

Cultures are essential for the accurate identification of most species. They should be carried out in specialized laboratories rather than by the medical practitioner. The latter should nevertheless know that cultures may be slow and that identification may take time, up to three weeks or more, as is the case with Tr. verrucosum, Tr. Schönleini, Tr. violaceum, Tr. rubrum, Tr. faviforme, M. ferrugineum, etc. The mycologist would be at fault if he made a premature diagnosis merely to satisfy the physician's understandable haste.
Laboratories use various organic or synthetic culture media. The most widely used is Sabouraud's medium, to which is usually added a broad-spectrum antibiotic, generally chloramphenicol, and actidione (cycloheximide) that prevents contaminant growth.
The colonies are submitted to macroscopic and microscopic examination.

Investigation of physiological characteristics

In some instances, clear-cut identification will necessitate a study of the physiological characteristics and metabolism of the isolated organisms.

Animal inoculation (Fig. 25)

In some conditions, especially the dermatomycoses and systemic mycoses, it will be necessary to inoculate laboratory animals with the pathological products. After an observation period which, as a rule, does not exceed 4 to 5 weeks, the pathogenic fungus may be isolated from the organs of these animals.

Examination under Wood's lamp

Used for the first time by Margarot and Devèze in 1925, examination under Wood's lamp remains an effective method of detecting microspora, especially in communities. It also shows the spread of pityriasis versicolor and erythrasma and facilitates follow-up after recovery.

Skin tests

Intradermal reactions (Fig. 26) using fungal antigens are highly specific in some diseases such as histoplasmosis, South American blastomycosis (Fava netto) and coccidioidomycosis. They are less specific for dermatophytes and of poor specificity in yeast diseases and aspergillosis.

Pathologic examination

While not always conclusive as regards the identification of the causative organism, biopsy is still of help in understanding the relationship of the organism with the invaded tissues (Figs. 27–32).

Serology

The serological reactions of agglutination, precipitation, complement fixation, immunoreaction in gelose, immunoelectrophoresis, immunofluorescence yield inconsistent results. This is largely due to the absence of standardized antigens. Results should therefore be interpreted with great caution.