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 that 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 *pedrosoi*, *Cladosporium carrionii*; *Blastomyces dermatitidis*; *Paracoccidioides brasiliensis*; *Rhinosporidium seeberi*; *Coccidioides 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 coronata* (submucous phycomycosis).

'Mycetoma' species

True fungi ('maduromycetoma'): *Madurella mycetomi*, *Allescheria boydii*, *Leptosphaeria senegalensis*. Actinomycetes ('Actinomycetoma') Streptomyces 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 (Actinomycetes, 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 'actinomycetoma' are made up of slender pseudohyphae. 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 phycomycosis. The hyphae of *Basidiobolus meristosporus* in subcutaneous and those of *Entomophthora coronata* 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. Nocardia species, Phycomycetes, 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 modifications, 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 demonstration of reticulins. The results are bright and crisp, an ideal method for demonstration and microphotography. It also differentiates *Histoplasma capsulatum* (positive) from leishmania and toxoplasma (both negative).

Gram's stain has a limited usefulness for staining the grains of Actinomycetes and Nocardia species and *Candida albicans*.

Southgate's mucicarmine stains *Cryptococcus neoformans* 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 leishmania 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 haematoxylin-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 pelletieri*; '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 body').

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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