

Atmospheric pollen grains and spores in relation to allergy. II

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The first article in the present series (Hyde, 1972) dealt with general considerations, Thommen's postulates were restated in the light of recent knowledge; the principal sources of airborne pollen grains and spores were summarized with special reference, in accordance with the trend of modern research, to the latter; and technical methods of pollen and spore survey were briefly but critically reviewed.

Further, the general British aerospora, its particular sources and its composition and phenology were described.

The present communication is concerned with diurnal (circadian) periodicity in the concentrations of various kinds of pollen grains and fungal spores outdoors during their respective seasons and with the indoor aerospora of houses, hospitals and certain industrial locations, and the problem of the distant transport of pollen grains and spores and its possible significance for allergy is discussed in the light of numerous observations in various parts of the world.

1. Diurnal ('circadian') periodicity

The 'daily count' of a particular type of spore as usually recorded is a measure of its mean 24 hr concentration on the day concerned: as Blackley (1873) long ago observed, the actual concentrations obtaining from hour to hour may be much greater or much less than the 'daily' figure indicates. Rempe (1937) made some observations on diurnal periodicity in cereal and tree pollens. Studies in the diurnal variation of grass and plantain pollen were made at Cardiff in 1943–45 (Hyde & Williams, 1945, 1946). The introduction of the Hirst trap (Hirst, 1952) gave a great impetus to research on the diurnal periodicity of both pollen grains and fungus spores; many such studies have since been made in England by Gregory and his associates (Gregory, 1952a, b; Hirst, 1953; Gregory & Stedman, 1958; Gregory & Sreeramulu, 1958; Hamilton, 1959a, b; Lacey, 1962; Sreeramulu, 1958a, b, 1959, 1962, 1963, 1964) and more recently in India (Sreeramulu, 1961; Sreeramulu & Ramalingam, 1962, 1963, 1964a, b, 1966; Sreeramulu & Seshavataram, 1962). Meredith (1961, 1962, 1964) has done somewhat similar work in Jamaica. Pady and his collaborators have investigated the

performance of various groups of fungi under controlled conditions as well as in the field (Pady, Kramer & Wiley, 1963; Kramer & Long, 1970; Haard & Kramer, 1970; Pady *et al.*, 1971; Pady & Kramer, 1971; Pady, 1971). Further observations have also been made by Adams (1964) and by Harvey and Walkey in Cardiff. General aspects of the subject have been dealt with by Hirst (1959), Ingold (1960, 1965, 1966) and Gregory (1961, 1966).

During fine weather in Southern Britain, each type of pollen or spore in season tends to be at its peak in the atmosphere at a particular time of day or night; it is not unusual for the hourly grass pollen concentration in central Cardiff to rise and fall to the extent of one hundredfold (viz from 10 to 1000 grains and back per m^3) in the course of 24 hr, and those of several kinds of tree pollen and of *Cladosporium* and *Sporobolomyces* spores may vary even more widely. These fluctuations are further reflected in the differences between some of the highest 24 hr mean counts and the highest hourly or 2-hourly counts registered in central Cardiff, e.g. grass pollen $2.9 \times 10^3/\text{m}^3$ and 9×10^3 respectively, nettle pollen (750 and 2×10^3), elm pollen (1.03×10^3 and 2.5×10^3) and *Cladosporium* (31.5×10^3) and 62×10^3). It is possible to distinguish broadly between the day spora and the night spora (Hirst, 1953; Gregory, 1961). The day spora at Cardiff includes birch, pine, *Polythrincium*, oak, ash, *Artemisia*, *Erysiphe*, *Torula* and plantain (*Plantago*) which reach their respective maxima during the morning hours from 08.00 hours onwards; *Botrytis*, *Cladosporium*, *Epicoccum* and *Pullularia* from noon onwards; elm and *Alternaria* in the early afternoon; and grasses (their principal peak see below) and nettle in late afternoon or early evening; some of the spring-flowering trees (elm, ash, oak) have been observed to reach their maxima towards midnight. The night spora proper comprises *Sporobolomyces*, *Tilletiopsis*, ascospores (both coloured and hyaline) and basidiospores (coloured and hyaline). Peak values in many instances are attained rapidly and are followed by a more leisurely fall in concentration. In some types they occur with almost unfailing regularity at the same hour every day but in others may come at different times. Some common grasses shed their pollen in the morning and so may bring about a marked morning sub-maximum; on sunny days the principal grass pollen maximum comes in the late afternoon, but this periodicity is broken if the sun remains even only overcast (Hyde & Williams, 1945). The complementary behaviour of representative members of the day and night spora respectively is exemplified by *Cladosporium* and *Sporobolomyces*. Though most observers are broadly in agreement on peak times in southern Britain some marked differences have been noted. Thus the average peak time for *Urtica* observed at Cardiff in 1955, viz 17.00 hours, was the same as at London in 1954 (Hamilton, 1959a) but Lacey (1962) working in Berkshire observed an average peak at 12.00–13.00 hours; and again Gregory & Stedman (1958) found that in late autumn the normal diurnal periodicity of coloured basidiospores was reversed, the peak time coming in the afternoon, not, as previously observed, in the early morning; this was perhaps a temperature effect. Pawsey & Heath (1964) noted a double daytime maximum of *Cladosporium* at 14.00 and 18.00 hours, thus confirming a previous observation by Gregory & Stedman (1958); Harvey (1967) also observed a double maximum pattern. In experiments at Cardiff peak concentrations of grass pollen in the general atmosphere coincided with times of maximum pollen release (Hyde & Williams, 1945); similarly in Berkshire with the basidiospores of *Ganoderma applanatum* (Sreeramulu, 1963); and probably much the same applies generally to places not too far from the main source(s) of supply, but

with a longer or shorter time lag according to distance (cf. Ogden, Raynor & Hayes, 1966), though the possible effect of periodic rises and falls in atmospheric turbulence on local spore concentration cannot be ruled out. Much work has been done in recent years on spore release in fungi in relation to external conditions (Ingold, 1965, 1966; Walkey & Harvey, 1967).

The normal 24 hr spore sequence in Britain is liable to be interrupted by rain; this may cause anthers to close and may also forestall emission by washing it off the flowers; either way, pollen is kept from being airborne. Spores already in the air are collected and carried down by falling rain; the degree to which the air is consequently scrubbed clean depends on the size both of the spores and of the raindrops and on the amount of rain which falls (Hirst, 1959). Most pollen grains are large enough to be washed out by all but the lightest showers of large (thunder) drops. Smaller spores are removed at lower rates. The onset of rain after fine weather may occasion a transient rise in the numbers of some dry weather types including *Cladosporium* (Hirst & Stedman, 1963) but the effect of prolonged rain is that the daytime spora disappears from the air; and during continuous damp weather *Sporobolomyces* and some other members of the night spora occur in abundance in the daytime also (Gregory, 1961; Hirst, 1953; McDonald, 1962).

The peak hours for various pollen types observed in southern Britain presumably apply elsewhere in north-west Europe. In Germany rye pollen (*Secale cereale*) has been observed at its maximum between 06.00 and 08.00 hours, hazel between 10.00 hours and noon, and alder and other trees about 14.00–16.00 hours (Rempe, 1937); and the peak time of day for birch (*Betula*) pollen near Helsinki, Finland, normally comes between 12.00 and 20.00 hours (Sarvas, 1952).

Outside Europe little is known about diurnal periodicity in pollen types other than those which occur in Britain. In Nebraska, Mexican firebush (*Kochia scoparia*) is at its maximum at 10.30 hours, and two species of ragweed (*Ambrosia trifida* and *A. psilostachya*) between 07.00 and 13.00 hours (Jones, 1952). In Iowa City the critical time of day for exposure to ragweed pollen is 09.00–13.00 hours (Smith & Rooks, 1954). Later observations in New York State showed a peak of ragweed pollen (*A. artemisiaefolia*) near the plants around 08.00–09.00 hours and at a distance an hour later (Ogden & Hayes, 1969). In regard to fungi, there is evidence that climate may affect diurnal spore-periodicity.

Thus in Maryland, U.S.A., *Cladosporium* and *Epicoccum* (both members of our day spora) are nocturnal (Panzer, Tullis & Van Arsdell, 1957); at Manhattan, Kansas, while basidiospores and ascospores are more abundant by night than by day and rusts and smuts vice versa, most other types of fungus spore were reported to behave inconclusively; but later work has demonstrated the general day and night pattern now familiar in Britain (Pady, Kramer & Clarry, 1967; Pady & Kramer, 1967a, b). Ogden & Hayes (1969) found that on Long Island, N.Y., *Ambrosia*, *Phleum*, *Zea* and *Ricinus* all exhibited characteristic daytime patterns of pollen emission. On the other hand, in the air over paddy fields in India, *Cladosporium* and various other moulds belong, as in England, unambiguously to the day spora and basidiospores and ascospores are nocturnal (Sreeramulu & Seshavataram, 1962; Sreeramulu & Ramalingam, 1964a, b). In Nigeria, *Cladosporium* and certain other types of spore, which during the rainy season are at a maximum there (as in Britain) in the afternoon, reach a daily peak during the dry season before noon (Cammack, 1955). In Jamaica, *Nigrospora* is at a peak at 08.00 hours and *Cladosporium* and *Alternaria* toward or soon after

midday while ascospores (especially *Leptosphaeria*) and basidiospores are most abundant during the night (Meredith, 1961, 1962, 1964).

Rice false smut (*Ustilaginoidea virens*) in Waltair, India, has a marked daily spore peak at the unusual time of 22.00 hours; atmospheric concentrations 1 m a.g.l. in a rice plot reached 328,000 spores/m³ (60 min mean) (Sreeramulu & Vittal, 1966). The various discrepancies referred to above may be in part due to the relative importance of the distant component of the spore catch as compared with the local one at the station concerned: this cannot as yet be assessed (Hirst, Stedman & Hurst, 1967b). But spore free Atlantic air, sweeping across Great Britain as it often does, must create a situation different from that which obtains, say, in the American Middle West, where previous 'fetch' of wind may at times bring in dense clouds of pollen and spores which may mask the effects of local pollen liberation (cf. Durham, 1938).

In general the dry weather day spora seems to comprise all those spore types (including pollens) which mature and are released under dry conditions and the night spora whose release is dependent on humidity: the apparent exceptions to this will probably be explained by further study.

The fact of diurnal periodicity is sufficient proof that spore sampling to be adequate must be continuous throughout the 24 hr. Diurnal variations in atmospheric spore content must affect patient's symptoms: if they can be forecast with any degree of probability, patients whose condition has been diagnosed as due to a particular kind of spore may be advised to take the appropriate steps to avoid exposure at the most dangerous hours. Patients often complain of symptoms occurring at particular times of day or in certain states of weather: conversely therefore, consideration of these observations in the light of diurnal spore periodicities may sometimes assist diagnosis.

2. Pollen grains and fungus spores indoors

(a) In houses and hospitals

The danger of inhaling pollen emitted from plants normally grown indoors is negligible: none of the popular house plants is anemophilous. The little pollen liberated by cut flowers (including those of the Compositae) is coherent and does not become airborne but can sometimes be seen to collect on surfaces immediately beneath the flowers. Pollen wafted in from outside may be another matter. The numerous attempts which have been made to filter out pollen (and spores) from the air entering patients' houses were reviewed by Rooks, Caplan & Shapiro (1956). The amount of ragweed pollen, as determined by gravity slide tests, present in the air of hospital rooms in Chicago, which had been fitted with one or other of two types of filter, varied between 2 and 3% of that outdoors (Unger & Myers, 1961). Air conditioning reduced the ragweed pollen content of a hospital room in Philadelphia to 2.5% of that outdoors and the addition of an air purifier made little difference; mould counts in an air-conditioned room were 5.5% of those in a room with open windows, and again the presence or absence of an air purifier made no significant difference (Spiegelmann, Blumstein & Friedman, 1961; Spiegelman, Friedman & Blumstein, 1963). More ambitious claims made for other apparatus apparently await investigation. Meanwhile we have found that the average grass pollen concentration over several days in a closed room in Cardiff was less than 2% of that on the roof of a high building near a public park; in other rooms and corridors adjacent to the closed room, with their doors and

windows open, the quantities of pollen deposited on gravity slides were in some instances comparable with those deposited outdoors. In another experiment the average pollen concentration over 41 days in a closed indoor room was 1.3%, and that of fungus spores over 68 days 1.2%, of the corresponding concentrations on the roof of the same building (Adams & Hyde, 1965). Grass-pollen hay fever subjects at the Cardiff Asthma Clinic are advised to sit quietly in a closed room at times when the grass pollen concentration is likely to be high, i.e. on sunny days and especially in late afternoon, and, if they wish, to open their windows for a short period after rising in the morning. Patients who are hypersensitive to other kinds of spore having different peak hours are advised accordingly (cf. Lacey, 1962). Closing the doors and windows appears to be at least as effective as are some kinds of filtering and air conditioning. Kailin (1964) confirms the protective effect of staying indoors with doors and windows closed.

Pollen encountered indoors comes from without and is readily avoided, but some kinds of fungus spore, on the other hand, are liberated indoors, at least in damp houses, and so render such places potentially unhealthy for allergic subjects. Storm Van Leeuwen (1924) postulated the presence of atmospheric 'miasmata' in damp districts in Holland where, according to his impression, asthma was especially prevalent. Several later workers sought to identify these miasmata with the fungus spores to be found in damp houses: indeed fungus spore allergy was generally regarded at one time as largely, if not entirely, a local risk attributable to spores present in the patients' homes. This view was supported by the earliest reports (Hopkins, Genham & Kesten, 1930; Bernton, 1930; Credille, 1933) but it was shaken by Feinberg's discovery in 1935 that allergy to mould spores present in the general outdoor atmosphere was by no means rare (in Chicago at least), and later by the results of Richards' (1954) long-term comparison between the atmospheric spore content of a normal clean dry dwelling and that of the open air outside it. Richards sampled, by means of Petri plates, the air in a bedroom in such a house, and also outdoors at a site a few yards away, every weekday for 12 months. The window of the room concerned was kept closed throughout the period and precautions were taken to avoid raising dust before the plates had been exposed, which would doubtless have affected the results (Maunsell, 1952; Swaebly & Christensen, 1952). He found that the air inside the house contained the same kinds of moulds and in much the same proportions (though on average in one fifth the numbers) as the air outside, and that the indoor mould aerospora fluctuated in size and composition along with the outdoor one, of which it was in fact merely a rather pale reflection. He concluded that in a normal dry house the most important source of airborne mould spores is the outside air and that therefore the most important botanical prerequisite for the study of allergy to airborne mould spores is a thorough understanding of the outdoor mycospora. The mould spore content of indoor air has also been investigated in greater or less detail by workers in several other parts of the world (Copenhagen: Flensburg & Samsøe-Jensen, 1950; Sweden: Rennerfelt, 1947; Ripe, 1962; Nilsby, 1948; U.S.A.: Newton, Scherago & Weaver, 1948; Wallace, Weaver & Scherago, 1950; Gordon, 1957; Colen & Van Arsdel, 1961; New Zealand: Di Menna, 1955). All of whose findings, together with those from the Hirst trap census of spores in and out of doors at Cardiff (Adams & Hyde, 1965) described above, are consistent with Richards' conclusions: indeed these have not been seriously controverted except perhaps by Van der Werff, who has emphasized the greater importance of mould spores indoors. He made numerous counts simultaneously both within

and immediately outside (mostly damp) homes in Holland (Van der Werff, 1958) and subsequently sampled by means of Petri plates atmospheric moulds within and immediately outside a damp old house at Amersfoort, Holland, over a period of 4 months, 1 August to 30 November. *Cladosporium* and *Penicillium*, which were both growing in number on damp surfaces inside the house, were much more abundant in the indoor atmosphere; *Fusarium* and *Pullularia*, which were caught in relatively small numbers outdoors, did not appear at all indoors; and yeasts were on average about six times as numerous outdoors as indoors (Van der Werff, 1963). The atmospheric spore content of a damp mouldy house is likely in general to be higher than that of a dry healthy one. The moulds which grow on wallpaper, distemper, books, etc., and which therefore contribute to the indoor aerospora, are all of almost all such as occur also outdoors. Van der Werff's findings on damp houses do not therefore invalidate Richards' conclusions on dry ones. But that indoor moulds can be important is illustrated by an investigation in New York where in a group of thirty-nine mould-sensitive patients all of the thirty-eight sensitive to *Alternaria* had the fungus growing in their homes; no moulds of any kind were found in fifteen out of a control group of twenty-four homes (Sherman & Merksamer, 1965).

One kind of fungus spore is peculiar to damp dwelling houses, viz that of the dry rot fungus *Serpula* (*Merulius*) *lacrymans*, a Basidiomycete* which is extremely common in England (Cartwright & Findlay, 1958) and Holland (Varekamp, 1957; Van der Werff, 1958) and doubtless elsewhere in the temperate zones (it does not grow at temperatures above 80°F). Active fruiting bodies, though apparently most common in late summer and early autumn, can probably be produced at any time of year (Richards, 1953) and spore concentrations of up to 360,000 per cubic metre (24 hr mean) have been observed in England in the air of a cellar containing such fruiting bodies (Gregory, Hirst & Last, 1953). *Serpula lacrymans* satisfies all Thommen's postulates (Frankland & Hay, 1951) and an extract of its spores can evoke symptoms of asthma (Huxheimer, Hyde & Williams, 1966).

The air in hospitals, which in the past has been surveyed mainly in relation to bacteria, was sampled for fungi in a London Hospital ward and found to contain, in addition to *Aspergillus fumigatus* (0.35–2460 colonies per m³ of air sampled), also other fungi (including *Cladosporium*, *Aureobasidium* (*Pullularia*) *pullulans*, other *Aspergillus* spp. etc.) averaging 0.106/m³ and enormous numbers of *Candida albicans* (Noble & Clayton, 1963).

(b) *Fungus spores in industrial locations*

Since moulds will grow on almost any kind of dead or damp organic matter it would not be surprising if in special factories, workshops and other places where such materials are normally present the inhalation of mould spores were an important occupational hazard. This subject was reviewed and investigated afresh by Van der Werff (1958) who found that particular organic substrates were affected by more or less characteristic associations of moulds. Thus cotton and linen fabrics are commonly inhabited by *Pullularia*, *Cladosporium*, *Alternaria*, *Epicoccum* and *Stysanus*; textiles generally are associated with *Mucorales*, *Penicillium* and *Cladosporium*. *Mucorales* also occur very abundantly in bakeries, flour mills and other vegetable grinding mills; butchers' shops yield *Aspergillus niger*, *Aleurisma carnis* and *Cladosporium* [it has

* Certain other Basidiomycetes sometimes attack timber in houses but they do not cause dry rot properly so called and are not confined to houses.

long been known that these will flourish on meat in cold storage (Brooks & Hansford, 1923)]; vegetable stores and fruit shops are constantly infected by *Mucorales*, *Penicillium* spp. *Botrytis* and *Cladosporium* (Van der Werff, 1958; Liebeskind, 1964). Workers in tomato houses are liable to become allergic to the spores of *Cladosporium fulvum* (a fungus parasitic on the tomato plant and the cause of stem rot) to which they may be exposed in concentrations of $10^6/\text{m}^3$ or more (Gregory & Hirst, unpublished) while remaining unaffected by those of saprophytic *Cladosporium* spp. (Rackemann, Randolph & Guba, 1938). During the citrus packing season in Israel the incidence of spores of *Penicillium digitatum* and *P. italicum* is very high in citrus packing-houses and their vicinity (Barkai-Golan, 1961). *Cladosporium* and yeasts flourish on fish flour manufactured in Lima, Peru, in such abundance that their spores have come to affect to a deleterious extent not only the air in the fish flourmills but also the general atmosphere of the city (Vargas Morales, 1964). The aerospora of an open cattle shed in India, though probably reinforced by fungi growing on cattle fodder, differed only quantitatively from that outside (Sreeramula, 1961).

(c) *Farmer's lung and related conditions (extrinsic allergic alveolitis)*

It has long been known that farm workers were especially liable to evince respiratory symptoms and in consequence of the frequency of this condition (now known as farmer's lung and since 1964 recognized as an industrial disease), the aerospora of farm buildings has since 1950 attracted special attention. Spore concentrations up to $1600 \times 10^6/\text{m}^3$, the highest ever recorded in air, have been observed in such buildings in southern England where mouldy hay was being shaken. Actinomycetes formed 98% of the total; the five species of fungi proper which were isolated most frequently are all potentially pathogenic to man and animals (Lacey & Lacey, 1964).

Farmer's lung (thresher's lung, Dreschkrankheit) which affects farm workers during late autumn, arises from the inhalation of dust from hay or straw which has been stacked while damp and in consequence has subsequently become overheated. Batches of hay which had 'moulded' and heated spontaneously to 60–70°C after baling at about 45% moisture content have been found closely to resemble 'farmer's lung hay' (FLH): investigation showed that such experimentally 'moulded' hay had passed through a stage during which it was acted on by a succession of bacteria, moulds and actinomycetes (Gregory & Bunce 1961; Gregory & Lacey, 1963a, b; Gregory, Lacey & Lacey, 1963). Mouldy hay associated with farmer's lung has been found to contain up to 10^9 actinomycete spores to 1 g dry weight (Corbaz, Gregory & Lacey, 1963). Two thermophilic species of actinomycetes, viz *Thermopolyspora polyspora** and *Micromonospora vulgaris*, are able to form FLH antigens and it is suggested that their spores and perhaps those of related species are the effective cause of the disease (Pepys *et al.*, 1963; Corbaz *et al.*, 1963; Pepys & Jenkins, 1964; Wenzel *et al.*, 1964). The two species named above have both been recovered from lung biopsies on farmer's lung patients (Emanuel & Wenzel, 1965). The spores of various thermophilic actinomycetes are so small that they must readily be deposited in the alveolar apparatus (Austwick, 1966). Inhalation tests show that the symptoms and signs of the acute illness in farmer's lung may be produced in farmer's lung patients by extracts of mouldy hay but not by extracts of the fungi found in mouldy hay (Williams, 1963). The process of composting in mushroom-growing farms favours the growth of thermophilic actinomycetes and the inhalation of their spores is responsible for sensitizing certain

* Now termed *Micropolyspora faeni*.

mushroom workers and producing 'mushroom worker's lung' (Sakula, 1967). Fog-fever in cattle appears to have a similar aetiology (Jenkins & Pepys, 1965). Bagassosis is a disease (similar to farmer's lung) due to inhaling dust from mouldy bagasse: the antigens concerned are similar to those of FLH and may perhaps prove to be due to the moulds present (Pepys, 1966). The symptoms of maple bark disease (caused by spores of *Cryptostroma corticale*) are said to resemble those of farmer's lung; the fungus has been reported on sycamore logs in England (Kessler & Ohman, 1965; Nash-Wortham, 1967).

(d) *Aspergillus fumigatus* as a cause of asthma

The mould *Aspergillus fumigatus* occurs in the general atmosphere in Holland, Britain and elsewhere in the temperate zones, though it does not seem to be recorded anywhere in large numbers; it may sometimes be missed in the course of Petri plate surveys because some strains grow best when incubated at a temperature of 37°C rather than at ordinary room temperatures (De Vries, 1960). The spores of *Aspergillus* spp. including *A. fumigatus* are liberated into the air in enormous quantities when mouldy hay is shaken out to feed stock in winter (Lacey & Lacey (1964) recorded up to $21.6 \times 10^6/\text{m}^3$ in cowhouses) and the numbers of *Aspergillus*-like spores in the air are correspondingly high in the general atmosphere in winter (Hyde, unpublished). Counts of *A. fumigatus* in a London hospital ward ranged from 0.35 to 2470/ m^3 with a marked peak during autumn and winter (Noble & Clayton, 1963). The fungus causes a violent lung infection in birds; air counts up to 7/ m^3 have been noted in the penguin enclosure at the London Zoo; the size of its spores (average 3 μm) is very near to that which favours maximum alveolar retention (Austwick, 1963). *A. fumigatus* is sometimes isolated from patients' sputum even in the apparent absence of active aspergillosis. The fungus is a relatively common cause of intractable asthma; some patients may be allergic to the spores liberated in their lungs (Pepys *et al.*, 1959).

(e) House dust

'House dust' is a mixture which may include the skin products of man and domestic animals, fragments of moths, flies, mites and spiders, other plant and animal fragments derived from carpets, curtains, upholstery, clothing and bedding (wool, camels' hair, horse-hair, feathers, straw, coir, rushes, kapok, cork dust), pollen grains and plant hairs and last, but not least from the present point of view, fungus spores. There is plenty of evidence that house dust normally contains numbers of viable fungus spores: Schwartz (1948), working in Copenhagen, found that nearly half the 239 mould colonies he got from his house dust samples were *Penicillia* and that these, together with *Aspergillus*, *Mucor*, *Alternaria*, *Fusarium*, *Rhizopus* and *Cladosporium* (in descending order of frequency), made up 94% of the total. Swaebly & Christensen (1952) found that house dust from vacuum cleaners in seventy-six houses in Minnesota contained, as a rule, between 10,000 and 1,000,000, or exceptionally as many as 3 million, viable fungus spores to the gram, the great majority being *Penicillium* or *Aspergillus*. Swaebly & Christensen (1952), using a Wells air centrifuge, found that the kinds of fungus spore present in house dust in New Jersey were all included among the commonest in the outdoor atmosphere there: *Cladosporium*, the predominant outdoor mould, coming rather low, however, in the scale of frequency in house dust samples (cf. Davies, 1960, below). Van der Werff (1958) examined the fungus spore content of fine dust samples, from door tops, window sills and the horizontal surfaces

of furniture, as well as of obviously mouldy materials, derived from 140 dwelling houses in Holland, and also sampled the moulds in the atmosphere of forty of these and eleven other houses, and obtained the following: *Phycomycetes*: twenty-one species, representing eight genera, the most frequent being *Rhizopus* (in eighty-eight houses), *Mucor* (in eighty-four), and *Thamnidium* (in nine); *Ascomycetes*: five species, representing four genera, fungi imperfecti: sixty-six species representing twenty-seven genera, the most frequent being *Penicillium* (in 130 houses), *Cladosporium* (ninety-five), *Trichoderma* (thirty-seven), *Fusarium* (thirty-three), *Botrytis* (thirty-one), *Aspergillus* (thirty), *Alternaria* (twenty-two), *Stemphylium* (sixteen), *Pullularia* (fourteen) and yeasts (nineteen) and one *Basidiomycete* (*Serpula* (*Merulius*) *lacrymans*). Frey, Cross & Durie (1963) found *Mucor* and *rhizopus* to be relatively far more abundant in house dust than in the air, *Cladosporium* less so.

R. R. Davies isolated moulds from ten house dust samples from various parts of Great Britain: all the genera among his isolates had previously been observed in the outdoor aerospora (Richards, 1956) and, except that *Cladosporium* (the conidia of which presumably do not long remain viable in house dust) was relatively rare, the commonest moulds found in house dust were those most frequently deposited from outdoor air (Davies, 1960, cf. Schwartz, 1948, and Swaebly & Christensen, 1952, above).

Numerous attempts have been made to inculcate fungus spores as the bearers of the house dust antigen(s); this simple hypothesis appeared to be contradicted by the results of Reyman & Schwartz (1947), who at Copenhagen tested patients with dust from their own homes and with moulds obtained therefrom; they found that most of the patients who were sensitive to the fungi were also allergenic to house dust but not vice versa and concluded that fungus allergens were unlikely to be the cause of house dust allergy. Nevertheless, the investigations of Rimington *et al.* (1947) strengthened the presumption that fungus allergy is in some special way connected with house dust allergy: they showed that substances chemically similar to their 'purified house dust antigen(s)' are secreted by several species of mould: and they did not observe any case of fungus spore allergy in the absence of house dust allergy.

A further suggestion, viz that the moulds present in house dust might be presumed under humid conditions to colonize some of its ingredients and so to act on them as to give rise to the characteristic house dust antigens, gained colour from the apparent association of allergic disease with damp houses and/or damp areas (Maunsell, 1952, 1954; Ordman, 1956; Reymann & Schwartz, 1947; Fiori & Tosco, 1955) and the likelihood of this hypothesis appeared to be strengthened by Davies's estimates of the numbers of *Penicillium* spores present in dust from various dry houses and from a very damp house, viz 60,000–1,100,000 and 23,200,000 per g respectively. But his further investigations showed that the hypothesis was the reverse of true; although his dust samples, when kept at various degrees of humidity ranging from 60 to 97.8% RH, were readily colonized by the moulds present (notably by *Mucoraceae*, *Penicillium* spp. and *Aspergillus* spp., but hardly at all by *Cladosporium*) the allergenic potency of the house dust concerned either was no greater after than before colonization or was significantly less (Davies, 1960). He concluded (a) that the allergenic potency of house dust is unrelated to the numbers of mould spores it contains, (b) that moulds developing on house dust actually consume the specific antigens (water-soluble polysaccharides) which it contains, and (c) that mould allergens and house dust allergens are distinct (Davies, 1960; Maunsell, 1960, Kantor & Wasserman, 1966, also concluded that house dust contains a specific antigen). The antigenic activation of kapok and boll

cotton, which according to Wagner & Rackeman (1937) took place as the result of colonization by moulds and therefore occasioned reactions in their patients, may have been due to the formation of mould antigens, not of house dust antigens. It has recently been shown that the human bed-mite (*Dermatophagoides pteronyssinus*) occurs very commonly in the house dust and is an allergen; it is not yet clear in what form antigenic material derived therefrom is usually inhaled (Cunnington & Gregory, 1968); a given patient may be sensitive to house dust but not to the mite and vice versa (D. A. Williams, personal communication).

3. Distant transport of pollen and spores

The dispersal of pollen and spores is only a particular example of the scattering of discrete particles following their liberation into the atmosphere (Gregory, 1945, 1961). If a quantity of pollen is released into perfectly still air its constituent grains, being acted on only by the force of gravity, will fall vertically and (following Stoke's law) they will attain almost immediately a limiting velocity and will fall at a uniform speed towards the ground. But under natural conditions pollen grains and fungus spores take off into air which is in motion at a rate compared with which their falling velocities are very small though not quite negligible (Hirst, 1959; Ingold, 1960, 1966; Schrodter, 1960; Gregory, 1966). This motion takes various forms according to the conditions obtaining at a particular time, viz mass movement in a more or less definite direction, eddying or turbulence (produced by friction with obstacles on the ground) and upward convection. The conditions which normally accompany overcast weather, the combined effects of wind, eddy diffusion and gravity on the succession of puffs of pollen emitted from a point source (for example, a tree) result in the formation of a sort of conical cloud similar to the trail of smoke issuing from a chimney and shaped like a cone, more or less horizontally flattened according to conditions and likely to have a vertical angle of around 20° ; the concentration of pollen along the axis of such a cone will diminish rapidly downwind and that at the edges of any given cross section will be much less than at the centre (the boundary of the cloud being drawn by definition where the concentration at the periphery of the cross section is one tenth that at the centre). For a pollen source close to the ground (compared with the relevant distance downwind) the lower boundary of the trail or cloud will be formed by the earth's surface, and pollen concentration will diminish with height as well as with distance downwind. The height of the cloud and concentrations within it at a given distance downwind, will depend on the intensity of turbulence at the time, vigorous turbulence promoting high clouds and low concentrations and feeble turbulence the reverse. In general the degree of turbulence is least on clear calm nights and greatest on clear sunny days. Since the conical cloud is in contact with the ground, some of the spores will be sedimented on to the earth's surface, a process which will reduce the total numbers in the cloud. Experiment has shown that this reduction takes place very rapidly (Stepanov, 1935); and the atmospheric concentration due to a given source therefore falls off rapidly with a distance from it (Gregory, Longhurst & Sreeramulu, 1961); nevertheless according to Gregory (1962) a fraction of the order of 10% of the quantity emitted 'escapes' into the general atmosphere; presumable this 'escape' is in part due, at least during sunny summer weather, to the strong convection currents ('thermals') which at such times rise from heated land surfaces carrying with them pollen and spores to a height of possibly several thousand feet. Airborne particles are carried therefore not only outward but also upward: plant spores have been observed

in abundance up to 11,000 ft above ground (Stakman *et al.*, 1923) and in greatly reduced numbers up to between 60,000 and 90,000 ft (Bruck, 1967).

Numerous observations confirm the bare fact of distant dispersal. Pine pollen grains are carried in small numbers from south-west Europe into southern Britain regularly during early spring every year (Hyde & Adams, 1961). Significant numbers of pine, spruce and fir pollen grains have been trapped off Greenland (800 miles from the nearest possible source in Labrador; Dyakowska, 1948). Erdtman (1937) operated his vacuum cleaner trap, fixed at 18 m above sea level on the ship's bridge during a voyage from Gothenburg to New York during May and June 1937, and found pollen grains in the air all the way across the Ocean: concentrations varied from 18/100 m³ over the North Sea and 15/100 m³ off the coast of New England down to 0.7/100 m³ in mid-ocean, though with temporary rises due to pollen 'rains' at distances up to 600 km from land. Pollen grains and spores can in fact, though in only very small numbers, cross not only the Atlantic Ocean, but also the North Pole (Polunin, 1951; Pady & Kapica, 1955). Pollen from Africa has been found in 'recent' ice in a Swiss glacier (Bortenschlager, 1965). What is more important from the present point of view is the distance from the source to which spores may be carried in quantities sufficient to evoke allergic symptoms, and the likelihood that this will happen regularly rather than only occasionally. Gigantic numbers of spores (millions upwards) must be released in order to produce a measurable effect at a distance. A single tree or a small grass field is likely to have only a negligible effect a mile away, but the combined trails from the trees in a large forest or from hundreds of acres of meadowland should and do make themselves felt for many miles. Durham (1938) recorded exceptionally high concentrations of *Alternaria* spores in the eastern half of the U.S.A. on 6 and 7 October 1938, Actual counts in many instances were 100 times the mean daily value for the month (exclusive of the day of the 'storm'). He attributed the phenomenon to the transport 'by large air masses' of spores originating probably in southern Minnesota; this was, even in Durham's 10 years' experience, a unique occurrence. But large numbers of spores have several times been observed at distances of between 7 and 40 miles from any possible source and in circumstances which must regularly recur. Hesselman (1919) trapped pollen on two lightships in the Gulf of Bothnia, distant respectively 18½ and 30 miles east of the coast of Sweden, and in the course of 41 days (16 May to 25 June) registered over the whole period catches composed almost entirely of coniferous and birch pollen and equivalent to daily averages of 40 and 22/cm² at the two stations respectively. Wiseman *et al.* (1954) trapped pollen on the Ambrose Lightship off Sandy Hook, 9 miles from the nearest land; they found that the total seasonal count there was 46% in 1949 and 60% in 1950 of the average count for New York City, the daily counts on some days being higher than at their land stations. Sreeramulu (1958a), using a Hirst trap at 70 ft above sea level on a voyage in the Mediterranean in October–November 1956, found a variety of fungus spores and pollen grains of terrestrial origin; in Malta Harbour he recorded up to 222 fungus spores and seventeen pollen grains per cubic metre, though when the ship was travelling at a distance of about 50 miles from shore total concentrations fell to about 13/m³. Barkai-Golan (1958) trapped moulds at intervals during the whole of a sea crossing between Haifa and Crete (600 miles). Durham made a flight of 60 miles at 1500 ft over Lake Michigan in the latitude of Milwaukee, sampling the aerospora every few minutes: he obtained almost equally heavy ragweed pollen counts all the way across; this confirmed his previous impression that the high ragweed pollen concentrations normally experi-

enced in Chicago were due at least as much to a heavy influx from the surrounding states as to local sources (O. C. Durham, personal communication, 1962). An 8-year pollen census (1953–60) has been carried out by means of slides exposed daily at 125 ft above sea level on Bishop Rock Lighthouse, which stands on an isolated rock to the south-west of the Isles of Scilly, 32 miles from Land's End. Pollen catches were related very closely to wind direction: with surface winds from any quarter between south and west they were negligible, but with north-east winds they rose at times to levels comparable with those at Cardiff and over the whole period averaged nearly 30% of the latter. In June 1953 and July 1955 the average daily catch of all kinds of pollen was equivalent to 44 and 35 grains per cm^2 respectively and therefore closely similar to Hesselman's (quoted above). The largest 24 hr catch of grass pollen recorded for any station in Britain during the whole of the Cardiff based gravity slide survey (1942–60) was 1679 grains on 5 cm^2 at Bishop Rock on 29 June 1953. The relatively high counts obtained at this station (as in the Gulf of Bothnia and on the Ambrose Lightship) are probably to be attributed in part to the method of trapping: relatively high winds and intense turbulence in such situations would cause abnormally large numbers of spores to impinge on the slides, so giving an exaggerated impression of high concentrations: nevertheless they are evidence of large-scale transport over at least 30 miles across the sea (Hyde, unpublished). A census of ragweed pollen in the several boroughs of New York City showed similar counts in all the boroughs, notwithstanding the fact that ragweeds had been systematically eradicated from one borough only; it was inferred that ragweed pollen concentrations in New York City are primarily dependent on pollen which drifts in from outside it (Cohart & Kandle, 1959). The concentrations of plant spores observed in central London (Hamilton, 1959a; Davies, 1962) and Liverpool (Davies, Denny & Newton, 1963) are evidence of considerable 'drift' from the large and widespread sources outside; and the same applies to our catches in central Cardiff (Hyde, 1972). The relative importance of background pollen ('drift') and locally produced pollen respectively has been discussed by Raynor & Ogden (1965) in the light of their experimental researches.

All the observations of massive long-distance transport just cited, with the possible exception of Durham's (1938), have been made under somewhat similar conditions, viz (1) the existence of very large and productive sources such as extensive forests or grasslands or cereal crops, (2) favourable winds, and (3) transport over the sea, or over land not much above sea level. Continuous long-term gravity slide observations (1945–49) at two stations located at altitudes of 1400–1600 ft among the mountains in South Wales have given rather different results. These stations were both situated above the zone of cultivation; but massive sources of grass pollen existed no farther than 10 and 20 miles respectively to the east of them, and yet here among the mountains daily pollen catches averaging $20/\text{cm}^2$ over a month were exceptional (Hyde, unpublished). It may be that passage across the sea, or over land which is not much above sea level, is more conducive to 'drift' than is passage involving a rise of 1000 ft or more in altitude (cf. Meade, 1959).

The conclusion that in certain circumstances spores may be carried many miles in quantities sufficient to evoke allergic symptoms has been amply confirmed by recent work (Hirst & Hurst, 1967). Spore trapping over the English Channel in summer showed that spore concentrations of over 10,000 per cubic metre (of which on average 80% were *Cladosporium*) were common at 600 m usually decreasing to hundreds per cubic metre at 3000 m (Hirst *et al.*, 1967a). Distant dispersal of extensive spore clouds

over the North Sea is a daily occurrence during fine weather and steady south-westerly winds. After such clouds had spent a night over the sea the greatest concentrations were recorded between 500 and 1500 m; these were several times greater than those measured at 30–60 m. Under such conditions counts registered with surface traps must grossly under-estimate total spore transport. Pollen grains sediment faster than do the much smaller spores of *Cladosporium*; buoyancy therefore affects the extent of distant transport (Hirst *et al.*, 1967b).

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