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FUNGI IN THE SPUTUM OF NORMAL MEN[1]

by

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Abstract

A standardized survey of respiratory and cardiovascular findings was conducted among male telephone workers in Baltimore, Maryland who were over 40 years of age. Sputum produced within 1 hour after arising was requested as part of the examination. All but 1 of the 246 eligible employees returned the specimen bottles, but only 103 were able to produce sputum in time for examination. All suspected fungal growth was isolated and insofar as possible identified as to genus.

Some fungus was isolated from 83 of the 103 specimens. *Penicillium* was found in about 40 per cent of the specimens, *Hormodendrum* in about 30 per cent, and *Alternaria* in about 10 per cent. The association of chronic wheezing and subsequent respiratory illness with the presence of *Hormodendrum* or *Alternaria* in the sputum was sufficiently marked to suggest further investigations of this association for possible etiologic relationships.

Introduction

The role of fungi in bronchopulmonary disease is puzzling. Even when they are not dismissed out of hand as contaminants of the respiratory secretions, careful study may still fail to tell whether fungi caused the disease process as primary pathogens, aggravated it as secondary invaders, or as saprophytes merely found it to their liking.

No single approach is likely to unravel this tangled skein. In addition to clinical and laboratory studies which have been the principal methods of the past, epidemiologic studies are needed. If general populations with a broad range of characteristics are systematically examined for a broad range of fungi, associations may be found to point the way for more specific probing. Furthermore, findings among general populations will provide a background against which to assess results of studies among more selected groups of subjects.

This paper reports results from one of several possible epidemiologic approaches to the problem of fungi and chronic respiratory disease.

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Materials and Methods

In May, 1967, a standardized survey of respiratory and cardiovascular findings was conducted among a group of telephone company employees in Baltimore, Maryland. Eligibility for the survey was restricted to men between the ages of 40 and 65 years, who worked in jobs related to installation, construction, maintainance and repair of telephone facilities in districts selected to represent both the center and the periphery of the city. Men who had met these criteria at the time of a similar survey 5 years earlier and who were still working in the Baltimore area were also invited to participate. Of the 389 men invited, 307 or 79 per cent participated, 47 or 12 per cent were unable to participate because of distant work assignments, vacations or illness, and 34 or 9 per cent declined the invitation.

The survey procedure consisted of a standardized questionnaire of respiratory and cardiovascular symptoms, physical measurements including height and weight with the subjects stripped to the waist, blood pressure determinations, electrocardiography, forced expiratory volume in 1 second, and peak expiratory flow rate. These procedures were performed as similarly as possible to telephone company surveys described previously (Holland, Reid, Seltser & Stone, 1965). In addition, each man was given a small sterile glass bottle and instructed to use it to collect all phlegm that could be coughed up from the chest during the first hour after arising on the day after the examination. If no sputum could be produced, the bottle was to be returned unused.

The survey was conducted on 5 successive days, Monday, May 22 through Friday, May 26, 1967. Of the 246 men examined during the first 4 days, 245 returned sputum bottles. The one man who did not do so was hospitalized for emergency treatment when his survey electrocardiogram suggested an acute myocardial infarction. There was no sputum in 131 bottles, and 11 containing sputum were returned too late to be examined for fungi. The 103 specimens available for culturing were taken to the laboratory as soon as volume and gross characteristics of the sputum could be recorded by the survey staff.

Sterile saline solution was added to very scanty specimens and to those consisting of thick and tenaciously mucoid sputum. All specimens were shaken for 30 minutes on a Kahn reciprocal shaker. Approximately 1 ml was streaked onto each of three plates containing the following media: (1) Sabouraud's dextrose agar; (2) Sabouraud's dextrose agar with 20 units of penicillin, 40 units of streptomycin and 0.5 mg actidione per ml of medium; and (3) blood agar with 5 per cent human blood and the same concentration of antibiotics as in (2). The plates were held at room temperature until they could be transported to the laboratory at Missouri State Sanatorium, Mt. Vernon, Missouri where they were incubated at 30 °C. Very little hyphal growth had occurred prior to incubation.

Representative colonies of all filamentous morphological types appearing within streaked areas of the plates were transferred to slants

of Sabouraud's dextrose agar. Isolates not identifiable on lactophenol cotton blue preparations were transferred to Sabouraud's slide cultures. Identification of fungi was carried only as far as the genus. The presence of bacteria or yeast was recorded, but no identification of these organisms was attempted.

Illnesses occurring among the study subjects for the 4-year period after the survey were determined from the records of the medical department of the telephone company. All persons with an illness absence 8 days or more must present a physician's certificate stating the diagnosis. The number, duration and reported causes of illnesses lasting for 8 days or more were recorded for the portion of the 4-year period during which the study subjects were employed.

Results

The gross appearance of the sputum specimens as determined by independent inspections by the survey and laboratory teams is shown in table 1. The survey team followed the classification suggested by the British Medical Research Council (Miller & Jones, 1963). They were coached in this procedure by Drs. Walter Holland and A. E. Bennett from St. Thomas's Hospital Medical School, London, England. Seven specimens were considered to be saliva. Of those considered to be sputum, 77 were mucoid with no pus, 12 mucoid with a trace of pus and 5 mucopurulent with the purulent component less than one-third of the total. Two specimens were taken to the laboratory before classification by the survey team. Although different criteria were used by the laboratory team, the agreement was reasonably good. The results showed a general tendency for Americans to classify sputum as more purulent than British workers.

The presence of bacteria was noted in cultures from 50 of the 103 specimens; yeasts were found in 69. Neither type of organism appeared to be associated with the presence or absence of fungi nor with smoking history, body build or cardio-respiratory findings, except that persons with poor ventilatory function were more likely to have bacterial growth noted. Some fungus was isolated from 83 of the 103 specimens submitted.

Table 1. Gross characterisitics of sputum specimens as determined by two independent inspections

Classification by laboratory personnel	Classification by survey personnel					
	Total	Saliva	Mucoid	Mucoid with trace of pus	Muco-purulent	Not examined
Total	103	7	77	12	5	2
Mucoid	70	7	56	5		2
Mucopurulent	27		19	6	2	
Purulent	6		2	1	3	

The genera identified from more than 1 subject are listed in table 2. *Penicillium* was found in one-half of the specimens from which fungi were isolated and *Hormodendrum* in one-third. In addition, 13 genera were represented by only a single isolation and 27 individuals showed some type of growth suggestive of fungi which could not be identified. The 13 single isolations were *Calcariosporium*, *Catenularia*, *Chaetomium*, *Chrysosporium*, *Geotrichum*, *Graphium*, *Papularia*, *Phoma*, *Piricauda*, *Pullularia*, *Sporotrichum*, *Trichoderma* and *Tritirachium*.

Table 2. Fungi isolated from more than a single subject among 103 subjects with sputum available for culturing

Genus	Number
Penicillium	42
Hormodendrum	29
Spicaria	17
Aspergillus	16
Scopulariopsis	10
Alternaria	9
Beauvaria	5
Cephalosporum	3
Rhizopus	3
Gonatobotrys	2

There were no significant variations in the frequency or type of isolates by day of examination or age of subject. Fungi were isolated from only 1 of the 7 specimens classified by the survey team as saliva, and from slightly more than 80 per cent of the others, with no significant variation according to type of sputum. Fungi were found in only about 60 per cent of the 38 sputum specimens with a volume less than 1 ml and were present in about 90 per cent of the 65 specimens of more than 1 ml.

The likelihood of isolating some identifiable fungus from sputum appeared to be associated with a history of cough, phlegm, breathlessness on exertion, wheezing in the chest during most days and nights, and effort pain suggestive of angina pectoris. It was not associated with nasal catarrh. Associations were also noted with a history of smoking at the time of the survey, but not with the number of years smoked, depth of inhalation or type of tobacco. Body build as reflected by ponderal index and subscapular skinfold thickness, and blood pressure levels did not appear to be related to finding fungi in the sputum.

Because of the relatively numerous fungus genera identified, further analysis was carried out using combinations of genera based on morphological similarities. This morphological classification was developed without knowledge of the foregoing results. Two groups occurred with reasonable frequency, group A *[Penicillium, Spicaria, Aspergillus* and *Scopulariopsis]* represented by 57 subjects and group B *[Hormodendrum* and *Alternaria]* by 34 subjects.

The relationships of group A and group B fungi to characteristics of

the study population were limited to the seven initially found to be associated with isolations of some identifiable fungus. These seven characteristics were cough, phlegm, breathlessness on exertion, chronic wheeze, angina, current smoking, and sputum volume. To examine the effect of each characteristic independently of the effects of the other six, isolation rates were adjusted by binary multiple regression (Feldstein, 1966).

The adjusted rates are shown in table 3. With the effects of other variables held constant, group A fungi were somewhat associated with breathlessness and wheezing, and more definitely with angina and sputum volume. Cough, phlegm and smoking showed no association with group A organisms. Group B fungi also had little association with cough and phlegm; they did show moderate association with sputum volume and more with wheezing. They were less likely to be isolated from persons who were short of breath on exertion or who smoked than among persons without these characteristics. Individual genera within each group tended to show correlations with these clinical characteristics which were similar to those for the group as a whole.

Table 3. Frequency of isolation of Group A and Group B fungi from men with and without specified respiratory findings

Respiratory finding		Number of men	Group A fungi[1]			Group B fungi[2]		
			No.	%	Adjusted[3] %	No.	%	Adjusted[3] %
Total		103	57	55.3	–	34	33.0	–
Cough	Present	54	34	63.0	57.1	14	25.9	27.6
	Absent	49	23	46.9	53.5	20	40.8	39.0
Phlegm	Present	63	37	58.7	51.7	18	28.6	29.3
	Absent	40	20	50.0	61.1	16	40.0	38.8
Breathlessness	Present	11	10	90.9	75.0	2	18.2	14.6
	Absent	92	47	51.1	53.0	32	34.8	35.2
Chronic wheeze	Present	18	13	72.2	67.7	9	50.0	63.5
	Absent	85	44	51.8	52.7	25	29.4	26.6
Angina	Present	12	11	91.7	85.3	3	25.0	33.2
	Absent	91	46	50.5	51.4	31	34.1	33.0
Smoking	Present	70	40	57.1	55.6	17	24.3	24.2
	Absent	33	17	51.5	54.7	17	51.5	51.6
Sputum	1+ml	65	44	67.7	67.9	25	38.5	41.0
	< 1 ml	38	13	34.2	33.9	9	23.7	19.3

[1] Penicillium, Spicaria, Aspergillus, Scopulariopsis.
[2] Hormodendrum, Alternaria.
[3] Adjusted for effects of all other respiratory findings by multiple regression analysis.

Of the 246 subjects, 211 were still employed at the end of the 4-year observation period. Only minimal differences in the average lengths of follow-up were found between persons with and without sputum examined, and between persons with group A, group B or other fungi isolated from their sputum. In this 4-year period, 39 subjects had at least one illness absence of 8 days or longer caused by acute respiratory disease or an

acute exacerbation of chronic obstructive respiratory disease. There were no illness absences caused by asthma or by the chronic manifestations of obstructive respiratory disease. Group A fungi were not associated with subsequent respiratory illness. Persons from whom group B fungi were isolated had nearly twice as high a frequency of respiratory illness and twice as much lost time from these illnesses as other groups of subjects. The presence or type of fungi in the sputum was not related to the frequency of other types of illnesses.

Discussion

With only 103 subjects producing sputum, the positive findings of this study can be no more than suggestive. But on the other hand, the chances of detecting real associations were also limited because less than half of the available population – those producing sputum – could be studied. If a sputum inducer had been used for all subjects, the search for associations could have extended over a much wider spectrum of clinical conditions.

With no significant variation in fungus isolations over the 4-day collecting period, it is unlikely that some isolated event was responsible for their presence. However, the degree of seasonal variation in this population is unknown. Although little variation from month to month was noted in Tucson, Arizona with respect to numbers and kinds of fungi isolated from the air (Dworin, 1966), considerable changes have been noted in places with more variable climates (Schaffer, Seidmon & Bruskin, 1953; Grose, Szekessy & Munoz, 1967; Noble & Clayton, 1963). Seasonal differences have also been found in the frequency of fungi isolated from the sputum of tuberculous patients (Goodman & Collins, personal communication).

Fungi isolated from the subjects in this study probably came from the lower respiratory tract. Isolations were most common from specimens with an appreciable volume of mucoid or mucopurulent sputum. If oral contamination had been more important, fungi should have been most frequent in saliva or in scanty specimens. Nor is the nasopharynx a likely source, because nasal catarrh was not related to fungus isolations. Although the slight negative association with a history of phlegm and the positive association with sputum volume appear contradictory, it is possible that a history of phlegm in these subjects may represent a mixture of upper and lower respiratory tract secretions.

The most marked association was found between the presence of *Hormodendrum* or *Alternaria* in the sputum and a history of wheezing in the chest. Whether or not these organisms play a part in causing this symptom cannot be determined from the evidence in this study, but the association is sufficiently marked to warrant further investigation. In a series of 92 patients in Venezuela with asthma or rhinitis, *Hormodendrum* was found in the sputum of only 8 per cent, while *Aspergillus* was isolated from 20 per cent and *Penicillium* from 16 per cent (DeMontemayor, Rodriguez & Salas, 1965). It should be noted,

however, that very few subjects in the present study who had a history of wheezing admitted to having had a diagnosis of asthma.

Also striking in this population was the association of group A fungi *[Penicillium, Spicaria, Aspergillus, Scopulariopsis]* with a history of angina. Again, whether or not this is a chance observation cannot be determined with certainty. But in this instance, there is other internal evidence to indicate that these fungi are probably not associated with arteriosclerotic heart disease. The group A fungi were isolated with equal frequency from persons with and without a history of a previous heart attack, among persons with and without electrocardiographic evidence of coronary artery disease, and among persons with and without subsequent illness from coronary heart disease. It does not seem likely that a real association could exist with angina pectoris and not with other manifestations of arteriosclerotic heart disease.

The association of *Alternaria* and *Hormodendrum* with respiratory illnesses in this small study is only suggestive at best. Because the presence of these fungi was also associated with a history of chronic wheeze, it is noteworthy that in a follow-up study of nearly 1400 telephone employees, the symptom of chronic wheeze was more strongly related to subsequent respiratory illness than any other cardio-respiratory symptom (Comstock, Stone & Tonascia, unpublished data). There is thus a certain degree of internal consistency which emboldens us to venture the opinion that the triad of *Alernaria* or *Hormodendrum* in the sputum, chronic wheeze, and respiratory illness warrants further investigation to see if either of these organisms plays an etiologic role in respiratory disease.

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