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Digestion of Tissues by Invading Fungi: Questions and mechanisms

- Bob Stoddart

Microfungi are ubiquitous in air and soil, except in the most extreme of conditions. Most are saprophytes or harmless symbionts: very few of the total are pathogenic. The principal reasons for the variety of fungal pathogens, either in animals or plants, are twofold. First, many fungi have such exacting requirements for growth that they cannot proliferate at body temperatures or obtain all their nutritional requirements as pathogens. Second, *living* organisms have a variety of protective mechanisms, some passive and others active, which are very efficient in defence against fungi.

When a tissue dies, its passive anti-fungal defences may still function to some degree, but its active protective mechanisms soon cease to operate and so dead tissues are potentially vulnerable to fungal attack. In natural history collections attempts are made to afford compensation for the loss of active defence by using exogenous, additional protection through fixation, drying and preservatives such as are used in fluid collections and in tanned specimens. In general, such protection is reasonably effective, but specimens do, periodically, show evidence for the presence of fungus and this raises several questions as to the source of the fungus, whether it is alive, what it has done to the specimen – or will do to it, the source of the fungus and whether other specimens are at risk. There are, occasionally, considerations of human risk also to be made. The central question, however, is whether the fungus has been acquired after the death of the tissue, or was present in life, before the specimen was prepared.

Fungi are pleomorphic and their appearance can be greatly affected by the site and conditions of their growth. In general, yeasts are not invasive in their yeast forms, but tend to grow in suspension or on surfaces. They are likely to affect the surface of a specimen and to spread only locally, unless they are able to survive in preservative fluids or spread across cavities. They may disperse in dusts, as may fungal spores, which often resemble yeasts when seen histologically. Hyphal fungi are potentially invasive of tissues, though they do not necessarily invade and may simply produce surface growth which may be dense. They may spread easily through fluids and extend across cavities, but do not always do so. If their growth conditions become adverse, for example by drying, they may die or form spores and the mechanisms of spore formation are variable between species with some being able to form arthrospores simply by the fragmentation of hyphae. As a general rule, if the fungal growth on a specimen, when examined microscopically, shows evidence of hyphae or pseudohyphae invasive growth should be presumed as a starting point, even if no hyphal invasion can be seen. If any sporangia or possible arthrospores are visible, there should be a presumption that they have dispersed into the immediate environment of the specimen and may be present on nearby surfaces or in surrounding fluids. If clear-cut sporangia are seen, it is sometimes possible to identify the species of fungus concerned, or, at least, to place it within its taxonomic group and this may aid in predicting the types of damage that it may have done.

If fungal infection of a specimen is suspected, a sample should, if possible, be taken for microscopic and histological examination to determine, if possible, whether invasive fungus is present. In this, care should be taken to distinguish between fungal hyphae and features such as blood capillaries or small vascular channels in plants, which can imitate dead or dying hyphae. If fungus is confirmed, a series of questions follows.

a. Was fungus present in the living tissue?
Animal tissues generally respond to fungal invasion by a variety of cellular mechanisms. In mammals these include responses by neutrophil polymorphs, lymphocytes and macrophages, which lead to collections of some or all of these cells around fungi and the engulfment of fungus by macrophages. In lower animals there are functionally equivalent cellular responses, though the separate cell type may not be identifiable. However, the presence of fungus inside phagocytic cells is a clear indication of its presence before tissue death and implies that it was part of the specimen *ab initio*. In plants, the tissue responses are different, but the presence of polyphenols (which may be blue or brown) around the fungus is an indication that the fungus was present before tissue death, or soon after.

In mammals and several other vertebrates, fungi can induce the generation of immunoglobulins directed against them and this can lead to the precipitation of amorphous immune complexes, which stain pink with

haematoxylin and eosin, on the fungal surfaces. Such precipitates (the Hoeppli-Splendore phenomenon) occur only in living tissues and also provide evidence for a fungal presence in life.

b. *Is the fungus still alive?*
This may be very hard to determine. Sampling by an experienced microbiologist and subsequent culture may provide an answer, but care is needed to ensure that the fungus is not a recent exogenous contaminant. Careful morphological comparison with that in the tissue may be required, but the pleomorphism of many fungi is a difficulty.

Many fungal hyphae appear to show considerable lengths of dead material even under condition favourable for growth and demonstration of metabolic activity can be problematic *in situ*.

c. What sort of fungus is it?
Culture may provide the answer to this, given the caveat in 'b)' above. Histology may also help.

Aspergillus spp. Generally show quite narrow hyphae of reasonably uniform diameter, with frequent and complete septa. Where growth is confined, dilated 'flask' cells may be seen. Branching is usually dichotomous and even, with adjacent hyphae often branching in synchrony to produce 'fairy ring' effects. If sporangia are seen (eg in cavities or at surfaces) they resemble holy water sprinklers (aspergilli) and their shape, size and number of ranks of spores (sterigmata) may indicate the species. *Penicillium spp*. Are closely related to *Aspergillus* and their hyphae are quite similar, but their sporangia are simpler.

Fusarium spp. Also have narrow hyphae, but few septa and do not branch so often or so regularly.

The Zygomycetes, which in Britain are chiefly represented by species of *Mucor* and *Absidia*, are common and rather aggressive fungi. Their hyphae are often broader than those of the species above, less even in diameter, branch rather irregularly (and often at right angles) and tend to look rather vacuoled or empty. They have a bluish stain with haematoxylin and eosin and may show zygospores. In specimens from the USA species of *Rhizopus* may be found, which show even more irregular and broad hyphae. Zygomycetes are commonly found on waste foodstuffs. A wide range of rusts and other common plant pathogens my also be encountered as may dermatophytes (the organisms of ringworm and athletes foot). These last usually fluoresce under ultra-violet light and the colour of the flourescence may indicate the species.

Stuffed mammals and skins may grow organisms such as *Trichosporon capitatum,* which causes piedra in humans. These invade hair shafts, causing the hair to become brittle and break. Organisms of this type are known also to grow on house dust and rotting wood.

A wide range of organisms grow on timber and in soil. They may occur, for example, in archaeological bone and are abundant in earth from graveyards and cemeteries. Several of them are inherently coloured (generally brown). They may grow as masses of parallel hyphae ('rhizoids') and show mating between hyphae, indicated by the presence of crescent shaped unions (termed 'clamps').

In general, it is difficult to identify fungal species without culture, but recognition of the main taxonomic groups is often easier and may indicate the source of the fungus.

3. Where does the fungus orginate?
Though fungi are effectively, everywhere, particular species are associated with particular environments and with certain sources of specimens, so that it can be possible to identify likely sources of infection and to respond accordingly.

Museums are often in or near old buildings, in which there may be decaying wooden window frames, roof timbers – and sometimes floor timbers – affected by dry or wet rot and lawns (and grass clippings) may be nearby. All of these may harbour fungi, as may the dust between floorboards or underneath floor coverings. Visitors may introduce dermatophytes and the waste from cafes in or near the museum may harbour zygomycetes. In country towns, dusts from cornfields or from hay may carry large burdens of fungal spores over considerable distances. In general, this does not seem to matter as much as might be supposed, provided that the fungi and their spores are not disturbed too much, but building operations, such as re-roofing, re-flooring, demolishing walls or replacing decayed window frames *are* a real hazard, as they are for leukaemic patients in hospitals. Even building operations some hundreds of yards from museums pose a risk.

Air conditioning systems afford a further problem in that they may disperse fungal infections from specimen to specimen, especially if they are not adequately filtered. The position of their air intakes relative to dustbins, garden waste and building work should be considered. In particular, thought should be given as to whether the outfall from venting a hazard, such as a kitchen, waste store or even – in the author's experience - a mortuary lies too close to, or upwind of, the intakes of an air-conditioning plant.

4. What has the fungus done to the tissue or what may it do?
Where fungi invade tissues, they generally do so along natural routes of access and cleavage planes. In animal tissues they tend, even in life, to invade airways (especially in birds, reptiles and some insects) and they can spread rapidly through air-filled cavities such as lungs, the stomata of leaves or the empty marrow cavities of archaeological bone. They will also track through vascular systems in plants and, sometimes, in animals and commonly enter bone by this means. In general, fungi do not invade cartilage at all easily and they are slow to penetrate the matrix of cancellous bone. Indeed, it is often striking that dense mats of fungal rhizoids may be within the trabecular bone of marrow cavities while sparing the hard cancellous bone. Zygomycetes are notably more able to penetrate soft tissues, whatever their texture, than are most other fungi.

The nature of the damage done depends on the enzymes produced by the fungus and this is often variable, even within strains of a given species. In some forms of *Aspergillus spp*., *Mucor spp*. and in a wide range of plant pathogens, there is production of pectolytic enzymes. These are, variously, pectin methyl esterases, pectin transeliminases and polygalacturonases of several types, which together degrade simpler pectins, including those of the middle lamella and cause individual plant cell walls to separate from each other. This process is termed 'maceration' and can leave the cells themselves reasonably intact. In contrast, celluloses and some xylanases will damage the entire wall and make it swell or fragment, often with injury to the cells within. Proteolytic enzymes are common fungal products and tend to be of broad specificity, so that they can degrade a wide range of proteins, especially in animal tissues. Fungi can also produce a wide range of lipases and nucleases, but most species only produce a limited range of lytic enzymes. For this reason – and because of the inhibiting effects of fixatives and preservatives – only a limited range of fungi are likley to cause major damage to natural history specimens and most of these are Zygomycetes, Aspergilli and related groups or fungi such as dry rot (*Merulius lacrymans*).

5. Is the fungus hazardous to humans?
Apart from their tendency to induce allergy in susceptible individuals, most of the fungi encountered as problems in collections are unlikely to cause serious hazards for museum staffs or the public, but there are some exceptions. Anyone with an immune deficit or a known hypersensitivity to a fungus should avoid contact with them and, especially, avoid inhaling spores. Care should be taken in handling any fungus growing on an animal tissue until the species or major taxonomic group is determined and culture should be attempted only by persons with appropriate microbiological training and is a suitable laboratory, though this is as much for the safety of the collections as of the staff.

Some few specific fungal species present a high level of hazard in general and they should be borne in mind, even though the chances of their being encountered in Britain are *very* low. *Coccidioides immitis* could possibly be present in specimens of desert-swelling mammals from the South-West USA and a few parts of West Africa. On no account should any attempt be made at culture, even if its presence is suspected, without the use of high-grade containment facilities. Other potentially dangerous species that might be encountered include *Blastomyces dermatitidis* (on animal tissues and decayed wood, especially from the area around Tennessee, USA), *Paracoccidioides brasiliensis* (from similar sources in the south of the USA, Central America and some areas of South America) and *Sporothrix schenkii* (on old softwood from various sources). If museum roofs are being inspected, areas fouled with old pigeon droppings should be avoided or protective clothing should be used in order to avoid inhaling *Cryptococcus neoformans*, which can be a dangerous pathogen and has an almost world-wide distribution. Finally, any decaying specimen, especially a dried-out pot, that smells of garlic when opened should be handled with care and in a fume cupboard. It may have been exposed to an arsenical preservative, such as sodium arsenite or arsenious oxide and be releasing arsine (arsenic trihydride), a highly poisonous gas, as it putrefies, whether by fungal or bacterial action.