Allergy 1984, 39, 183–193

Environmental Aerobiological Studies in Allergic Bronchopulmonary Aspergillosis

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An aerobiological pilot study was undertaken to measure airborne concentrations of Aspergillus spores in and around the dwellings of two patients with allergic bronchopulmonary aspergillosis (ABPA). The aim of the investigation was 1) to find a specific source of Aspergillus antigen, and 2) to find out whether disease activity, or exacerbation, paralleled Aspergillus spore prevalence. In the environment of the first patient heavy spore contaminated air was found in a cow-shed, and the adjacent hayloft and scullery. The patient experienced no typical exacerbation during the study period, probably because she now scrupulously avoided this source. The environment of the second patient contained lower spore concentrations in and around the house, and the patient had no typical exacerbation, possibly due to low-intensity exposure. Both patients, however, had increased bronchial obstruction during periods with high Aspergillus spore content in the outside air. The results suggest that avoidance of Aspergillus spore sources and low overall exposure may play a major role in preventing exacerbation of the disease.

Key words: aerobiology; Andersen Sampler; allergic bronchopulmonary aspergillosis; Aspergillus spores.

Accepted for publication 17 October 1983

CLINICAL ASPECTS

Aerobiological investigations in the micro-environment of a patient with allergic bronchopulmonary aspergillosis may reveal a particular mould source. Avoidance and, if possible, removal of this source may be an important factor in preventing new exacerbations of the disease.

Allergic bronchopulmonary aspergillosis (ABPA) is a dynamic pulmonary disorder, first described some 30 years ago by Hinson (7) and associates. Since that time, many studies have been made of the clinical, immunological and roentgenological features of this disease (5, 6, 13, 18, 20, 21, 25). For the diagnosis of ABPA a set of criteria has been developed (20), which will enable the clinician to assess the disease as "definite", "probable" or "possible" (Table 1). An important aspect of the disease is the occurrence of exacerbations, which are accompanied by increased total immunoglobulin E and eosinophils and roentgenologically visible infiltrations in the lungs (17, 20, 25). Vernon (24) postulates that environments rich in *A. fumigatus* spores may influence the development of ABPA, suggesting that exogenous sources may be important. That ABPA is often exacerbated in the winter months, when airborne spores are more frequent (5, 14, 15, 16), suggests an exposure factor. Another possibility is the germination of persistent spores, or pieces of mycelium, trapped in the damaged and obstructed bronchial tree. In most papers dealing with the epidemiology of ABPA these two matters are left undecided (5, 13, 14, 15, 22, 24). Two questions are essential to the approach: 1) is there a high prevalence of Aspergillus spores in the indoor and outdoor surroundings of pa-

tients with ABPA, and 2) is the course of the disease correlated with changes in airborne Aspergillus spore concentrations. An aerobiological pilot investigation of the environment of two patients with ABPA was made, and comprised weekly or monthly samplings of in- and outdoor air for at least 1 year, daily peak flow tests, and regular roentgenological and serological tests. The results of this study are now reported.

MATERIAL AND METHODS

Aerobiological, environmental investigations

Carefully timed sampling in periods of 10 min were performed with a six-stage Andersen (1) microbial air sampler, with one Petri dish placed below the sixth stage, according to a previously described method (23). The sampler was calibrated to process 28 l air/min.

The culture medium used was Yeast Morphology agar (YM agar), containing per litre: 3 g yeast extract; 5 g pepton, 10 g glucose and 20 g agar. The plates were incubated at 28°C and examined after 3, 5, and 10 days. Colonies were counted according to the "positive hole method", which corrects for the probability of multiple spore deposits on one deposit area (1). All results are expressed as colony forming units (i.e. viable spores) per cubic meters of sampled air. Form genera of fungi were identified macro- and microscopically. If necessary subcultures and slide preparations were performed, in order to study morphological aspects in more detail. In case of doubt, monocultures were sent to the Centraalbureau voor Schimmelcultures in Baarn, The Netherlands (Dr. G. de Vries) for further determination. In general, no attempt was made to determine the colonies at species level. From April 1981 until April 1982 monthly samples were taken from several places in the house of patient A. The dwellings of patient B were sampled approx. every week from March 1981 until December 1982.

On each occasion an outside sample was taken, in order to compare indoor and outdoor collections. Colony counting and determination was carried out not only for Aspergillus, but included all recognizable genera growing on the plates. The latter will not be discussed here.

Clinical and laboratory investigations

ABPA was diagnosed on the basis of the criteria mentioned in Table 1. Exacerbation of ABPA was defined by the appearance of new infiltrative pulmonary lesions, together with a two or threefold increase in total IgE and number of eosinophils. Sputum was regularly tested for the presence of hyphae and was subsequently cultured on Sabouraud's medium. Skin testing was performed with a battery of common fungial allergens consisting of: mixed Penicillium, mixed Cladosporium, mixed Aspergillus, *Alternaria tenuis* and a mixed "rest-group" with Mucor, Botrytis, Pullularia, Absidia and Epicoccum. The results were read after 30 min, 8 and 24 h.

Inhalation provocation with *A. fumigatus* (and in patient B also Penicillium mixture) was carried out according to a previously described procedure (2), using an antigen concentration of 5 mg *A. fumigatus* per ml Coca's solution. The test was considered postive as there was a fall in FEV_1 of $\geq 15\%$ of the initial value.

Immunological parameters. Total and specific immunoglobulin E were measured by PRIST and RAST (Pharmacia Diagnostics).

The number of precipitation lines against *A. fumigatus* extract by double-diffusion, and spe-

Table 1

ABPA criteria according to Rosenberg (20)

1. Chronic obstructive lung disease
2. Positive skin test with Aspergillus extract
3. High total immunoglobulin E
4. Typical roentgenographic findings, like "fleeting shadows" and central bronchiectasis
5. General malaise with fever and/or weight loss
6. Productive cough with typical sputum plugs, and/or hyphae, and/or hemoptisis
7. High peripheral blood eosinophils (≥ 600 per m^3)
8. Positive precipitins against Aspergillus and/or positive specific anti-Aspergillus immunoglobulin E

Disease certain: criteria 1–4, and three of the last four
Disease probable: criteria 1–3, and four of the last five
Disease possible: criteria 1–2, and three of the last six

cific IgG antibodies by ELISA were determined according to previously described procedures (9).

Antigenic extracts of *A. fumigatus* used for skin tests and inhalation procedures were obtained from Diephuis laboratories (Groningen, The Netherlands). Antigens used for double-diffusion and ELISA are a metabolic antigen of *A. fumigatus* prepared as described previously (8).

Lung function during the stay in hospital and at the regular out-patient visits was recorded spirographically, together with vital capacity (VC) and forced expiratory volume in 1 sec (FEV_1). At home the patients recorded their daily peak flow (PF) rates with a Wright peak-flow mini-meter, 3 times daily. Additionally, the patients made a note of their pulmonary condition.

Eosinophils were quantitatively expressed as numbers per mm^3.

Roentgenological examination of the chest was performed every 3 months, and, additionally, whenever pulmonary complaints were present.

Case histories

Patient A, a non-smoking female, born 1962: in 1966 she had a diagnosis of "paroxysmal bronchitis". In the next 14 years she was hospitalized several times, mainly because of exacerbation of "bronchitis", but also recurrent "pneumonia" and signs of infiltration on her chest roentgenogram. In 1976 she had a strongly positive skin test for different fungi, including *A. fumigatus*. In that period a bronchogram showed centrally located bronchiectasis of the right upper lobe. Because of an exacerbation of her pulmonary disorder, she was admitted to our hospital in June 1980. Additional information included shortness of breath while running, and occasional wheezing in the chest. The family history of obstructive lung disease was negative. She lived on a modern-housed farm with three barns, one of which was adjacent to the back of the house (Fig. 1), and used for storing hay during the winter for cattle feed. A large peat stack was situated opposite the house. At the time of admission she was using no medication. At physical examination the patient appeared well; some rales were heard over the chest region. The chest roentgenogram showed consolidated shadows in the right lower lobe and lingula (Fig. 2). The right upper lobe showed irregular broad linings, suggesting bronchiectases. Table 2 shows the most important clinical data on admission and at follow-up.

A diagnosis of ABPA was made on the following grounds:

1. a history of obstructive lung disease;

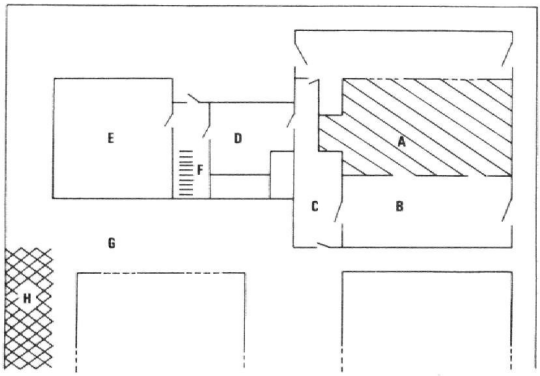

Fig. 1. Ground-plan house: patient A. A = hayloft; B = cattle barn; C = scullery; D = kitchen; E = livingroom; F = stairs to bedroom first floor; G = outdoors; H = peat stack.

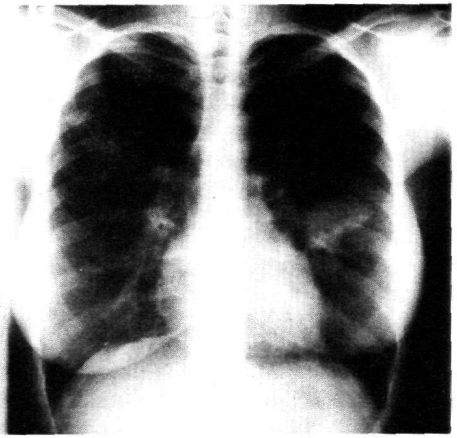

Fig. 2. Chest radiograph of patient A (June 1980), showing patchy infiltrations of right upper field (RUL), and sharply demarcated infiltration of left middle field (lingula).

2. positive skin test and early positive inhalation-provocation with *A. fumigatus* extract;
3. high total and specific IgE to *A. fumigatus* allergen;
4. positive precipitins and elevated ELISA IgG titers to *A. fumigatus* antigen;
5. signs on the chest roentgenogram compatible with this diagnosis, and bronchograpically proven central bronchiectasis;
6. peripheral blood and sputum eosinophilia;
7. on one occasion a positive sputum culture with *A. fumigatus*.

She was initially treated with corticosteroids, cromoglycate, and antibiotics. Inhalation therapy with amphotericin-B was also given. The corticosteroids were rapidly tapered to 7.5, 5 and 2.5 mg on alternate days, and finally stopped in June 1981.

Patient B, male, smoker, born 1912. About 30 and 20 years ago he suffered from "bronchopneumonia". The patient has been treated since 1971 for the recurrent infiltrations seen on the chest X-ray, with purulent sputum production, and bronchial-obstructive complaints. The family history of obstructive lung disease is positive (father and one son). He enjoys gardening; and at home he has a large greenhouse and two compost heaps in the yard (Fig. 3). He attended our pulmonary department for the first time in 1979 because of abnormalities on the chest roentgenogram in connection with a pre-operative screening. Additional information included recurrent cough with expectoration of yellowish-green sputum, sometimes wheezing in the chest, and dyspnoea on exertion. At physical examination, some rales were heard over the thorax. Compared with former radiographs, his chest roentgenogram (Fig. 4) showed progressive abnormalities of the right upper lobe with broad bronchial linings and signs of peribronchial infiltrations.

Pertinent laboratory and lung-function data are shown in Table 2.

A diagnosis of ABPA was made on following grounds:

1. evidence of bronchial-obstruction, especially in the last 10 years;
2. positive skin test, and a strong dual reaction with *A. fumigatus* extract on inhalation provocation;
3. high total IgE and strongly positive RAST with *A. fumigatus* allergen;
4. positive precipitins and elevated ELISA IgG titers to *A. fumigatus* antigen;
5. signs on the chest radiographs compatible with this diagnosis, and centrally located bronchiectases;
6. peripheral blood and sputum eosinophilia;
7. positive sputum cultures with *A. fumigatus*.

RESULTS

Home situation patient A, aerobiological data

Aspergillus spp. including *A. fumigatus* were present in all seasons, but consistently higher concentrations were recorded between December and March (Fig. 5). There were, however, also high concentrations of Aspergillus in April 1981 and at the end of September 1981, especially in the barn and the adjacently situated scullery. In the barn, stored hay was probably the main source of Aspergillus. The concentration of Aspergillus spores decreased with increasing distance from the barn, indicating that the spores spread from the barn to the scullery and onwards to the kitchen and livingroom (Figs. 1

Table 2

Clinical features of the two patients with allergic bronchopulmonary aspergillosis

Patient	Year	Month	Blood eos./ mm^3	Total IgE u/ml	Specific IgE *A. fum.*	No. precipitins	Infiltrations on chest X-ray	FEV_1 % pred.	Medication
A	1980	June	726	2850	+++	7	yes	65	none
	1980	Sept.	88	2255	+++	7	minor	98	prednisolone 40 mg/day, cromoglycate, antibiotics
	1980	Nov.	66	857	++	5	no	98	prednisolone 7.5 mg/day
	1981	Jan.	187	437	++	4	no	95	prednisolone 7.5 mg every other day
	1981	March	319	350	++	3	no	100	idem.
	1981	June	231	288	++	1	no	98	hereafter prednisolone stopped
	1981	Sept.	22	315	++	3	no	N.D.	none
	1982	Febr.	79	220	+++	2	no	N.D.	none
	1982	May	143	204	+++	2	no	99	none
B	1979	April	671	27.000	++++	3	"fixed" shadows RUL	67	none
	1980	Dec.	704	>10.000	+++	4	idem	95	none
	1981	April	671	20.000	++++	5	idem	72	short course of steroids
	1981	Sept.	308	10.000	++++	5	some infiltrations right subhilar region	N.D.	none
	1981	Nov.	979	N.D.	N.D.	N.D.	N.D.		none
	1982	May	198	27.320	++++	5	as former	100	none
	1982	July	1562	21.331	+++	N.D.	idem	72	short course of steroids
	1982	Oct.	1089	28.678	+++	4	idem	97	none
	1982	Dec.	594	N.D.	N.D.	N.D.	some infiltrations RLL	57	short course of steroids
	1983	Febr.	759	20.675	+++	5	as former	90	none

FEV_1 = forced expiratory volume in the first second.
RUL = right upper lobe.
RLL = right lower lobe.
N.D. = not done.

and 5). In August 1981 the barn showed surprisingly low Aspergillus concentrations, whereas in the scullery they were very high. The bedroom, situated on the first floor, showed consistently low spore concentrations.

The outdoor counts of Aspergillus were, in general, much lower than the indoor, with the exception of October 28, 1981.

Clinical follow-up patient A

During the sampling period, pulmonary complaints were minimal with the exception of some audible ronchi and a cough in August 1981. Most peak flow recordings were within the normal range of 375–425 l/min (Fig. 6). There were, however, some days with lower scores,

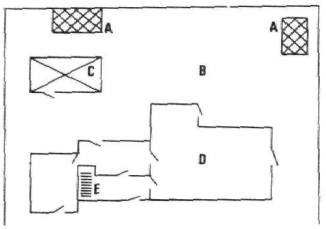

Fig. 3. Ground-plan house: patient B. A = compost heaps; B = outdoors; C = greenhouse; D = livingroom; E = stairs to bedroom first floor.

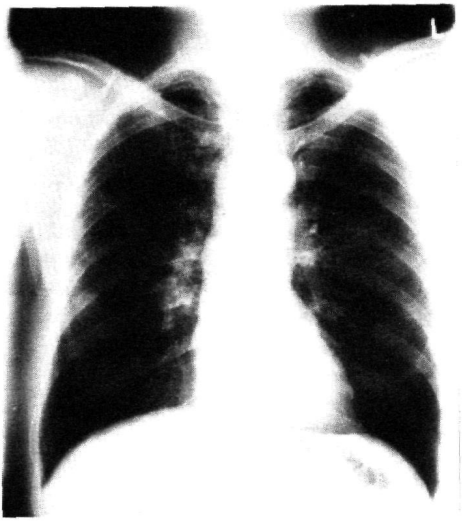

Fig. 4. Chest radiograph of patient B (April 1981), showing patchy infiltrations right apex, right hilum, left apex, with suggestion of central bronchiectasis left and right suprahilar region (ring shadow).

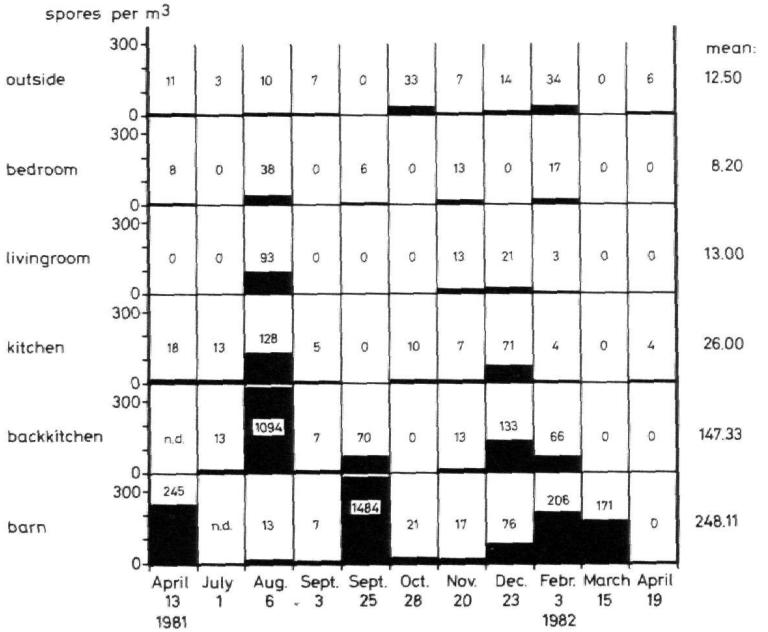

Fig. 5. Monthly figures of Aspergillus in the environment of patient A (September 1981 was measured two times), showing highest prevalence of fungus in the barn. Lower concentrations are noted with increasing distance from the barn.

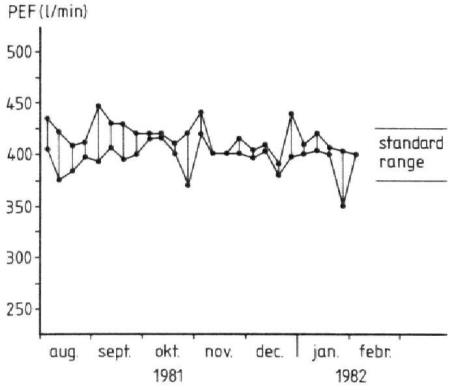

Fig. 6. Peak flow recordings of patient A during part of the aerobiological investigation. Registration per week. Upper line = each point represents the best day average of that week; lower line = the worst day average of that week.

e.g., August 16, 17, September 27 (all 1981), and January 23, 1982. Only cromoglycate and, occasionally, salbutamol inhalations were used in these periods. Corticosteroid medication, started September 1980, had been stopped in June 1981. Aspergillus spores were abundant in early August and at the end of September 1981 (Fig. 5), the lower peak flow rates at that time, however, were not associated with typical exacerbation of ABPA. There was no increase in eosinophils, total and specific IgE, or new infiltrations on her chest roentgenogram. Because of clinical improvement, peak flow recordings were stopped after January 1982. In May 1982, there were no symptoms and signs of active ABPA, except for a persisting specific IgE score of 3+. Shortly after May 1982 she moved to another part of the country.

Home situation patient B, aerobiological data

Aspergillus made up less than 1% of the total catch, with 904 colonies. In the outside air Aspergillus was most prevalent during autumn and winter; inside the house, especially in the living room, the fungus was present during most months of the year (Fig. 7). The overall concentrations were lowest during spring and early summer. In the greenhouse only three peak counts (March 1981, February 1982 and October 1982) were measured.

Clinical follow-up patient B

Peak flow recordings showed very low values in April-May 1981 (Fig. 8). Just previously, high concentrations of Aspergillus were found in the greenhouse (Fig. 7). At that time the criteria for diagnosing an exacerbation of ABPA were not met. The already elevated eosinophil count,

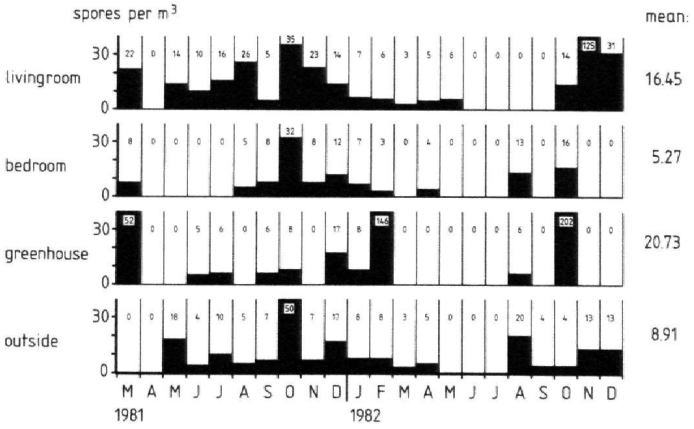

Fig. 7. Peak flow recordings of patient A during part of the aerobiological investigation. Registration per week. Upper line = each point represents the best day average of that week; lower line = the worst day average of that week.

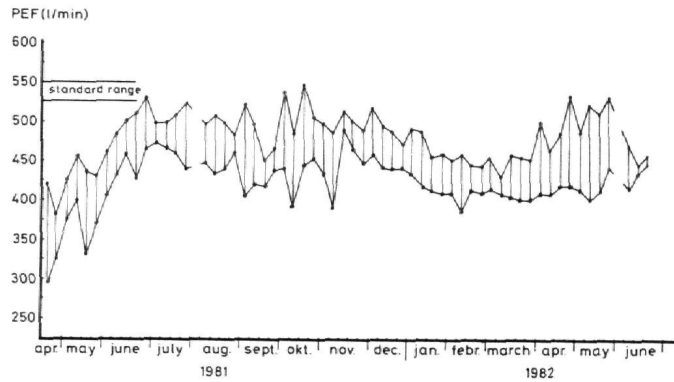

Fig. 8. Peak flow recordings of patient B during part of the aerobiological study. Registration per week. Upper line = each point represents the best day average of that week; lower line = the worst day average of that week.

total and specific IgE, and number of precipitins remained unchanged, and no new infiltrations were seen on the roentgenogram. Aspergillus was not cultured from the sputum. Thereafter, lower peak flow rates and higher Aspergillus counts were recorded occasionally, e.g. in May, October, and November 1981, and February 1982. At that time, the only clinical signs were some rales and a cough; there were no serological or radiological changes. The peak flow curves showed a slowly downward course between medio November 1981 and the end of March 1982 (Fig. 8). This period coincides with higher concentrations of Aspergillus spores in the outside air. The patient, however, did clinically well during this period. Productive cough with fever were present in October 1981 and May 1982, but without any further rise in serological parameters, or new infiltrations. PF recordings were stopped after June 1982 because of persistent well-being.

During the sampling period only three short courses with corticosteroids and an antibiotic were prescribed.

DISCUSSION

In this study an attempt was made to answer two questions, a), are high concentrations of Aspergillus spores found in the surroundings of patients with ABPA. and, b), is there a correlation between intensity of exposure to exogenous Aspergillus spores and exacerbation of the disease. In this pilot study, regular air sampling with an Andersen sampler was carried out in and around the dwellings of two patients with ABPA.

Adjacent to the house of patient A, a barn filled with hay proved to be the prime source of Aspergillus spores. This is in accordance with previous findings (3, 11, 14), in which particularly hay and straw contained many of these spores. A large peat stack next to the house, originally suspected to harbour large quantities of Aspergillus (12, 22), appeared to be of minor importance, showing mainly sterile mycelia. It is likely that the former unawareness of patient A about the mould source in the barn contributed to the development of her disease.

The surroundings of patient B showed no specific source of Aspergillus spores. Air sampling in the greenhouse did show two Aspergillus peaks, but mostly *Botrytis* and *Penicillium* were found here. In the garden, notwithstanding two compost heaps, only low spore concentrations were recorded.

It might be possible that the greenhouse and compost heaps had contributed to the development of his ABPA in the past. The livingroom of this patient showed fairly constant, but low, concentrations of Aspergillus spores, except in the autumn of 1981 and 1982. These findings

are in agreement with indoor mould studies of Kozak (10).

No definite answer can be given concerning the role of exogenous spore load for exacerbation of the disease. During the sample period from April 1981 to April 1982 patient A experienced no exacerbation, although rather high concentrations of Aspergillus spores, mainly in the barn, were found.

Before we started our study, this patient was not aware of the mould source in the barn. Since the results of the sampling became known, she scrupulously avoided the barn and the adjacent scullery. In August 1981 this patient had a cough and some rales were heard. It was in this period that there was an abundance of Aspergillus spores in the outside air, suggesing a relationship. However, no typical signs and symptoms of ABPA were found. Patient B also had no typical exacerbation during the sampling period from March 1981 to August 1982; Aspergillus, however, was frequently found at each sampling site, although never in high concentrations. It is possible that a peak concentration of spores was missed because sampling took place only 1 day a week, but his stable clinical condition makes this unlikely. The occasional worsening of his pulmonary condition (Fig. 8), sometimes accompanied by slight fever and cough, could have been evoked by Aspergillus spores, but no clear signs of ABPA were found.

That both patients did so well during the study could be explained by the following:

a. in the case of patient A, avoidance of the pertinent mould source in the barn;
b. the actually inhaled number of Aspergillus spores was too small to evoke overt ABPA exacerbation, at least in these two patients.
c. Species, other than the *A. fumigatus*, may, at times, have been more prevalent. Occasional species determination, however, did show fumigatus as the most prevailing fungus. Still, because of the non-existence of cross reactivity between the Aspergilli, this possibility must be borne in mind.

Our findings that the overall Aspergillus counts were low, except for the barn of patient A, are in good agreement with the figures of Vernon (24), who sampled the homes of urban dwellers with and without ABPA. Also Mullins (14) found no more than an average of 10 spores/m^3 in the air of Cardiff. That high concentrations of spores in barn, greenhouse and compost heaps, were responsible for the development of ABPA in these two patients, could not be proven but seems plausible. It is also plausible that the exacerbations the patients experienced before the sampling took place, were caused by inhaling high concentrations of spores in their direct neighbourhood. Otherwise, this study shows that the spore concentrations actually measured were not capable of illiciting exacerbation of ABPA. That some of the patients in Vernon's study (24) had exacerbations in periods of low spore concentrations, seems to indicate, either a difference in host susceptibility, or the possibility that peak concentrations were missed by these investigators.

Our finding that Aspergillus spores are found in the air especially during the winter months is in agreement with other studies (13, 14). However, although the fungus seems to be airborne more frequently in that season, the concentrations are not much higher than in the other seasons. In contrast to another study (13), our patients showed no disease activity in the winter months, possibly due to the reasons discussed before. The peak flow recording of patient B, however, showed a slight, but constant, decline from November 1981 until March 1982, possibly related to slowly increasing bronchial obstruction, caused by the persistent presence of Aspergillus spores in the outside air.

The absence of exacerbations in our patients was not likely to be due to medication. Patient A only used a small amount of prednisolone until June 1981, and patient B had only two short courses of corticosteroids, at the start of the sampling period.

The hypothesis that exacerbation of ABPA could originate endogenously, because of new fungus growth from persistent endobronchial spores or pieces of mycelium, could be neither proven or rejected by this study. The observation that in patient A all diagnostic criteria for ABPA, except a positive RAST, disappeared,

does not support the idea of endogenous persistence of the fungus.

Patient B showed Aspergillus in the sputum only once and no new pulmonary infiltrations were apparent. On the other hand, he persistently showed high total IgE, and specific antibodies against *A. fumigatus* (IgE and IgG, Table 2), which may be ascribed to the continuous presence of fungal spores in his indoor environment. However, some persistent fungus in the bronchial tree cannot be excluded.

In conclusion, the answer to question (a) can be "yes" in the case of patient A, and "probably" in patient B. As no real exacerbations of ABPA, according to the applied criteria, were found, evidence of an exogenous cause for these exacerbations (question (b)) can only be circumstantial.

Only continuous sampling can supply an answer to the question of how many spores are needed to bring about an exacerbation. Continuous viable sampling of Aspergillus, however, does not seem possible, while non-viable spore sampling, with morphologic recognition of Aspergillus spores, gives disappointing results (4). Possibly, newer immunochemical methods (19) for detecting Aspergillus allergens in the environment of ABPA patients could provide important information about exogenous allergen load, and disease behaviour in patients with ABPA.

ACKNOWLEDGEMENTS

We are grateful to Mrs L. Koese for her excellent technical assistance; Mrs U. Bosma and Mrs S. W. Elting for typing the manuscript; Dr. J. Franssen for studying one of his patients; the Centraalbureau voor Schimmelcultures in Baarn, the Netherlands, supervised the mycological data.

This study was supported by the Dutch Asthma Foundation, Leusden, The Netherlands (Grant No. 80–10).

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