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AEROBIOLOGY AND THE RELIEF OF ASTHMA IN AN ALPINE VALLEY

By

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INTRODUCTION

During the past decade, a steadily increasing number of British children with severe asthma have received treatment in Alpine sanatoria. Among these children, 110 with perennial asthma were sent to Davos on the advice of Dr. J. Morrison Smith at whose suggestion this study was undertaken.

Many patients with bronchial asthma are relieved of their symptoms in the high mountains. This has become increasingly recognised since *Storm van Leeuwen, Varekamp & Bien* (1924) took three asthmatic patients on a journey through Switzerland to study their condition at Basel, Lugano, Ragaz, Vulpera, Davos and St. Moritz, and at Davos provoked an attack of asthma in a symptom free patient by exposing him to dust from Holland. Among the factors advanced to explain the relief of bronchial asthma in the high mountains, *van Geuns* (1956) includes lowered O_2 concentrations and altered respiration, a changed psychical and social environment, and either a lower level or absence of bronchus irritants such as inhalant allergens. The present study is concerned only with this third factor and describes an investigation into the fungal spore and pollen content of the air of Davos (altitude 1,560 m) and its comparison with that in the air over Central London (altitude 29 m).

METHODS

A spore trap (Hirst 1952), which is volumetric and continuously recording, was installed on the flat roof of the Nederlands Sanatorium en Astmakliniek. The methods used were described by Davies, Denny & Newton (1963). The greased slides, prepared in this laboratory and sent to Davos in polythene staining jars with screw caps, were exposed for 24 hours each day from 10th June to 31st October 1966, and 8th May to 5th August 1967, and, after exposure, returned to London and mounted in glycerine jelly. They were scanned as previously described (Davies *et al.* 1963) and the mean concentrations for the pollens and spores trapped each day were calculated.

Records for Davos were compared with those obtained with a similar trap on a roof top at St. Mary's Hospital in Central London. Sampling from roof tops, at 20 and 25 m above ground level respectively, ensured that in each case the air flow was not obstructed by adjacent buildings and the catches would typify a wide area and be unbiased by pollen or spore sources in the immediate locality.

POLLENS

The tree pollens which are airborne in early spring were not sampled, but in comparison with the pollen of the *Gramineae* they are relatively unimportant as allergens. The mean daily concentrations of grass pollen in the air at Davos and London in 1966 and 1967 are shown in Figs. 1 and 2. Sensitivity to grass pollen varies from that of the patients with pollen asthma, who experiences symptoms from concentrations as low as 1 grain/m³, to that of patients with hay fever who may be symptom free when exposed to concentrations of 20–30 grains/m³. However, since it has been found (Frankland & Davies, unpublished observations) that when concentrations over Central London rise to 50 grains/m³ or more, all patients in the area who are clinically sensitive to grass pollen experience pollinosis, a base line of 50 grains/m³ has been drawn in Figs. 1 and 2. Days when the mean concentration of grass pollen rises to 50 grains/m³ or more may be described as one hundred per cent symptom days, and in 1966 during the periods studied there were 31 such days in London and 24 in Davos. In 1967 the slides exposed in Davos from 17th–25th June disappeared in transit, but taking comparable

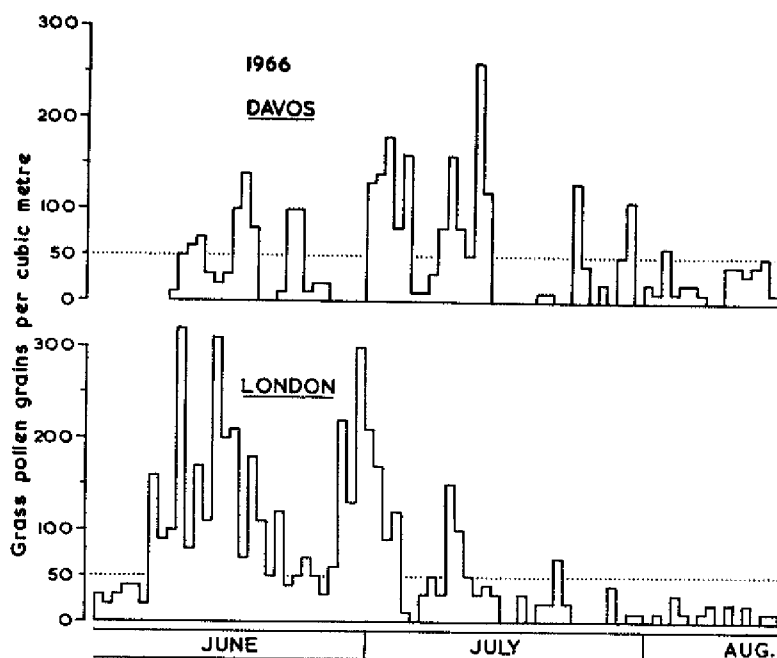


Fig. 1.

Daily concentrations of grass pollen grains in the air at Davos and London in 1966.

days only there were 5 one hundred per cent symptom days in Davos and 10 in London. Comparative data including the numbers of days when grass pollen was recorded at both sites is given in Table 1. On the basis of this evidence patients with pollen asthma would have been exposed to a similar challenge with grass pollens in Davos as in London in 1966, but would have been exposed to a reduced challenge in 1967.

Fungal spores. Only a few representative of the many types of fungal spore commonly trapped from the air will be considered in detail.

Cladosporium. The spores of this genus are ubiquitous and have been recorded in the atmosphere from the N. pole (Pady & Kapica 1953) to the extra tropical desert of Arabia (Davies

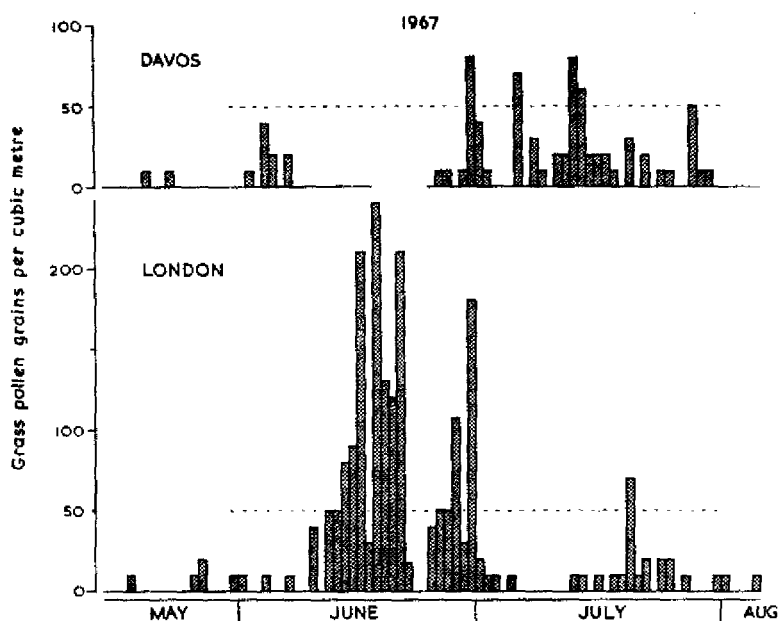


Fig. 2.

Daily concentrations of grass pollen grains in the air at Davos and London in 1967.

1969). Sensitivity to *Cladosporium* was first described by Cobe (1932). Hyde, Richards & Williams (1956) who tested 627 patients by injecting 0.02 ml. of a 1/100 extract reported 8 per cent gave positive reactions and that 4 per cent were clinically sensitive to this mould because their seasonal asthma occurred in the summer when the spores are most abundant in the air. Since Frankland & Davies (1965) report that in patients with hypersensitivity to this spore, symptoms are precipitated when the mean daily concentrations above central London rise to 3,000 or more /m³, concentrations at this level and above are given in Figs. 3 and 4. On this basis, in the periods studied clinically significant concentrations were recorded on 41 days in London and 6 in Davos in 1966, and 26 in London and 2 in Davos in 1967. Clearly, patients whose

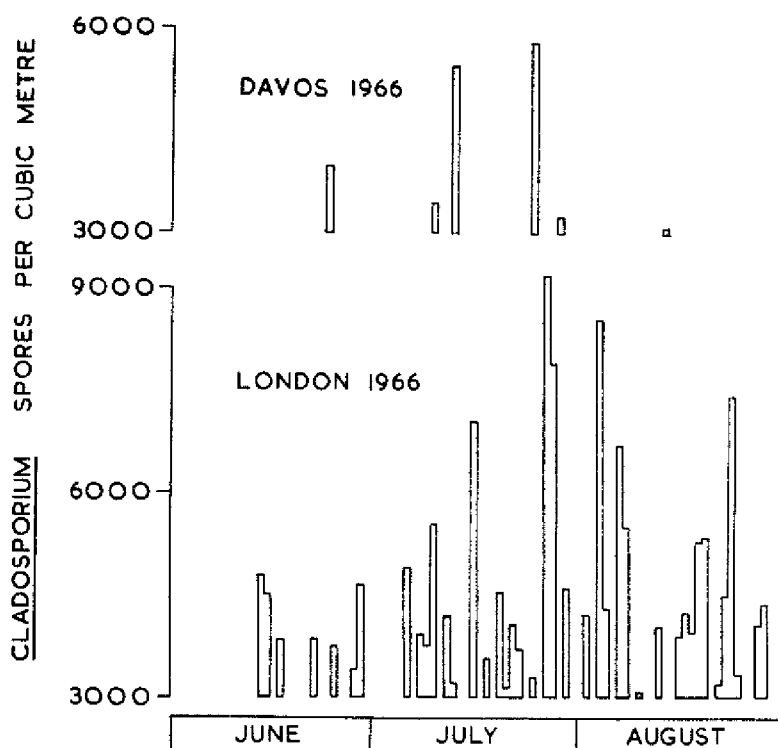


Fig. 3.

Daily concentrations of *Cladosporium* spores above 3000/m³ in the air at Davos and London in 1966.

TABLE 1

Mean Daily Concentrations and Numbers of Days Grass Pollen Recorded.

	1966		1967	
	London	Davos	London	Davos
Number of days no grass pollen recorded	14	15	18	41
Number of days grass pollen trapped	52	51	52	29
% days grass pollen trapped	79	77	74	41
Mean daily concentration (grains/m ³) for the period	56	45	35	10

Cladosporium spores 1967

Concentration in thousands per cubic metre

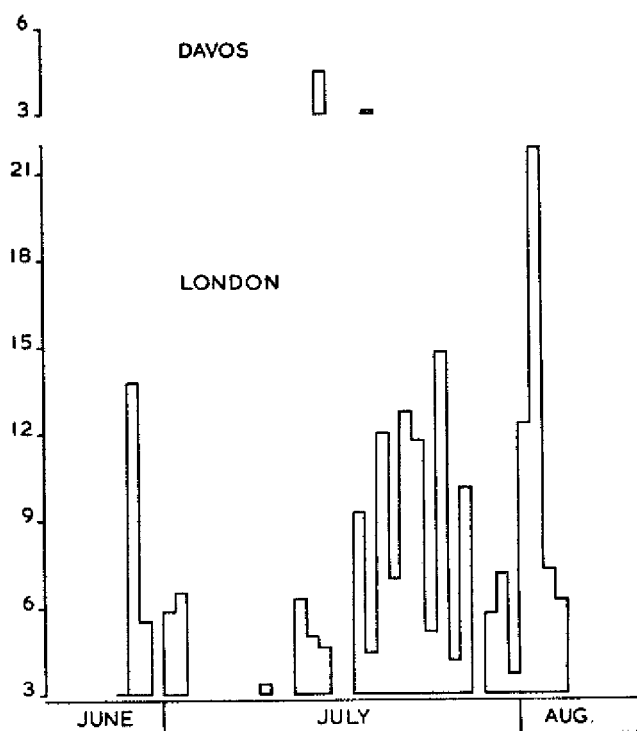


Fig. 4.

Daily concentrations of *Cladosporium* spores above 3000/m³ in the air at Davos and London in 1967.

sole sensitivity is the spores of this genus should be improved or relatively free from asthma in Davos.

Alternaria. Asthma due to this fungus was first described by Hopkins, Benham & Kesten (1930). Sensitivity to *Alternaria* is frequently present in patients allergic to *Cladosporium*. Frankland & Davies (1965) reported its clinical importance in Britain to be equal to that of the *Penicillia* and second only

TABLE 2

Alternaria, Concentrations and Days Trapped as a Percentage of the Total Number of Days Sampled each Month.

1966	Monthly average concentration spores/m ³		Number of days trapped		% of days trapped	
	Davos	London	Davos	London	Davos	London
June	5	15	6	14	28	67
July	8	32	6	24	51	78
August	10	125	12	29	39	94
September	3	30	8	22	3	73
October	0	4	0	8	0	38
1967						
June	1	4	3	7	14	33
July	4	39	7	25	22	81

to *Cladosporium* as a mould allergen. The results for this mould are summarised in Table 2.

During comparable periods of the appropriate seasons for *Alternaria*, the spores were trapped on 42 days in Davos and 129 days in London; concentrations of 50 spores/m³ or more were recorded on 4 days in Davos and 49 days in London. On only one day did the concentration reach 100 spores/m³ in Davos, whilst in London concentrations of 100 or more were recorded on 25 occasions.

Ustilago is an obligate plant pathogen and the cause of "Smut" in cereal crops. The chlamydospores of *U. nuda* are abundant in the air of Central London in June and July. The numbers trapped appear to have increased during the past few years and this may be associated with the increased area of Britain devoted to barley growing. Up to the present, however, this observation has been difficult to confirm because weather factors are important and highest concentrations are recorded in dry weather when the wind is from E. Anglia, an important cereal growing region. The suggestion that Smuts may be worthy of investigation as allergens was made by *Feinberg* (1949) in the U.S.A. since he found that patients quite fre-

quently gave positive skin tests to *U. zeae*. Positive prick tests to *U. nuda* in patients with clinical histories of asthma when the spores occur in the air have been reported by Frankland & McEwen (personal communications), but since no lung provocation tests have been performed in such patients *Ustilago* cannot be reported as an inhalant allergen with certainty. Comparable data for Davos and London is shown in Table 3.

TABLE 3
Ustilago, Concentrations and Days Trapped as a Percentage of the Total Number of Days Sampled each Month.

1966	Monthly average concentration spores/m ³		Number of days trapped		% of days trapped	
	Davos	London	Davos	London	Davos	London
June	73	1,512	19	20	90	95
July	57	224	21	26	67	84
August	51	8	18	14	58	45
1967						
May	5	6	5	6	20	25
June	31	610	13	20	59	87
July	189	368	29	28	94	90

In Davos, the maximum concentration of *Ustilago* in 1966 was 330 spores/m³ on 30th July, in London the peak concentration was 7,720 spores/m³ on 11th June, and on 12 days concentrations greater than 1,000 spores/m³ were recorded. In comparable periods of 1967, the peak concentrations of *Ustilago* at Davos and London respectively were 2,140 spores/m³ 9th July and 5,670 spores/m³ 26th June. Concentrations greater than 1,000 spores/m³ were recorded on one day in Davos and seven days in London.

Ascospores. The Ascomycetes comprise the largest group in the fungi and are characterised by the production of ascospores in special cells termed asci. In most the ascospores are explosively discharged, "squirted out" from the ascus by a mechanism described in detail by Ingold (1967). Highest con-

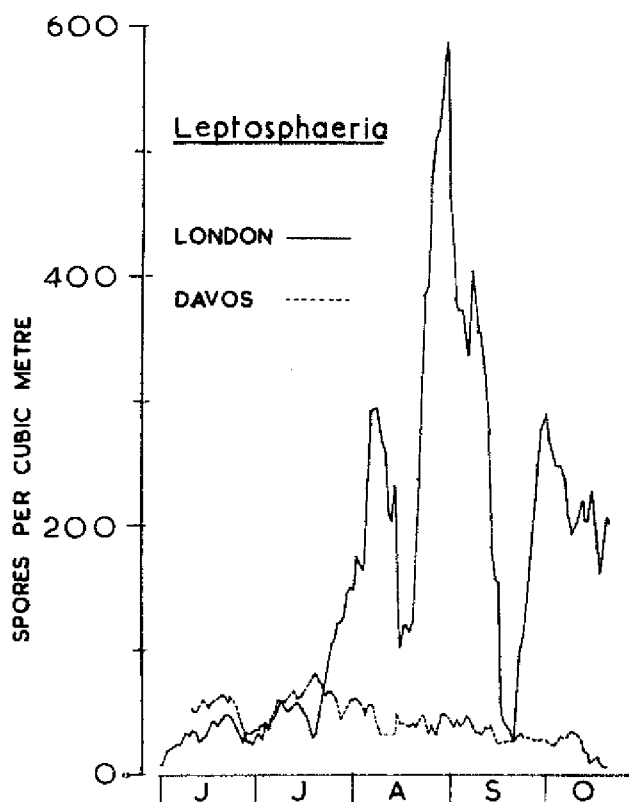


Fig. 5.

Concentrations of *Leptosphaeria* ascospores in the air at Davos and London in 1966, data presented as a 10 day continuous mean.

centrations are recorded during damp weather in the summer and autumn. Details of genera with morphologically distinct ascospores which are commonly trapped from the air in Britain are given by *Davies et al.* (1963). The role of ascospores in the aetiology of seasonal allergies has been scarcely investigated. Ascospores recognisable in deposits trapped from the air have either not yet been grown in artificial culture, or if developing in artificial culture are attributed to well known genera of the fungi imperfecti whose relationships to perfect

states have yet to be established. For example, *Leptosphaeria acuta* (Hoffm. ex Fries) Karst grows on the lower stem regions of the common nettle *Urtica dioica*. Linn. and produces yellow/brown ascospores which are fusiform and septate and characterise the perfect form of this fungus. When portions of nettle stem are planted in agar media, the fungus that develops is the imperfect form *Phoma acuta* which produces simple hyaline spores in flask shaped structures termed pycnidia. Since Ganderton (1968) has obtained positive prick tests with an extract of *Phoma* in patients with a clinical history of seasonal allergy when *Leptosphaeria* spores occurred in the air, data for *Leptosphaeria* is given in Table 5 and Fig. 5 and total ascospores in Table 4.

TABLE 4

Total Ascospores, Concentrations and Days Trapped as a Percentage of the Total Number of Days Sampled each Month.

1966	Monthly average concentration spores/m ³		Number of days trapped		Percentage of days trapped	
	Davos	London	Davos	London	Davos	London
June	139	68	21	21	100	100
July	183	101	31	31	100	94
August	244	429	31	31	97	100
September	121	314	30	29	100	97
October	126	385	26	26	81	100
1967						
June	95	25	22	17	100	77
July	197	100	31	25	100	81

In June and July, ascospore concentrations at Davos were higher than in London, but from August onwards higher concentrations were recorded in London. This may be explained by the facts that the ascomycetes occur on vegetation, the ascospores trapped in Davos have local origins, whereas in Central London most sources lie over 10 kilometers away and spore clouds are increasingly diluted with distance from the source. The higher concentrations recorded in London in the

late summer are partly related to higher temperatures and to the higher catches of *Leptosphaeria*.

TABLE 5

Leptosphaeria, Concentrations and Days Trapped as a Percentage of the Total Numbers of Days Sampled each Month.

1966	Monthly average concentration spores/m ³		Number of days trapped		% of days trapped	
	Davos	London	Davos	London	Davos	London
June	55	40	19	21	90	100
July	54	54	29	25	94	81
August	45	246	29	31	94	100
September	35	251	29	27	97	90
October	20	159	26	26	84	100
1967						
June	45	15	22	15	82	68
July	73	12	28	14	90	45

In 1966 maximum ascospore concentrations at Davos and London respectively were 650 spores/m³ on 3rd August and 1,850 spores/m³ on 29th August. In London concentrations greater than 1,000 spores/m³ were recorded on 8 days. Concentrations greater than 500 spores/m³ were sampled on 3 days in Davos and 23 in London.

Leptosphaeria. As with total ascospores, in June and July concentrations of *Leptosphaeria* in the air of Davos were higher than in London. From August onwards higher *Leptosphaeria* concentrations occurred in London as shown in Fig. 5. In 1966 maximum *Leptosphaeria* catches were 170 spores/m³ on 23rd August in Davos, and 1,220 spores/m³ on 29th August in London. Concentrations greater than 500 spores/m³ were recorded on 8 days in London.

In the spore clouds over Central London during the summer and autumn, the predominant species of *Leptosphaeria* is *L. eustoma* (Fries) Sacc. which has its origin on cereal crops. High concentrations of *L. eustoma* immediately above wheat

and especially above barley were reported by *Last* (1955). Crops of barley are grown within 12 kilometers of Central London and the peak concentrations of this spore in late summer and autumn (see Fig. 5) may first be associated with cereal harvesting and secondly with the development of *Lep-tosphaeria* on the stubble. Since Davos is situated in the high Alps, it is not only further away from arable land devoted to cereal growing than Central London, but lying in a deep valley is sheltered from the spore clouds borne by the prevailing winds. Furthermore, since spore clouds are diluted with increasing height and distance from the source, it is scarcely surprising that fungi associated with cereal crops such as *L. eustoma*, *U. nuda*, and *Alternaria* and *Cladosporium* occur in lower concentrations at Davos than in London.

Basidiospores. These spores, produced by the toadstools and bracket fungi of the Basidiomycetes are regularly encountered in the atmosphere and in London occur in high concentration between August and October. *Feinberg* (1949) considered basidiospores worthy of investigation as allergens because he obtained positive skin reactions with extracts from toadstools. Clinical sensitivity to the spores of a basidiomycete those of the dry rot fungus (*Merulius*) *Serpula lacrymans* was first reported by *Frankland & Hay* (1951). The occurrence of basidiospores in the air in high concentration was reported by *Gregory & Hirst* (1952) who also suggested they should be investigated as a cause of seasonal asthma. *Bruce* (1963), by skin test and respiratory provocation, demonstrated sensitivity to basidiospore mixtures containing either *Polyporus giganteus*, *Ganoderma applanatum* and *Stereum hirsutum*, or *Polyporus betulinus*, *Polystictus versicolor*, *Fomes annosus* and *Armillaria mellea*. Recently, *Herxheimer, Hyde & Williams* (1966) by skin-testing (intradermally with extracts 1: 1,000 W/V) and by inhalation tests, have clearly shown clinical sensitivity to the spores of individual basidiomycetes viz: *Agaricus bisporus*, *Armillaria mellea*, *Coprinus micaceus*, *Ganoderma applanatum*, *Hypholoma fasciculare* and *Polyporus squamosus*.

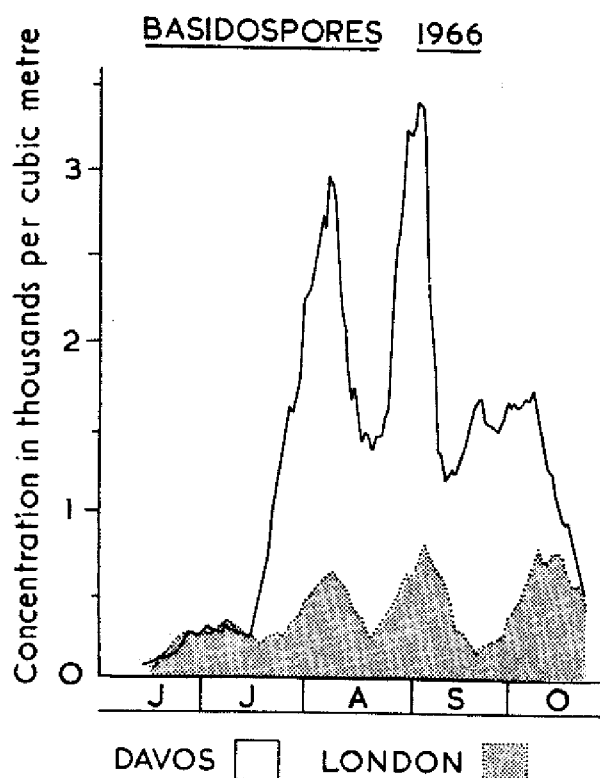


Fig. 6.

Basidiospores, total concentrations in 1966 plotted as a 10 day continuous mean.

Although a number of basidiospores have distinct features and may be assigned to genera in the Hymenomycetes, in the present study the spores were simply classified as hyaline or coloured and those of the genus *Sporobolomyces* and the Hemi-basidiomycetes are excluded. The results for the two sites are given in Table 6 and Fig. 6.

The maximum basidiospore concentrations recorded were 6,210 spores/m³ at Davos on 4th September and 1,170 spores/m³ on 7th October in London. Concentrations of more than 1,000 spores/m³ were recorded on 71 days in Davos and 7 in London. Basidiospores comprised 59 per cent of the total

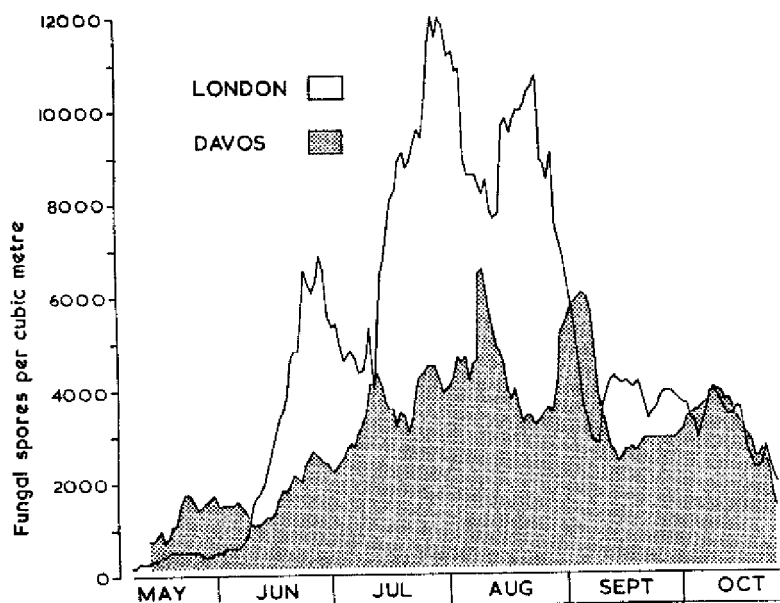


Fig. 7.

Total spore concentrations at Davos and London plotted as a 10 day continuous mean.

spores trapped in Davos, but only 12 per cent of the total sampled in London. It should be noted that the maximum basidiospore concentrations occurred earlier in the year at Davos than in London, and in 1966 the year of this study, the total catch in London was only 59 per cent of the mean

TABLE 6

Total Basidiospores, Concentrations and Days Trapped as a Percentage of the Total Number of Days Sampled each Month.

1966	Davos	London	Davos	London	Davos	London
June	127	158	21	21	100	100
July	589	263	31	31	100	100
August	2,838	442	31	31	100	100
September	2,662	452	30	30	100	100
October	1,541	566	26	29	100	100

for the years 1960–1967 inclusively. Hyaline basidiospores accounted for 69 per cent of the basidiospores trapped at Davos, and 21 per cent of those trapped in London.

Total Spores. The mean concentrations for the total spores trapped daily at each site in 1966 is given as a ten day continuous mean (Fig. 7) so that changes in the curves due to minor weather fluctuations are smoothed. Peak concentrations occurred earlier in the year at London than in Davos. The total spore catch at Davos was 71 per cent of that trapped in London, but if the basidiospores are excluded the Davos trap recorded 39 per cent of the spores trapped in London.

DISCUSSION

In a study into the content of the air at Basel and Samaden (near St. Moritz), *Wortmann* (1955) recorded a greater deposition of mould fungi and yeasts in Petri-dishes exposed at Basel. The maximum catch at Basel was obtained in July, at Samaden in August. In the present study maximum concentrations for total fungal spores and grass pollen (Figs. 1, 2 and 7) were obtained earlier at London than in Davos. The Basel exposures recorded three times as many *Cladosporium* colonies as were trapped at Samaden, and far higher *Cladosporium* concentrations were recorded in London than at Davos (Figs. 3 and 4). *Wortmann* trapped colonies of *Penicillium* in equal numbers at both sites, but found *Aspergillus* was twice as common at Samaden. Since *Gregory & Stedman* (1953) report the horizontally exposed Petri-dish has a low trapping efficiency, and in view of the importance of *Aspergillus fumigatus* in the aetiology of bronchopulmonary Aspergillosis (*Hinson, Moon & Plummer* 1952, *Frankland & Hamilton* 1958, *Pepys et al.* 1959, *Longbottom & Pepys* 1964, *Pepys* 1969) this result requires investigation with volumetric sampling equipment such as the multistage liquid impinger (*May* 1966) or the Andersen sampler (1958). Particles larger than spheres of 7 μ diameter and unit density are de-

posited in the upper respiratory tract, fifty per cent of those of 5 μ diameter reach the alveoli of the lung (Davies 1963, Druett 1967). Since the Hirst trap employed in this study has a retentivity for unit density spheres of one hundred per cent for particles greater than 7 μ diameter, and fifty per cent for particles of 4 μ diameter, it simulates the upper respiratory tract in which the naturally inhaled pollens and larger fungal spores are deposited and produce allergic symptoms, and like the upper respiratory tract retains less than fifty per cent of the morphologically indistinguishable spores of *Aspergillus* and *Penicillium* which may penetrate to the alveoli. Antigenic particles reaching the alveoli tend to produce alveolitis rather than asthma (Pepys 1969). "Spot sampling" at a set time each day may fail to record spore clouds due to variations in diurnal periodicity arising from variable weather factors. The Hirst trap has the advantages that it is a continuous sampler, it is quantitative, intake approximates to the isokinetic and it records the total air-spores including Pollens, Ascospores and Basidiospores regardless of viability.

The grass pollen concentrations at Davos are sufficient to produce symptoms of pollinosis, but whereas in Central London most of the pollen originates to the West and South West of the conurbation, in Davos the lower slopes of the adjacent mountains are grassed. Higher up the slopes and over much of the area coniferous forest occurs and here lies the source of basidiospores. The importance of woodlands as a source of basidiospores has been demonstrated by Adams, Hyde & Williams (1968) who report more than 75 per cent of the Hymenomycetes are tree-loving and 8 per cent are more or less exclusively associated with coniferous trees. The Alpine valley has an air-spore of its own which reflects the ecology of the valley.

In the selection of patients for treatment in Alpine sanatoria, sensitivity to basidiospores should be noted since, from the evidence described, they will occur in high concentration in Alpine valleys. On the other hand, patients with sensitivities to spores such as *Cladosporium*, *Alternaria*, *Ustilago*

and ascospores, including *Leptosphaeria*, should find a salubrious environment.

Finally, it must be noted that although regional change can benefit the allergic patient, who is either free from attacks or improved in the allergen reduced environment, his ability to produce antibodies remains. Even a temporary respite can be invaluable to the severely asthmatic child, but with adults Storm van Leeuwen (1924) reports hypersensitivity may be unchanged after six to ten years in an allergen free environment, and "... a complete absence of allergens may even set up a temporarily increased hypersensitiveness, as a degree of resistance present may be lost owing to the absence of any reaction."

SUMMARY

1. The pollen and fungal spore content of the air at Davos and London was studied by means of continuously recording spore traps in 1966 and 1967.

2. Grass pollen concentrations tended to be lower in Davos than in London. During comparable periods, mean daily concentrations greater than 50 grains/m³ were recorded on 29 days in Davos and 41 in London.

3. *Cladosporium* spores were trapped in clinically significant concentrations on only 8 days in Davos in comparison with 67 days in London.

4. Concentrations of Total Fungal Spores, *Alternaria*, *Ustilago*, Ascospores and *Leptosphaeria* were all much lower in Davos than in London.

5. High Basidiospore concentrations at Davos were associated with local coniferous forest. Mean daily concentrations for Basidiospores were greater than 10³/m³ on 71 days in Davos and 7 in London.

6. Patients with sensitivities to fungal spores other than those of the Basidiomycetes, should gain some relief from their symptoms at Davos.

7. Patients with grass pollen asthma are exposed to clinically significant concentrations of grass pollen at Davos.

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