

## Fungal spores in lung and sputum

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### Summary

A survey of fungal aerospora over a 2-year period was combined with an investigation of the fungi cultured from 295 samples of peripheral human lung and 2466 samples of sputum over the same period. 83% of lung and 88% of sputum samples contained culturable fungi, although in 16% of lung and 31% of sputum samples *Candida albicans* was the only one. Those fungi of small spore size tended to be found in lung tissue while those with larger spores were more likely to be found in the sputum. While their presence in the respiratory tract was related to their occurrence in the air, differences in their frequencies in the air and lung suggested that the lung had a selective inhibitory effect on the development of different spores. In particular, *Aspergillus fumigatus* was cultured more frequently than would have been anticipated from its profusion in the air.

### Introduction

The inhalation of pollen grains and fungal spores has been known for many years to provoke attacks of asthma. More recently, the syndrome of allergic alveolitis has been shown in many cases to be caused by the inhalation of fungal spores, while in addition lung infection by fungi has become a problem in immunosuppressed subjects and in patients with prosthetic heart valves.

Studies in our Unit over many years have shown that large numbers of fungal spores can be found in the air throughout the year. These spores are derived from many different genera and each has its distinct season (Hyde & Williams, 1961). Many are of a size and shape that allow them to be inhaled into the lung, and since the average number of spores in the air throughout the year is around 4000 per m<sup>3</sup>, it is inevitable that we inhale many all the time. On occasions, the spore count may rise to as many as 100,000 per m<sup>3</sup>, thus involving us in large scale unsuspected challenge tests.

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This study was planned to find out whether viable fungal spores can be found in the human lung and also to test whether theoretical concepts of particle deposition, dependent on size and shape, hold true in the living human lung. In doing so it was hoped to shed light on the significance of the isolation of fungi from sputum and lung biopsies in disease states.

## Subjects and methods

### *Air sampling*

A survey of the spore content of outside air was undertaken over a 13 month period from October 1974 to November 1975 on the roof of Sully Hospital (51°23' N, 03°14' W) 14 m above ground level. Air was drawn at 28.3 l/min onto six corn meal agar plates in an Andersen sampler (Andersen, 1958). Sampling was continued for 10 min and three samples were taken at 8 hr intervals 1 day per week. Additional information was available from a survey of the air run specifically for *A. fumigatus* (Mullins, Harvey & Seaton, 1976) and unpublished data from a continuously running survey of the air in the centre of Cardiff using a Hirst spore trap (Hirst, 1952).

### *Sputum survey*

All sputa sent to the Bacteriology Laboratory at St David's Hospital between February 1974 and July 1976 (a total of 2466) for routine bacteriology were plated directly onto malt agar, incubated at 37°C, and corn meal agar, incubated at 25°C. The population sampled had an average age of 67 years (s.d. 17 years) and the sex distribution was males 63% and females 37%.

### *Lung tissue culture*

Duplicate portions of peripheral lung from both upper and lower lobes of one lung from 295 patients were taken at necropsy, placed immediately in screw cap sterile jars and transferred to the laboratory where they were stored at 4°C until processed. 211 of the patients had died out of hospital and the necropsy had been ordered by the coroner.

Weighed samples of approximately 1.0 g fresh weight were taken from each specimen and homogenized with 10 ml sterile saline by an 'Ultra-Turrax' homogenizer. 1.0 ml aliquots were plated out with molten (48°C) malt extract and corn meal agar (Buckley, Campbell & Thompson, 1969) and incubated at 37°C and 25°C respectively.

Specimens were obtained from Sully Hospital, Penarth, where necropsies were carried out for the Vale of Glamorgan; Aberystwyth, from a mostly rural population, and Liverpool, from an urban population. In each case information on age, sex and cause of death was included.

The average age of the population sampled was 64 years (s.d. 17 years) and the sex distribution was males 63% and females 37%.

### *Control experiments*

Using the Andersen sampler, three 10 min samples of the air were taken in the mortuary and laboratory at Sully Hospital in March, July and November 1975.

To see if the spore content of the specimens varied over the storage period of lung tissue, ten specimens were kept at 4°C and sampled repeatedly at 2 day intervals.

## Results

### Aerospora

The main moulds found in the air are shown in Table 1; of these the Basidiospores, Ascospores and the plant pathogens *Ustilago*, *Puccinia* and *Erysiphe* are not culturable, and therefore could not be found in the lung and sputum by culture methods.

Table 1. Principal moulds found in outside air

| Spores                         | Average concentration (per m <sup>3</sup> ) | Average size (µm)   |
|--------------------------------|---------------------------------------------|---------------------|
| <i>Cladosporium</i> spp.       | 986                                         | 12 × 5              |
| Basidiospores                  | 792                                         | 5-20                |
| Ascospores                     | 694                                         | 2-40                |
| <i>Sporobolomyces roseus</i>   | 221                                         | 4 × 3               |
| <i>Penicillium</i> spp.        | 102                                         | 2-12                |
| <i>Tilletiopsis</i> spp.       | 54                                          | 6 × 1               |
| <i>Ustilago</i> spp.           | 39                                          | 7-12                |
| <i>Botrytis cinerea</i>        | 27                                          | 7-16 × 5-7          |
| <i>Aspergillus fumigatus</i>   | 25                                          | 2.5-3               |
| <i>Aureobasidium pullulans</i> | 21                                          | (Conidia) 4-6 × 2-3 |
| <i>Epicoccum purpurascens</i>  | 12                                          | 10-12               |
| Sterile                        | 11                                          |                     |
| <i>Alternaria</i> spp.         | 7                                           | 30-50 × 8-10        |
| <i>Phoma</i> spp.              | 6                                           | 5 × 2               |
| <i>Paecilomyces</i> spp.       | 5                                           | 3-9 × 2-5           |
| <i>Puccinia graminis</i>       | 5                                           | 36-26 × 22-15       |

Less than five per m<sup>3</sup>: *Erysiphe* spp., *Cephalosporium* spp., *Verticillium* spp., *Aspergillus niger*, *Geotrichum* spp., *Stemphylium* spp., *Fusarium* spp.

### Sputum culture

The moulds most frequently isolated from sputum during the survey are shown in Table 2. *Candida albicans*, found most commonly, was unique in not occurring in the aerospora. *Aspergillus fumigatus* was found more frequently than would have been anticipated from its occurrence in the air, while *Sporobolomyces* was found less frequently. 12% of sputa contained no culturable spores and a further 31% gave only *Candida albicans* in culture.

### Lung culture

The moulds isolated from peripheral lung tissue are recorded in Table 3. Again *Candida albicans* was found most frequently, though absent in the air, and species of *Aspergillus* and *Phoma* occurred disproportionately often. 17% of lung specimens were negative and a further 16% gave only colonies of *Candida albicans* in culture.

An analysis of the results showed no significant difference between males and females, between the specimens obtained from Aberystwyth, Liverpool or Penarth, or between left and right lungs or upper and lower lobes. Nor was there any significant difference between age groups, between those that died at home or in hospital or between the different causes of death.

**Table 2.** Prevalence of moulds in sputum

| Moulds                         | Percentage of sputa with positive culture |
|--------------------------------|-------------------------------------------|
| <i>Candida albicans</i>        | 67.2                                      |
| <i>Aspergillus fumigatus</i>   | 19.2                                      |
| Penicillium spp.               | 18.6                                      |
| Cladosporium spp.              | 17.0                                      |
| Sterile                        | 7.1                                       |
| <i>Aspergillus niger</i>       | 2.1                                       |
| Aspergillus spp.               | 1.9                                       |
| Alternaria spp.                | 1.2                                       |
| Phoma spp.                     | 0.9                                       |
| Mucor spp.                     | 0.9                                       |
| <i>Aureobasidium pullulans</i> | 0.8                                       |
| <i>Paecilomyces varioti</i>    | 0.7                                       |
| <i>Epicoccum purpurascens</i>  | 0.6                                       |
| <i>Trichoderma viride</i>      | 0.6                                       |
| <i>Botrytis cinerea</i>        | 0.5                                       |
| Cephalosporium spp.            | 0.5                                       |

Less than 0.5%: *Stemphyllium botryosum*, *Chaetomium* spp., *Geotrichum candidum*, *Fusarium nivale*, *Neurospora sitophila*, *Rhizopus stolonifer*, *Sporobolomyces roseus*, *Sporotrichum carnis*, *Trichothecium roseum*, *Harpographium delicatum*, *Humicola lanuginosa*, *Verticillium alboactrum*.

**Table 3.** Prevalence of moulds in lung specimens

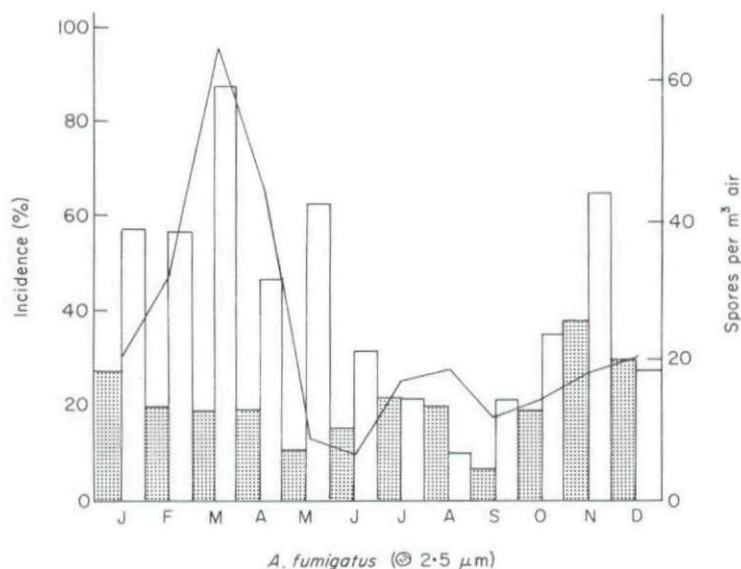
| Mould                          | Percentage of lung specimens with positive culture |
|--------------------------------|----------------------------------------------------|
| <i>Candida albicans</i>        | 62                                                 |
| <i>Aspergillus fumigatus</i>   | 44.3                                               |
| Phoma spp.                     | 21.4                                               |
| <i>Aspergillus niger</i>       | 12.4                                               |
| Penicillium spp.               | 7.4                                                |
| Cephalosporium spp.            | 7.1                                                |
| Cladosporium spp.              | 6.8                                                |
| Mucor spp.                     | 4.4                                                |
| Sterile                        | 4.4                                                |
| <i>Fusarium nivale</i>         | 3.4                                                |
| Alternaria spp.                | 2.7                                                |
| Aspergillus spp.               | 1.7                                                |
| <i>Stemphyllium botryosum</i>  | 1.4                                                |
| <i>Epicoccum purpurascens</i>  | 0.3                                                |
| <i>Verticillium alboactrum</i> | 0.3                                                |

**Table 4.** Average mould concentration recorded from post-mortem room, laboratory and outside air (per m<sup>3</sup>)

| Mould                          | Post-mortem room | Laboratory | Outside air |
|--------------------------------|------------------|------------|-------------|
| <i>Cladosporium</i> spp.       | 200              | 150        | 862         |
| <i>Penicillium</i> spp.        | 80               | 110        | 130         |
| <i>Sporobolomyces roseus</i>   | 3                | 0          | 130         |
| <i>Epicoccum purpurascens</i>  | 3                | 0          | 130         |
| <i>Botrytis cinerea</i>        | 5                | 2          | 6           |
| <i>Phoma</i> spp.              | 1                | 0          | 9           |
| <i>Aureobasidium pullulans</i> | 2                | 3          | 5           |

**Table 5.** Numbers of colonies cultured from sequential samples of ten lung specimens

| Mould                        | Samples |   |   |
|------------------------------|---------|---|---|
|                              | 1       | 2 | 3 |
| <i>Candida albicans</i>      | 4       | 7 | 9 |
| <i>Aspergillus fumigatus</i> | 3       | 4 | 4 |
| <i>Phoma</i> spp.            | 3       | 2 | 1 |
| <i>Aspergillus niger</i>     | 1       | 1 | 1 |
| <i>Aspergillus</i> spp.      | 0       | 3 | 0 |
| <i>Cladosporium</i> spp.     | 1       | 0 | 0 |
| <i>Penicillium</i> spp.      | 0       | 1 | 1 |

**Fig. 1.** Incidence of *Aspergillus fumigatus* in air (—), lung (□) and sputum (▨).

*Control experiments*

The census of moulds in the post-mortem room and the laboratory showed lower concentrations than were found in outside air (Table 4).

The fact that the most prevalent mould indoors was *Cladosporium*, which occurred infrequently from lung cultures, indicates that contamination had little influence on the results.

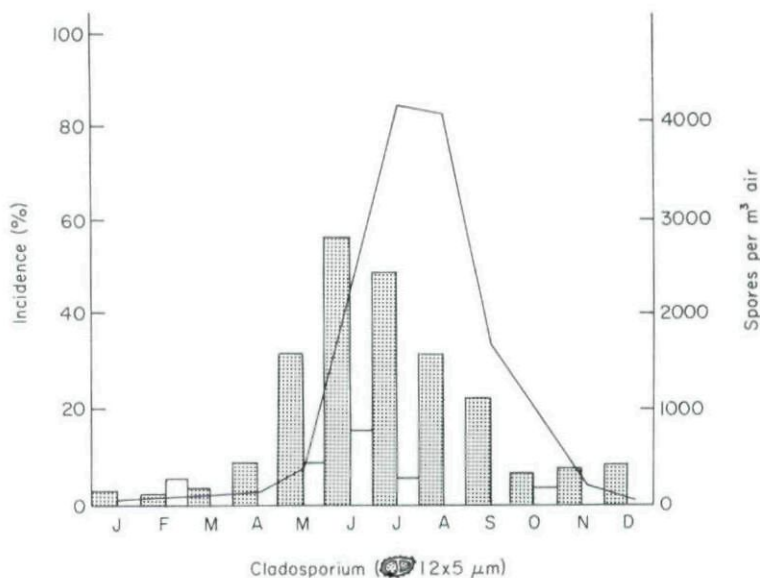


Fig. 2. Incidence of *Cladosporium* in air (—), lung ( $\square$ ) and sputum ( $\blacksquare$ ).

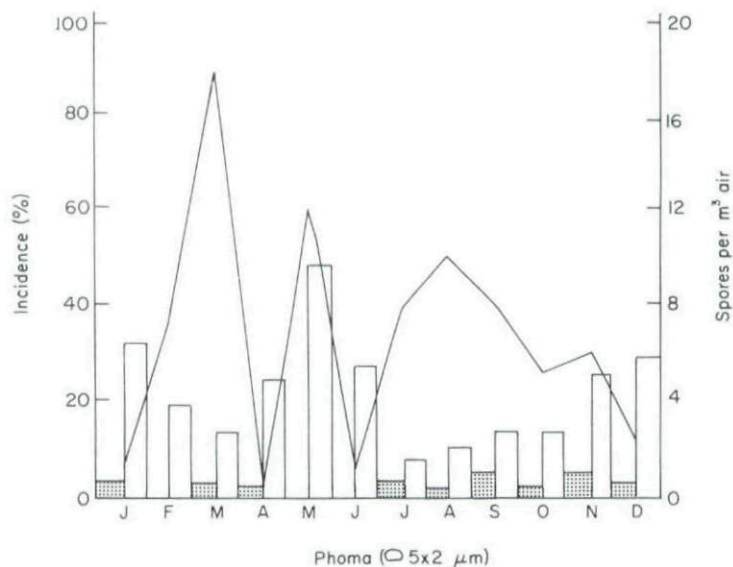


Fig. 3. Incidence of *Phoma* in air (—), lung ( $\square$ ) and sputum ( $\blacksquare$ ).

The moulds isolated from repeated sampling of the same lung specimens while they were stored at 4°C are recorded in Table 5. There is an increase in *Candida albicans* in sequential samples. Otherwise there was no evidence of fungal growth under the conditions in which we stored the specimens.

#### Seasonal variations

All moulds show some seasonal variation in the aerospora. A similar variation in positive cultures was seen in the sputum and lung samples. Fig. 1 shows the winter seasonality of *Aspergillus fumigatus* in the survey, both in the aerospora and from sputum and lung specimens. Fig. 2 shows similar changes in the case of *Cladosporium*, although in this case the mould has a summer season. Fig. 3 shows that *Phoma* is an exception in that it has a spasmodic occurrence in the air, occurs little in sputum and its occurrence in the lung does not obviously relate to its occurrence in air.

#### Discussion

Surveys of sputum, in particular for *Aspergillus fumigatus*, have given very variable results dependent upon the nature of the population sampled (Pepys, 1969; Henderson, 1968). Charpin *et al.* (1964) isolated very few fungi from bronchial secretions, but found significant spore retention in a later comparative study of the spore content of inhaled and exhaled air (Charpin *et al.*, 1971). Austwick (1966) found a wide variety of fungi in the lungs of dairy cows, reflecting the large numbers of fungal spores within a cowshed. These were restricted to those with an airborne diameter of 10 µm or less. In contrast, Michel *et al.* (1977) obtained pollen grains up to 20 µm diameter from samples of lung parenchyma. However, the airborne diameters of these grains may have been less due to dehydration prior to entering the respiratory tract. In a recent study of the fungal flora of the human lung in Japan, Okudaira, Kurata & Sakabe (1977) found fungi in 81% of examined lungs.

The potential of a particle for penetrating to the periphery of the lung is dependent upon its aerodynamic characteristics (Muir, 1972). In the case of spores, their abundance in the air and their seasonality will also affect their presence in the tracheo-bronchial tree. It has also been suggested that the electrostatic charge on a particle may influence the site of deposition in the lung, and such charges have been demonstrated on fungal spores (Gregory, 1957). Spores which reach the periphery of the lung are presumably removed by macrophage activity, and this may vary with different spores. The time found by Voisin *et al.* (1971) for the removal of *Candida albicans* and *A. fumigatus* spores from guinea-pig lungs was 5 and 12 days respectively after inhalation.

The occurrence of spores in the lung and sputum samples in our study appeared to be affected by their size. Thus *Cladosporium*, a large spore, was more abundant in the sputum than in the lung, but *A. fumigatus* and *Phoma*, with small spores, both occurred much more frequently in the lung than sputum samples. *Candida albicans* was the only fungus cultured which does not have airborne spores. As a yeast it lives saprophytically within the respiratory tract and was found with similar frequencies from lung and sputum. *Phoma* is an imperfect (asexual) stage of several different fungi (e.g. *Leptosphaeria* and *Didymella*), the sexual stages of which (Ascospores) are airborne and may be of respirable size.

It is conceivable that the vernalization effect of a period in the lung may increase the chances of spore germination on a culture plate, where they would grow as *Phoma*.

However, certain spores which on the basis of size and frequency in the air might have been expected to be isolated from lung samples were absent, viz: *Sporobolomyces*, *Tilletiopsis* and *Aureobasidium*.

The absence of *Sporobolomyces* and *Tilletiopsis* from the lung cultures and their very low levels in sputum raises the possibility that they may be removed very rapidly from the lung or that they are rendered non-viable. In fact, we have observed a high degree of inhibition of fungal growth on plates with incorporated lung tissue and a definite bias in the results which must be ascribed to this phenomenon, on which studies are continuing. On the other hand, the relative frequency of *Aspergillus* suggests that it may be more resistant to the lung's defences and this may be a factor in its pathogenicity.

Although it is well recognized that exposure to large numbers of fungal spores may provoke allergic reactions, as in workers on combine harvesters (Darke *et al.*, 1976) who may develop asthma, or as in allergic alveolitis (Edwards, Seal & Seaton, 1976), it follows from our study that the isolation of fungi from lung biopsy specimens should not be taken to imply pathogenicity. Since it appears that we all inhale large numbers of fungal spores throughout our lives, the significant factor is not the presence of the spore but the organism's response. In the case of immunosuppressed subjects (Rifkino *et al.*, 1967), or those whose defences are presumably impaired after open heart surgery (Petheram & Seal, 1976), fungi may become invasive pathogens. It is interesting that in these circumstances the fungi most commonly reported are *Aspergillus fumigatus* and *Candida albicans*, the two that we found most frequently in lung tissue. It is clear that these fungi are much less easily handled by the normal lung than most others. Further studies of the way in which lung handles different fungi should be of more than theoretical interest.

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