A guide to the histological identification of fungi in tissues

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SYNOPSIS Infections with fungi and fungus-like organisms have increased in recent years. The presence of a fungus is often unsuspected clinically and it may only come to light in the course of microscopic examination of tissues removed by biopsy or at necropsy. Subsequent culture is desirable but not always possible.

A simple scheme for identifying fungi and fungus-like organisms is presented based on general morphology, staining, and other special characteristics with notes on types of tissue reactions and common pitfalls.

Diseases caused by fungi may be divided into superficial mycoses and deep mycoses. Superficial mycoses are confined to the epidermis, hairs, and nails and can readily be diagnosed by clinical appearance and culture. Deep mycoses are usually cutaneous, pulmonary, or disseminated. They are most commonly seen in Britain in (1) patients whose immune defence mechanisms have been impaired either by disease or treatment; (2) immigrants or travellers; and (3) drug addicts who inject themselves. The existence of a deep fungal infection is often unsuspected and it may only come to light in the course of microscopic examination of a biopsy or of tissues removed at necropsy. Culture is desirable for a precise diagnosis but it may not be possible to obtain further material for this. The histopathologist must be prepared, therefore, to establish the presence of fungi in the tissues, to attempt identification of the species, and to exclude laboratory contamination and artefacts.

Diagnosis is made easier and the choice of alternatives narrowed by reasonable clinical information, the presence of any predisposing factors, and a working knowledge of the world-wide distribution of the various fungi. Descriptions of mycotic diseases are to be found in most standard textbooks of pathology. A bibliography is given at the end of this text.

Fungi of medical importance belong to the fungi imperfecti, that is, they have no discernible phase of sexual reproduction but produce asexual spores of various kinds. They grow either as budding yeasts or as filaments (hyphae or pseudohyphae) or as both. Terminology is cumbersome and the exact taxonomic position of some is in doubt. Actinomycetes, for example, resemble filamentous fungi morphologically and mycobacteria biochemically. Pneumocystis carinii is thought by some to be a protozoon rather than a fungus. These academic considerations apart, the problem presents itself to the clinical histopathologist is how to identify the organism from its shape, size, staining reactions, or other characteristics and the type of tissue reaction associated with it. A scheme for this process is given below. This necessarily is an oversimplification of a very complex subject but it should help to reduce a particular problem to practicable limits. The appearances described are those usually seen in the tissues.

What Is It Like?

ROUND BODIES (YEASTS)
Cryptococcus neoformans; Histoplasma capsulatum; Histoplasma duboisii; ‘Chromomycosis’ species: Phialophora verrucosa and pedrosii, Cladosporium carrionii; Blastomyces dermatitidis; Paracoccidioides brasiliensis; Rhinosporidium seeberi; Coccidiodes immitis; Sporothrix schenckii; Pneumocystis carinii.

FILAMENTS (TRUE HYphaE AND PSEUdOHyphaE)
Actinomyces israelii; Nocardia asteroides; Aspergillus species: fumigatus and niger.

Phycomycetes
Rhizopus, Mucor, and Absidia species (deep and
systemic phycomycosis), Basidiobolus meristosporus
(subcutaneous phycomycosis), Entomophthora corona,
ta (submucous phycomycosis).

‘Mycetoma’ species
True fungi (‘maduromycetoma’): Madurella
mycetomi, Allescheria boydii, Leptosphaeria senega-
ensis. Actinomycetes (‘Actinomycetoma’) Strepto-
myces and Nocardia species.

ROUND BODIES AND FILAMENTS
Candida albicans

What Size? (Round Bodies)

SMALL (1-5 MICRONS)
Histoplasma capsulatum, Pneumocystis carinii,
Sporothrix schenckii

LARGE (20-200 MICRONS)
Rhinosporidium seeberi, Coccidioides immitis

MEDIUM (5-20 MICRONS)
The rest.

Histoplasma capsulatum has to be distinguished
from the protozoa leishmania and toxoplasma (see
under staining below). The yeast forms of candida
species are egg shaped, 3-5 microns in size, bud
frequently and also form hyphae. The large sporangia
of Rhinosporidium seeberi and Coccidioides immitis
are quite characteristic. The differential diagnosis
of medium-sized yeasts rests on staining and other
properties and may be very difficult indeed.

What Shape? (Filaments)

Slender filaments or pseudohyphae, generally less
than 2 microns in diameter, are formed by organisms
that are not true fungi but bacteria (Actinomyces,
Streptomyces, Nocardia species). True filamentous
fungi consist of broad filaments or hyphae, usually
more than 2 microns in diameter. These may be
septate or non-septate and branching is a feature.
Various types of spores are formed, mostly in culture
and rarely in the tissues.

FELT-LIKE MASSES (GRAINS)
Actinomyces israelii (usually), Nocardia asteroides
(rarely), various ‘mycetoma’ species.

The grains of actinomycosis, nocardiosis, and
‘actinomyctoma’ are made up of slender pseudo-
hyphae. Grains made up of broad hyphae are formed
by true fungi responsible for ‘maduromycetoma’.
The distinction is a clinically important one in that
the former may respond to chemotherapy but the
latter have to be treated surgically.

REGULAR, SEPTATE HYphae WITH
DICHOTOMOUS BRANCHING

Aspergillus fumigatus and niger: these may form
large masses (‘aspergillomas’) in tuberculous or
bronchiectatic cavities in the lung.

Candida species also form regular, septate hyphae.
These are often club shaped, branch little, and yeast
forms are always present.

IRREGULAR, NON-SEPTATE HYphae WITH
HAPHAZARD BRANCHING

Phycomycetes: Rhizopus, Mucor, and Absidia
species responsible for deep and systemic phycomy-
cosis. The hyphae of Basidiobolus meristosporus
in subcutaneous and those of Entomophthora coro-
nata in submucous phycomycosis are clear and
difficult to see. They are, however, outlined by a
eosinophilic precipitate.

Any Special Features?

STAINING

The use of control material is essential with special
stains.

Haematoxylin-eosin cannot be relied upon to show
all fungi. Nocardi species, Phycymycetes, and
Pneumocystis carinii may remain invisible whilst
many yeasts stain poorly. The asteroid body of
Sporothrix schenckii is virtually never seen in tissue
sections. It is wise to do periodic-acid-Schiff and
methenamine silver stains on all suspect sections.

Periodic-acid-Schiff stain, or one of its modifi-
cations, such as Gridley’s method, is universally useful.
It stains fungi a purple colour of varying intensity,
sometimes rather faint in postmortem tissues. It
also stains many tissue components and the results
are not always clear cut.

Grocott’s modification of Gomori’s methenamine
(hexamine) silver stain shows all fungi black and
is superior to methods employed for the demon-
stration of reticulin. The results are bright and crisp,
an ideal method for demonstration and micro-
photography. It also differentiates Histoplasma
capsulatum (positive) from leishmania and toxo-
plasma (both negative).

Gram’s stain has a limited usefulness for staining
the grains of Actinomyces and Nocardia species
and Candida albicans.

Southgate’s mucicarmine stains Cryptococcus ne-
formans intensely brilliant red. This is practically
specific for this fungus.

Giemsa’s or Wright’s stains show Histoplasma
capsulatum in better detail. They also stain leish-
mania and toxoplasma.

Ziehl-Neelsen stain may show species of Nocardia
to be weakly acid-fast.
Birefringence
This helps to detect *Histoplasma capsulatum* and *duboisii* but is not specific: *Cryptococcus neoformans* and *Blastomyces dermatitidis* may also be birefringent.

Pigment
The yeast-like bodies of ‘chromomycosis’ species are golden-brown in sections stained with haematoxylon-eosin; this colour and an occasional septate form are quite characteristic. The grains of ‘mycetoma’ species are coloured whitish-yellow to black, occasionally even red, when looked at in the block. This helps in species identification: ‘actinomycetoma’ species (yellow-white grains) *Nocardia asteroides*, *Nocardia brasiliensis*, *Streptomyces madurae*, *Streptomyces somaliensis* (red grains), *Streptomyces pelletierii*; ‘maduromycetoma’ species (yellow-white grains) *Allescheria boydii* (brown-black grains), *Madurella mycetomi*, *Leptosphaeria senegalensis*.

The pigment may also be seen in the regional lymph nodes: this is not evidence of spread.

Capsule
Only *Cryptococcus neoformans* is truly encapsulated which is shown well by staining with mucicarmine or in India ink preparations. *Histoplasma capsulatum*, in spite of its name, has no capsule and the halo-like appearance is an artefact.

Budding
The following yeasts may be seen budding in the tissues: *Candida albicans* (filaments also present); *Blastomyces dermatitidis* (solitary and broadly based); *Paracoccidioides brasiliensis* (multiple and peripheral, ‘marine pilot’s wheel’ appearance); *Cryptococcus neoformans* (rarely); *Histoplasma capsulatum* and *duboisii* (rarely).

Any Help from the Type of Tissue Reaction?
Reactions to fungi vary widely not only between one species and another but in different cases of the same disease or even at different stages in the course of the same infection. The following is a guide to the patterns most commonly seen:

Little or No Reaction
*Cryptococcus neoformans* (particularly in the brain), *Candida albicans* (on mucous surfaces), *Aspergillus fumigatus* (in the lung and brain).

Predilection for Blood Vessels with Thrombosis and Infarction of Tissues
*Aspergillus fumigatus* (in the lung), systemic infection with Phycomycetes, occasionally *Candida albicans*. It is well to remember that thrombotic angiitis is also seen in *Pseudomonas aeruginosa* pneumonia.

Non-Specific Chronic Inflammation
*Rhinosporidium seeberi* (nose), *Cryptococcus neoformans* (lung), *Pneumocystis carinii* (‘plasma cell pneumonia’).

Suppuration
Actinomyces, *Nocardia*, ‘Mycetoma’ species particularly, but many others occasionally. The focus of suppuration may be surrounded by a histiocytic granulomatous border in infections with *Sporothrix schenckii*, *Blastomyces dermatitidis*, and *Coccidioides immitis*. Cat-scratch fever and lymphogranuloma venereum may have to be considered in the differential diagnosis.

Giant Celled Tuberculoid Granulomatous Reaction
*Histoplasma duboisii*, ‘Chromomycosis’ species, *Cryptococcus neoformans*, *Blastomyces dermatitidis*, and *Coccidioides immitis*. Caseation may occur, sometimes massive, with *Histoplasma capsulatum*, *Cryptococcus neoformans*, and *Coccidioides immitis*.

Eosinophilic Proteinaceous Precipitate Outlining Fungal Body
Subcutaneous phycomycosis (*Basidiobolus meristosporus*), submucous phycomycosis (*Entomophthora coronata*), *Sporothrix schenckii* (‘asteroid boyd’).

Pseudoepitheliomatous Hyperplasia of the Skin
‘Chromomycosis’ species, *Blastomyces dermatitidis*, *Coccidioides immitis*, *Sporothrix schenckii*, severe ‘granulomatous’ *Candida albicans* infection.

Pitfalls
Many artefacts and other structures may be confused with fungi. The most important are the common haematoxyphil spherules in the central nervous system (‘brain sand’), Russell bodies of plasma cells, conchoid and asteroid bodies of sarcoid granulomas, vegetable fibres and suture material, particularly when cross cut, partly laked red cells, nuclear debris, and the like. Tissues may be contaminated with starch powder from surgical gloves used by the pathologist on the cutting-up bench. These starch granules are PAS positive and birefringent. Finally, the possibility of contamination of paraffin wax, mounting media, and slides with real fungi should always be borne in mind.
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Bibliography

GENERAL ACCOUNTS

FUNGAL INFECTIONS IN THE TROPICS

PULMONARY INFECTIONS

TISSUE REACTIONS TO FUNGI (ILLUSTRATED)

TECHNICAL METHODS