

# A SYNDROME RESEMBLING FARMER'S LUNG IN WORKERS INHALING SPORES OF ASPERGILLUS AND PENICILLIA MOULDS

BY

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The effects of moulds on humans, and especially on the respiratory tract, have been of interest to many investigators (Virchow, 1856; Dévé, 1938; Riddell, 1956; Wegmann, 1956; Clayton, 1958; Staib and Ata, 1958). Some moulds are pathogenic, but there are others, such as aspergilli, which can produce lung disease only in certain circumstances. There is still no agreement about their relationship to macro-organisms, but roughly four types of activity are recognized (Dévé, 1938; Hinson, Moon, and Plummer, 1952; Frankland and Hamilton, 1958), namely:

- (1) *Saprophytic*.—Settling without any obvious reaction on the part of the host.
- (2) *Allergic*.—Most frequently manifested as bronchial asthma (Fawcitt, 1938; Törnell, 1946; Hinson *et al.*, 1952; Fuller, 1953; Studdert, 1953; Frankland and Hamilton, 1958).
- (3) Production of proper fungous lung diseases.
- (4) Doubtful fungal aetiology: farmer's lung.

We have investigated a group of subjects working for many years in environments containing great quantities of spores of aspergillus and penicillia, without any admixture of dust or other pathogenic organisms. From this investigation we tried to answer the following questions of general interest:

- (1) Does massive inhalation of moulds produce a fungous disease of the lungs, bronchial asthma, or farmer's lung syndrome?
- (2) Does inhalation of moulds influence the functional efficiency of the lungs?
- (3) Does inhalation of moulds produce specific antibody formation in the blood, positive culture findings in the respiratory tract, and skin hypersensitivity to them?

(4) Do the mycological and serological findings bear any relation to the clinical symptoms and are they of any diagnostic value?

(5) Are the objective findings and subjective symptoms caused by aspergilli or penicillia?

## MATERIALS AND METHODS

In a factory where molasses was fermented aspergilli for the industrial production of citric acid 61 men were examined who had worked or who still worked in an atmosphere containing great quantities of spores of aspergilli and penicillia. The fermentation is carried out in large open pans, and when the process is finished the thick layer of aspergillus culture is removed by the workers and at this time spore dust is produced.

Subjects were divided into two groups:

*Group I* comprised 38 men of average age 32, who had been working in this plant for from three months to 18 years. Every third day they worked in dense clouds of mould spores, protected by cottonwool filters applied to the nose and mouth. For the remaining two days they did other general work, and from time to time they were obliged to sterilize the cultivation vessels with formalin fumes.

*Group II* comprised 23 workers of average age 51.6 years, who had discontinued this work because of various symptoms. Nine of them, after an average exposure of 7.4 years to the mould dust, do general work in the neighbouring plant, where the moulds can only penetrate occasionally on the current of air from the main plant. The remaining 14 men left the work after an average exposure of 9.2 years, mainly because of increasing dyspnoea.

The *control group* consisted of 25 in-patients of our ward suffering from diseases other than lung diseases (Group A), another 25 patients with chronic bronchitis (Group B), and 54 healthy individuals (Group C). In all of them the bacteriological and clinical examination was made in the same way as in the tested groups.

All the tested subjects had their history taken, and were physically examined. Fluoroscopy was then carried out, chest radiographs taken, and the forced expiratory capacity measured and evaluated by the index of vital capacity (I.V.C.) (Kadlec and Vyskočil, 1954), the normal value being above 1 and the pathological value below 1. In addition, a blood cell count was made and the red cell sedimentation rate read for one and two hours. Sputum and laryngeal swabs were examined microscopically and cultivated on the usual bacteriological (including media for *M. tuberculosis*) and mycological media (Sabouraud, Czapek-Dox, and Littman's ox gall agar). In all patients we used the collodion agglutination method of Cavelti and Wagner (Cavelti, 1947; Wagner and Rejholec, 1952; Wagner, 1954; Seeliger, 1958) whereby antibodies against *Aspergillus fumigatus*, *A. niger*, and six species of penicillia found in the sputum or laryngeal swabs were sought. The collodion particles were mixed with collodion antigen 1:20. The mixture was added to the diluted serum of the patients to be examined, and two hours later the mixture

was centrifuged and after shaking read macroscopically. Flocculation was read as a positive reaction. (The detailed method will be published in *Zeitschrift für Immunitätsforschung*.) Skin sensitivity to these moulds was examined by the scratch test and by intradermal application (Mitze, 1953; Dyson and Evans, 1955; Kaplan, 1955; Hajos, 1956; Sternberger, Feinberg, Feinberg, Clarke, Benaim, and Warren, 1956; Epstein, Prince, and Morrow, 1957; Johnson and Hampton, 1957; Jillson, 1957). The antigen for skin and serological reactions was prepared from the filtrate of moulds by three methods: (1) freezing and defreezing, (2) warming up to 100° C., and (3) on ultrasonic destructive technique. The skin reactions were read after 30 minutes and 24, 48, and 96 hours.

The subjects were examined in our ward at least 24 hours after the last exposure to moulds. In addition 38 workers were tested in the plant while working with the moulds. The presence of spores in the air was examined by exposing mycological media for five to 60 seconds directly in the plant.

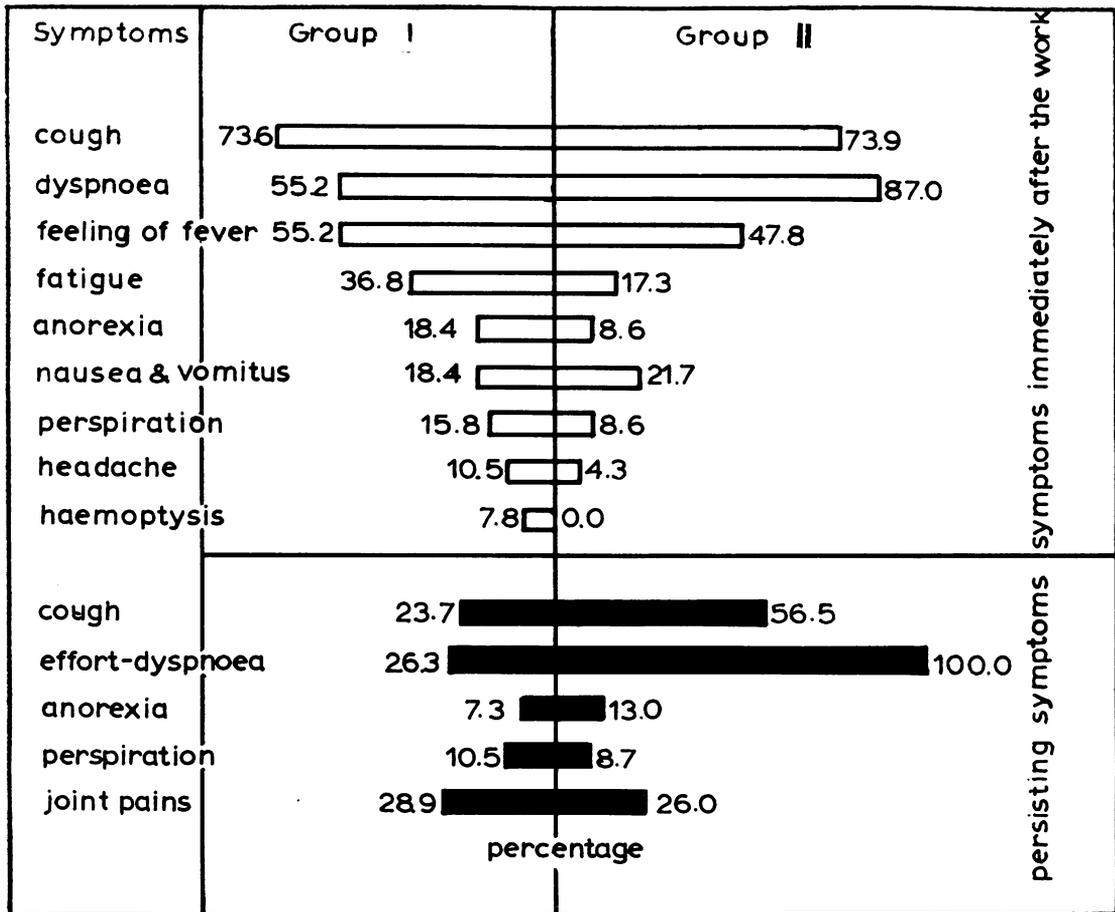


FIG. 1.—Comparison of persistent symptoms and symptoms immediately after work.

RESULTS

From 38 subjects of the first group, 93.5% reported symptoms when examined immediately after finishing the work with the moulds. These symptoms are summarized in Fig. 1.

Cough and dyspnoea and tightness of the chest were the principal symptoms in 73.6% and 55.2% respectively. Wheezing was heard even by the workers themselves, and some expectorated viscous greyish-black sputum. When the cough stopped, so did the dyspnoea, and after six to eight hours all symptoms had disappeared. In addition, 55.2% felt feverish, 36.8% were fatigued, and 18.4% had nausea and vomiting.

Complaints of those in Group II immediately after working with the moulds were similar, and dyspnoea in particular was more frequent (87%). All the symptoms described were absent in the same subjects on the days when other work was performed. On the other hand, the complaints were more pronounced after a longer interval between work (such as after holidays), and whenever "green spores" (contamination of *Aspergillus fumigatus* by penicillia) were inhaled. After the inhalation of

"black spores" (*Aspergillus niger*) the complaints were fewer.

Chest radiographs did not show any changes before and after the onset of symptoms (Table I). The forced expiratory capacity was reduced (average

TABLE I  
PATHOLOGICAL CHANGES IN GROUP I IMMEDIATELY AFTER WORK

Auscultatory signs of bronchitis	39.9%
Radiological "transient lung infiltration"	63.0%
Pathological I.V.C.	0
Raised E.S.R.	15.7%
Eosinophilia	13.1%
Mean I.V.C.	2.4

I.V.C. lower by 0.7) compared with the results obtained before working with the moulds.

Persistent symptoms (Fig. 1) had a different pattern from symptoms arising immediately after work. In Group I there were few complaints: joint pains in 28.9% and effort dyspnoea in 26.3%. All the subjects in Group II had effort dyspnoea and 56.5% had cough.

The results of examination of the same subjects (Group I and Group II) are given in Table II.

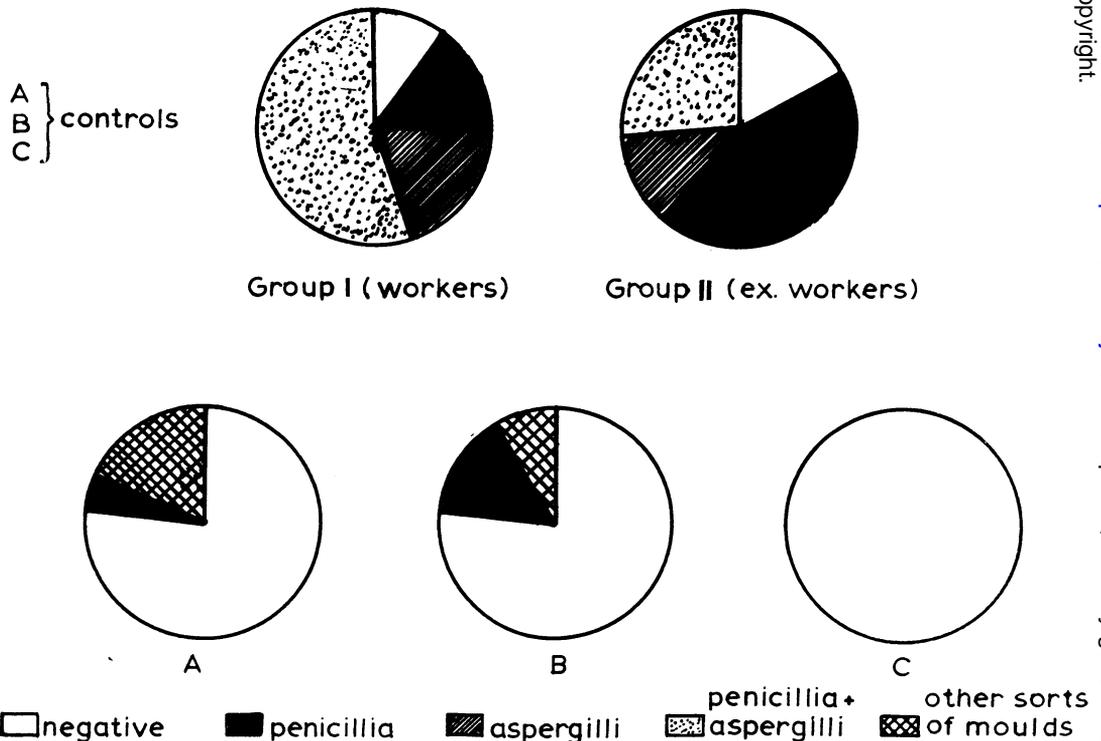


FIG. 2.—Proportion of moulds in respiratory tracts in Groups I and II and controls.

TABLE II  
PATHOLOGICAL CHANGES IN GROUPS I AND II  
DURING EXAMINATION IN THE WARD

Abnormal Signs	Group I (%)	Group II (%)
Auscultatory signs of bronchitis ..	39.9	71
Radiological .. .. .	63.0	85.7
Transient lung infiltration .. .. .	0	0
Radiological signs of tuberculosis ..	0	13
Pathological I.V.C. .. .. .	0	25
Raised E.S.R. .. .. .	13.1	11.1
Eosinophilia .. .. .	13.1	20
Epicermophytia .. .. .	39.4	18.1
Mean I.V.C. .. .. .	3.1	0.62

Chronic bronchitis was found in 39.9% of Group I and in 71% of Group II. The radiographs showed augmented interstitial and peribronchial tissue patterns in 63% of the first and in 85.7% of the second group. There was no specific granulation or any other radiological manifestation of a fungous lung disease. The lung function, estimated by the forced expiratory capacity, was always normal in Group I, and essentially impaired in 25% of Group II. The blood sedimentation rate was slightly raised in 13.1% of Group I and in 11.1% of Group II. Eosinophilia over 5% was found in 13.1% and 20% respectively. No other pathological changes in the blood were found.

The findings of moulds in the respiratory tract of the investigated groups and the controls are summarized in Fig. 2. There are striking differences in this respect between Groups I and II and the controls: completely negative in Group C, 76% negative in Groups A and B, and no aspergilli in any of the controls. Further, there is a marked difference between the occurrence of aspergilli and penicillia in Groups I and II.

From the species of aspergilli, *Aspergillus niger* van Tieghen, *A. fumigatus* Fresenius, and *A. flavus* Ling were found. From the species of penicillia *Penicillium simplicissimum* Thom, *P. herquei*, *P. rubrum* Stoll, *P. caseicolum*, *P. Miczinski-Zaleski*, and *P. italicum* occurred in different combinations. They were identified according to the methods given by Thom and Raper (1945), Lewis and Hopper (1948), and Raper and Thom (1949). The yeast-like fungi were present in 39.4% of Group I and in 17.8% of Group II. The common bacteriological flora of the respiratory tract were not changed. Cultures of *M. tuberculosis* were negative in all cases.

The skin sensitivity tests to the moulds mentioned above were more frequently positive in the second group of workers. In all positive tests an allergic skin reaction was immediate. The detailed results are given in Table III.

Specific antibodies against *A. fumigatus*, *A. niger*, and six species of penicillia in Groups I and II

TABLE III  
SKIN HYPERSENSITIVITY TESTS IN BOTH GROUPS

Group	Antigens		
	<i>Aspergillus niger</i> (%)	<i>Aspergillus fumigatus</i> (%)	Penicillia (%)
I	20.6	6.8	17.2
II	30.0	15.0	25.0

TABLE IV  
ANTIBODIES TO MOULDS IN GROUPS I AND II AND  
IN CONTROLS

Titres	Neg- ative (%)	1:2	1:4	1:8	1:16	1:32	Total
<i>Aspergillus niger</i>							
Group I ..	7	7	13	6	1	—	34
" II ..	7	5	8	2	1	—	23
Control ..	38	13	11	2	6	—	70
<i>Aspergillus fumigatus</i>							
Group I ..	16	9	7	2	—	—	34
" II ..	12	7	4	—	—	—	23
Control ..	53	7	8	—	2	—	70
Penicillia							
Group I ..	3	1	9	9	13	2	37
" II ..	15	1	2	4	1	—	23
Control ..	68	1	1	—	—	—	70

and in 70 individuals of the control group are given in Table IV. The finding of antibodies against aspergilli is similar in Groups I and II, most of the positive findings being up to a titre of 1 in 4. An interesting finding is that of antibodies against penicillia; they were found in a greater number and in higher titres in Group I, whereas in Group II they were found in smaller numbers, and in the controls they were negative in 97.1%.

#### DISCUSSION

Throughout our examination of 61 men employed in a plant where great masses of spores of aspergilli and penicillia whirl in the air, we were not able to find any person suffering from a specific fungous disease of the lungs. This is in agreement with usual experience that the pulmonary aspergilloses and penicillioses are rare diseases (Wegmann, 1956; Fragner, 1958). Some intrinsic factors seem necessary in their development, in addition to extrinsic factors, such as a primary lung disease which facilitates the settling of moulds and their further pathogenic activity in the organism (Staib and Ata, 1958).

The continuous inhalation of spores of moulds results, however, in their settling down and growing on the mucous membrane of the respiratory tract (Riddell, 1958; Staib and Ata, 1958). In confirmation of this hypothesis there were repeatedly positive cultures from the bronchi in 90% of workers

examined, and positive cultures of the same species of moulds in 87% of the subjects of Group II (see Fig. 2) who discontinued working with these moulds months or years before.

In our series, 55.2% and 87% in Groups I and II respectively of workers employed or previously employed in the mould dust suffered from acute attacks of dyspnoea, very similar to those described previously by investigators such as Fawcitt (1938), Törnell (1946), Fuller (1953), Studdert (1953), as the first stage of the farmer's lung syndrome. There is a definite relation between the symptoms and the period of work with the moulds, shown by the positive cultures from the respiratory tract, specific antibodies against the moulds in the blood of workers, and positive skin sensitivity tests. The literature concerning the original farmer's lung syndrome supports this opinion, and there also the same associations have been found.

There is, however, a difference in the objective clinical picture between our patients and those with the farmer's lung syndrome. Since we were not able to find any infiltration of lung, there are presumably some other factors in farmer's lung, such as organic dust or a constitutional factor (Studdert, 1953; Essellier and Jeanneret, 1956). Our dust consisted of pure cultures of moulds as opposed to hay and straw dust in farmer's lung, and this substantial difference may account for the different clinical picture.

On the other hand, there was a high percentage of chronic bronchitis in the workers examined. In the first group, comprising subjects of average age 32 years, 39.9% suffered from this condition. This is significantly more than the incidence of chronic bronchitis in the general population of Czechoslovakia. According to the extensive investigations of Mecl and Vyskočil, the incidence of this disease averages from 8 to 12.6% of women and men respectively. In the second group of older workers the incidence of chronic bronchitis was as high as 70%. Thus it seems very likely that the massive inhalation of pure moulds does not cause any damage to the lung tissue, but the damage is to the mucous membrane of the bronchial tree. We suppose that it starts with a spasm of the bronchi in response to the invasion of moulds. The repeated attacks of hyperaemia, hypersecretion, and spasm of the bronchi are consequently followed by damage to the epithelium of the mucous membrane. Thus the seeding of moulds, their growing and biological activity, are facilitated (Hinson *et al.*, 1952; Riddell, 1958). The reaction of the tissue is inflammation, and in this way chronic bronchitis develops.

The emergence of specific antibodies proves a more widespread activity of moulds on the macro-organism. This is evident from the comparison with the controls, when, in spite of a positive culture finding, very few antibodies were recognized. A higher finding of antibodies to penicillia in Group I would show a group of individuals with a good capacity to produce antibodies to prevent the pathogenic effect of moulds. In Group II, however, without this advantage, penicillia increase in the respiratory tract at the expense of aspergilli (Fig. 2) and produce symptoms which compel the affected persons to give up their work. This result might have been anticipated by a higher sensitivity to moulds in Group II (see Table III). A different pattern of antibodies against penicillia than against aspergilli and a different culture finding in both tested groups suggest rather that penicillia are the cause of symptoms in these patients in spite of long-term inhalation of spores of both species of moulds.

The relationship of moulds to the pathogenic and saprophytic flora of the bronchi is not well known. The presence and activity of moulds may influence the growth of other agents. In our groups the common bacteriological flora were not changed. On the other hand, there was a strikingly low occurrence of streptococcal infections in both groups examined, evidently caused by the antibiotic effect of the moulds. Also a lower occurrence of *Candida* in comparison with patients suffering from other diseases of the respiratory tract was confirmed by extensive sensitivity of yeasts to the filtrate from *A. niger* (results to be published elsewhere).

We were not able to prove any specific fungous disease of the lungs in the workers examined, and there was no real asthma, but the asthma-like attacks resembling the farmer's lung syndrome and caused by the direct irritation of bronchi were very frequent.

The massive inhalation of moulds caused the transitory spastic reaction of the bronchi, but not a permanent reduction in the functional efficiency of the lungs in most subjects.

The inhalation of moulds caused the production of specific antibodies, permanent positive culture findings in the respiratory tract, and skin hypersensitivity to them.

The mycological and serological findings bear a definite relation to the clinical symptoms.

The signs and symptoms are caused by penicillia rather than by aspergilli.

#### SUMMARY

In a group of industrial workers we studied the influence of inhaled spores of moulds from the

species of aspergilli and penicillia on the occurrence of diseases of the respiratory tract. Even after long-term inhalation we failed to find any mycetoma of the lungs. The incidence of chronic bronchitis was increased, and in the older group all subjects suffered from chronic bronchitis. In most subjects directly after contact with the moulds, we found a condition resembling the first stage of the syndrome of farmer's lung. The positive mycological and serological examination showed that long-term inhalation of moulds causes their permanent settling down on the mucous membrane of the respiratory tract and the production of specific antibodies against them.

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