

From King's College Hospital, London, Great Britain
(Delivered at the Dutch Allergy Society, Leiden, The Netherlands,
February, 20th, 1971)

THE STORM VAN LEEUWEN LECTURE
THE IMPACT OF AEROBIOLOGY
ON ALLERGY

By

KATE MAUNSELL

INTRODUCTION

Already in 1676 it was suggested by *Anthony van Leeuwenhoek* (44) that microbes not discernible by the naked eye may be carried by wind up into the air. His ideas and conclusions were discussed by *P. H. Gregory* (22) in the *Leeuwenhoek Lecture* 1970 at the Royal Society, London, in the light of modern aerobiology (21). The fascinating lecture stimulated me to choose as my subject on this occasion "The Impact of Aerobiology on Allergy".

Leeuwenhoek believed that small "animalcules", a hundred million times and more smaller than a coarse grain of sand, can become airborne and carried by the wind over long distances, and eventually, through gravity, return to earth. He considered that these "animalcules" might be living organisms. He, the constructor of microscopes, lacked sampling devices of the air spora and therefore could not study his animalcules on slides, where he would have identified many of them. Physicians at his time had already put forward the hypothesis that certain particles in the inhaled air might precipitate asthmatic attacks. *Johann Baptiste van Helmont* (32), doctor and chemist in Brabant related asthmatic attacks in one group of his patients to certain atmospheric conditions in late summer and autumn, and to dusty air raised by the demolition of

houses, and *J. Floyer* (17) in 1698 incriminated the inhalation of fog in marshland areas as a cause of asthma. However, the thoughts of *Leeuwenhoek* on the floating matters in the air and the clinicians' intuitions of their precipitation of asthma remained in separate fields.

The first systematic research on airborne particles was carried out in 1860, 1861 by *Louis Pasteur* (56, 57). He proved that there existed in the air some living matter which could induce fermentation and putrefaction when introduced into sterile infusions of broth. In order to identify the types he sucked air through filters of gun-cotton which were subsequently dissolved in solutions of alcohol and ether. The sedimented particles were identified under the microscope as bacteria, moulds and yeasts, and rough calculations of their concentrations in the air of Paris showed several thousands of micro-organisms per cubic metre of air. He suggested that this method might be useful in outbreaks of infectious diseases which were the main interest of the contemporary microbiologists, *Tyndall* (76), *Miquel* (54), *Hesse* (34) and others.

At the same time *C. Blackley* (4), a medical practitioner in England, conceived the idea of airborne particles causing allergic reactions. He showed that present in the atmosphere were pollen which could induce symptoms of the respiratory tract in certain individuals. Blackley himself suffered from hayfever, then called "catarrhus aestivus". The fields and country lanes were his laboratories and his trapping devices were glass slides coated with viscous substances and exposed to the wind—a method still in use. He followed the journey of pollen from ground level to higher altitudes by fixing the slides to kites. He proved by self-experiment that his rhinitis and asthma was due to inhalation of pollen. In addition to pollen, mouldy hay and some moulds gave him rather frightening attacks of asthma lasting for several days. It is apparent in the last paragraph of his book "Catarrhus Aestivus" (1873) that he realised their significance. "I am sure" he wrote "that other agents apart from pollen may yet be found to produce symptoms not unlike hayfever. Among the great

number of particles there are with functions similar to pollen, it would not be surprising if we should find some that have a similar kind of action . . .". Whereas *Louis Pasteur's* work on aerobiology and bacteriology started a new area of research, *Blackley's* studies on aerobiology and allergy were hardly noticed, or met with disbelief during his lifetime. Eighty-six years after the first publication, *Blackley's* book was reprinted.

Pollen and mould surveys on a larger scale started about 1930 but mould allergy was not accepted as readily as pollen sensitivity. In 1923 *Sheppegrell* (64), a pioneer in studies of airborne pollen, said: "As spores are not responsible for hayfever, no special effort was made to count or identify them on our slides". In 1932 *Patterson & Gay* (58) considered that the dominant moulds on their slides, *Alternaria* and *Cladosporium*, were "problematic in relation to hayfever".

In contradistinction, in Holland already in 1925 *Storm van Leeuwen* (71, 72) and *Varekamp* (78) showed by clinical observations, skin tests and exposure of plates that moulds, mites and housedust could cause sensitization in addition to pollen. *Storm van Leeuwen* believed firmly in climatic allergy and his work laid the foundation of the relation of aerobiology and allergy. After *Storm van Leeuwen's* untimely death this research did not progress until *Voorhorst* and *Varekamp* resumed these studies in 1954 and extended their scope.

In America moulds were investigated from 1935 onwards as a cause of hayfever and asthma (*Rackemann et al.* (65), *Durham* (14, 15), and others).

No exact information on allergy and aerobiology was available in Great Britain down to the outbreak of the First World War, and respiratory allergy was not accepted as a science until *Hyde & Williams* and their team from 1942 onward linked aerobiology and allergy by their pollen and mould counts at Cardiff and eighteen other stations varying from town and rural situations, from Atlantic Islands to high mountain tops (38, 39, 40, 41, 66, 37, 1).

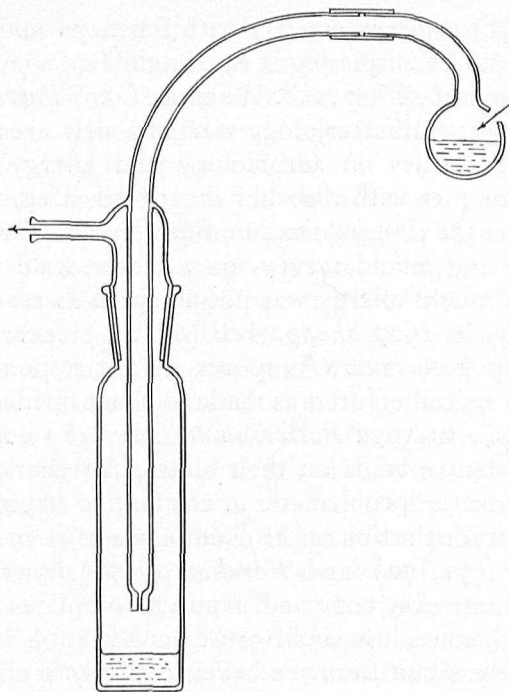


Fig. 1.

Liquid Impinger (From R. R. Davies (12)).

AERO-ALLERGENS

The presence of allergenic airborne particles, now termed "aero-allergens" was recognised by *Storm van Leeuwen* who was aware of their regional and seasonal fluctuation in the atmosphere out-of-doors and in-doors. They comprise microbial dusts, such as particles from arthropods, pollen, spores of fungi and actinomycetes, small algae, bacteria, and possibly viruses, and non-microbial dusts of plant and animal origin. Whereas plant pathology and biological warfare deal with pathogenic viable airborne microbes, allergy is concerned with both, pathogens and saprophytes, and not only with viable but also with dead material, since all of these can carry allergens.

To become airborne, all particles must be discharged from their sources by some form of energy. This may be active through ingenious methods of spore bearing organs, or passive through water droplets (9) or wind which can blow pollen from anthers, and spores from mycelia. Intramural dust is raised by air currents and our domestic activities, and the fine particles, including human scales, can float in the air for a considerable time. Bacteria do not become airborne as single units, but get attached to other particles, such as scales, using them as rafts (13). Aero-Allergy studies the airborne allergens from their discharge to their deposition in the respiratory tract where they can produce sensitisation.

Systematic investigations are still lacking to establish whether or not the inorganic pollutions, such as the irritant tarry matter, droplets of sulphuric acid and the gaseous constituents of air pollution render the mucous membrane of the respiratory tract more prone to sensitization.

SAMPLING DEVICES

The air spora can be examined by the gravity method which allows sampling by sedimentation, either on to sticky slides or to plates coated with suitable media. Both have their disadvantages, visual examinations of slides do not allow identification of all spore types, and sampling on media does not

TABLE 1
Rate of Fall of Spherical Particles of Unit Density in Air.

Particles (examples)	Diameter microns	Terminal velocity cm/second
Small spores	3	0.027
Larger spores	10	0.3
Small pollens	20	1.2
Larger pollens	50	7.2

Abbreviated from C. N. Davies 1949, 1952 (7, 8).

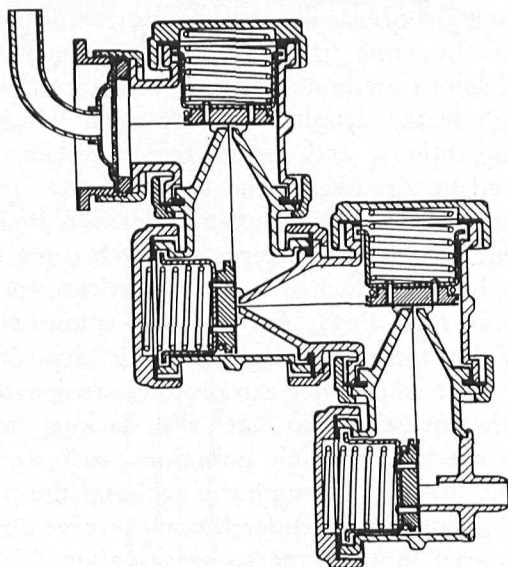


Fig. 2.

Cascade Impactor (May). From R. R. Davies (12).

allow estimation of non-viable spores. Neither method is advisable for quantitative comparison of sedimented particles of different sizes because they fall at different rates, and their numbers will be biased in favour of those of larger sizes. *C. N. Davies* (7, 8) calculated that small spores of 3 micron diameter fall at a terminal velocity of only 0.027 cm/sec and spores of 10 micron at 0.3 cm/sec, small pollen of 20 micron at 1.2 cm/sec and larger pollen of 50 micron reach a speed of 7.2 cm/sec. These figures relate to fall in still air and to particles of unit density and spherical shape (Table 1).

Modern techniques employ suction traps sampling by impaction which increase the catch of smaller spores, and also allow the estimation of the sampled volume of air. In the Impinger (Fig. 1) air is sucked at high speed through a narrow orifice, and the particles it entrains are impinged on the surface of a sampling fluid in the bottom of the tube. It is one of the oldest methods and still used in improved designs (*May*

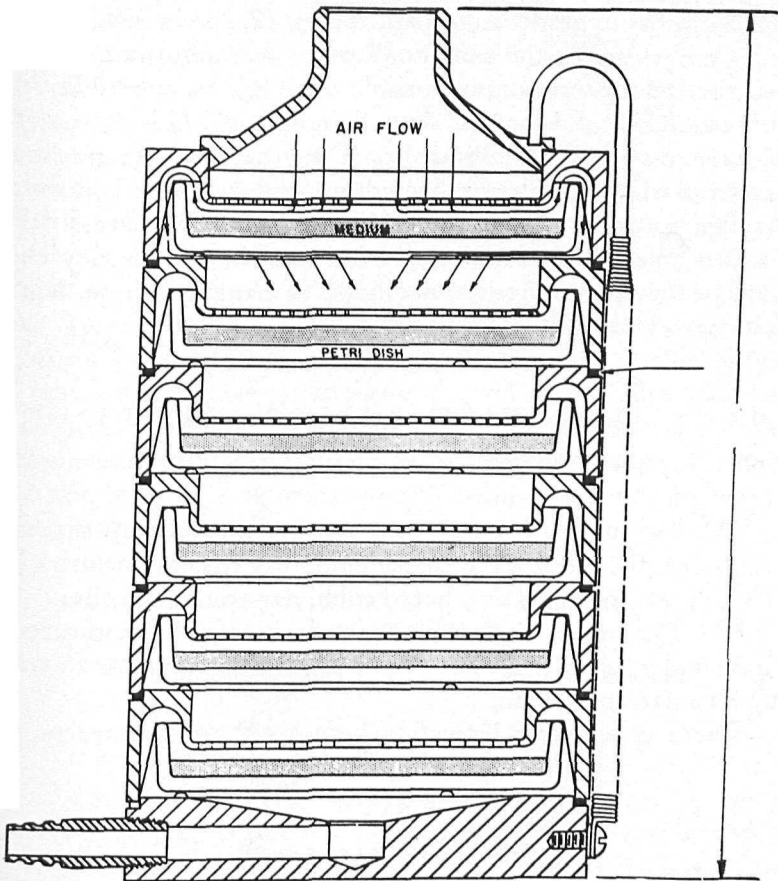


Fig. 3.

Anderson Sampler. From R. R. Davies (12).

& Druett, 51). The Cascade Impactor designed by May (50), (Fig. 2) is connected to a pump drawing in air at a known volume per minute, through four successive orifices of narrowing jets, depositing the spores on sticky slides behind them. In such a way they are graded into four size fractions. The band of dust is examined visually or by culture methods. An improvement on this spore trap was developed by Hirst

(36), the Volumetric Automatic Spore Trap, in which the slides moves across the sampling orifice at 2 mm per hour so that continuous trapping is possible. The Slit Sampler (*Bourdillon et al.*, 5) and the *Anderson* Sampler (3), (Fig. 3) catch microbes on media in Petri dishes. The latter is a size grading impactor which draws air through a circular orifice and then through a succession of perforated plates with progressively smaller holes. Larger amounts of air can be sampled by the Cyclone Sampler. Modern methods of sampling have been fully reviewed by R. R. Davies (12), in 1971.

THE UPPER AND LOWER RESPIRATORY TRACT

Following the definition of *Hatch & Gross* (31) we divide the respiratory tract into:

(A) The upper respiratory tract, extending from the entrance of the nose to the terminal bronchioles inclusively. These parts are lined by ciliated columnar epithelial cells.

(B) The lower respiratory tract, including the respiratory bronchioles and the alveoli. They are lined in part or in toto by alveolar epithelium.

There is a sharp distinction between these two tracts, in

TABLE 2
Deposition of Inhaled Particles.

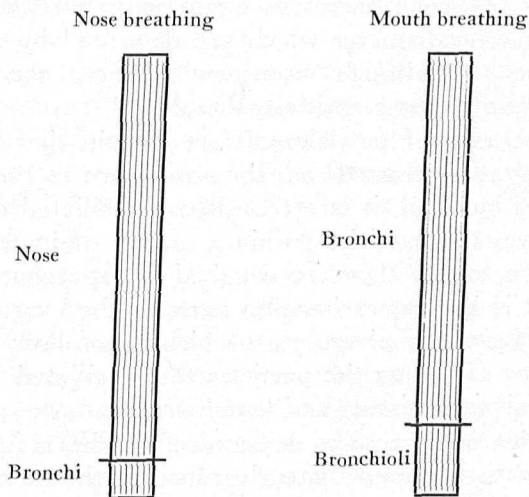
Site	Size (microns)	Physical forces	Clearance by
Nose, trachea	100-10	Inertia	Ciliated epithelium
Bronchi, terminal bronchioli	10- 5	Inertia	Ciliated epithelium
Respiratory bronchioles and alveoli	below 5	Sedimentation Molecular bombardment	Phagocytes

Based on C. N. Davies 1952 (8) and Hatch & Gross 1964 (31).

regard to the deposition of particles inhaled, their clearance, the immune response and the clinical syndrome.

Particles larger than 100 micron diameter are caught by the vibrissae of the nasal vestibule and do not enter the respiratory tract. Only those smaller than 100 micron are inhaled. Their *deposition* in the upper tract is effected mainly by inertia which impacts the particulate matter of the air stream on the walls of obstacles, such as the irregularities of the nasal conchae and the bifurcations of the bronchi. Down to 10 micron diameter, they become impacted on the nasal mucous blanket, and in this range are pollen, larger spores, debris and pellets of arthropods and larger dust particles. Between 10 and 5 micron diameter, *i.e.* the size of smaller spores and dust particles, impaction takes place mainly in the lower bronchi and bronchioles (Table 2). The trapping is fairly complete. However, the respiratory tract, which is the

Blockage of nasal cavities leading to mouth breathing of pollen grains
(20 micron diameter).



Particles of 20 micron diameter reaching bronchi and bronchioli after blockage of nasal airways.

Adapted from O. M. Lidwell, 1970 (45).

most complicated of sampling devices, does not always follow the laws of deposition. The most important interfering factors are changes in air velocity and the partial and complete blockage of the nasal cavities, leading to mouth breathing. When this occurs the larger particles can no longer be trapped in the nose and therefore reach the bronchi and even bronchioli (45) (Diagram). Those patients suffering primarily from atopic rhinitis can get, during phases of complete nasal blockage, asthma attacks.

The laws of deposition are different in the lower respiratory tract. As the airstream flows downward from the trachea to the bronchioles, the air velocity decreases and only small microbial particles, smaller than 5 micron diameter, such as small fungal spores, actinomycetes, bacteria attached to finest dust particles and non-microbial dusts reach the respiratory bronchioles and alveoli. Here the air is relatively still. Now deposition can occur by gravity sedimentation, but the efficiency is less than in the upper respiratory tract since gravity deposition and inertia decrease with particle size, *Sudlow* (75). The efficiency increases again with particles of less than 0.5 micron diameter which are deposited by molecular bombardment (Brownian movement). These are probably not of interest in hypersensitivity diseases.

The *clearance* of particles differs also in the upper and lower respiratory tract. From the nose down to the terminal bronchioles included, it is effected by the ciliated epithelium which moves the particles within a matter of hours towards the pharynx where they are coughed up, spat out or swallowed. But in the gas exchanging parts of the lungs the clearance is effected by phagocytes, which can follow two pathways. They can carry the particles to the ciliated epithelium or to the alveolar tissues and lymphstream.

Here clearance requires days, even months, a fundamental distinction to the immediate clearance in the naso-bronchial tract.

The immune mechanism in the upper respiratory tract is set into motion by the high water solubility of the atopens

which are extracted from the inhaled particles by the moisture of the mucous blanket. The freed atopens with their molecular weight of 10,000 to 40,000 can pass through the epithelium and react with the cell bound IgE antibodies which have been identified as a carrier of reaginic activity (42). In contradistinction, precipitins are believed to mediate the hypersensitivity reaction of the lower respiratory tract such as "extrinsic alveolar alveolitis" (*Pepys* 59, 60, 61). although in some instances the appearance of precipitins may be related more to exposure to the allergens than to the clinical syndromes. Precipitins are also involved in "allergic pulmonary aspergillosis" a syndrome first comprehensively reviewed in 1952 by *Hinson, Moon & Plummer* (3, 5) who found *aspergillus fumigatus* in mucous plugs and considered that this sensitivity had occurred by inhalation of the fungus.

CLINICAL EFFECTS OF AERO-ALLERGENS

A. *The Upper Respiratory Tract*

The symptoms caused by inhalation of atopens are based on a hereditary factor and affect the atopic group of individuals. The reactions are of a functional and reversible nature, and the manifestations are rhinitis, sinusitis, polypi, bronchitis and asthma. We define asthma as airflow obstruction of a variable nature, confirmed by a difference of at least 30 per cent in the FEV_1 of the predicted value at times (*Meisner & Hugh-Jones* 53).

A knowledge of the type of inhaled particles, their sizes and concentration in a given volume of inhaled air, and their allergic potency is needed to understand sensitization and subsequent changes of respiratory function.

The concentration of airborne allergens varies in different seasons and in different localities. We related the concentration of the airspora in London in three consecutive years, from March to October, to the incidence and severity of asthmatic attacks in pollen and mould sensitive patients. The survey was based on *E. D. Hamilton's* (28) assay of continuous

sampling with the Hirst spore trap and on *K. Maunsell's* (46) daily follow up of asthmatics with pollen and mould allergies. A definite relation between the rise of incidence and severity of attacks and the concentration of the airspore was found only in the grass pollen group. The recurrent late summer and early autumn attacks, which had intrigued many allergists, suggested a reaction to ascospores and these findings were also noted by *Ganderton* (20) for the ascospore stage of phoma. Another explanation for the late summer asthma came from Cardiff where basidiospores from toadstools, mushrooms, coprinus and shadow yeasts were shown to be allergenic by skin- and inhalation tests. 14 out of 19 patients with late summer asthma responded to basidiospores. 8 out of 14 had status asthmaticus in those months (33, 37).

The concentration of the atopen cloud is greatest near the source and it is obvious that the patient's exposure to a local cloud may have a greater bearing on his attacks than the cloud in the general atmosphere. For example, mowing of grass showed a heavy increase of liberated mould spores (70). Counts of *Cladosporium* in a London park were approximately 80 times as high than in a nearby street (10).

The *intramural air*, if undisturbed and doors and windows closed, will become practically free from particulate matter because the dust particles settle by gravity, as shown already in 1881 by *Tyndall* (76). If the windows are open, the in-door air-spore reflects the outer air-spore, but when dust is raised, for instance by bedmaking, the picture changes. A heavy increase of airborne moulds has been registered (48). Also fluff, human scales and mites as well as mite detritus (6) can become airborne. Human scales, which are weak allergens, may become highly allergenic because mite secretions coat the scales and thus add a strong allergen to a weaker one (68, 79). Indoor air can be loaded with spores of *Merulius lacrymans* (dry rot) if a fruiting body develops. The shed spores can reach a concentration of more than 300,000 per cubic metre of air (24). Other risks of inhaling allergens occur by exposure to moulds cultured for commercial purposes (80),

to bacillus subtilis used in the manufacture of the so-called biological washing powders (62), to insects, such as the grain weevil and locusts cultured in laboratories (18, 19).

Concentration of the inhaled cloud is not the only factor which accounts for the severity of rhinitis and asthma. *Hyde & Adams* (37) stressed that the relative importance of inhaled allergens cannot be estimated by numbers alone. For example, their average daily mean counts per cubic metre for one year at Cardiff were 13.4 grass pollen grains and 295 *Sporobolomyces*, but the approximate mean daily volume (m^3/m^3) was 300,000 for grass pollen and 3000 for *Sporobolomyces*. Such calculations are needed for basic knowledge of the response to aero-allergens. It should be taken into account that the recipient's response depends also on other factors, such as the potency of the aero-allergens and the degree of the patients allergic state, which varies from patient to patient, and in the same patient can vary at different periods of his life. The biological estimation of these factors falls no more into the realm of aerobiology, but it should be mentioned that inhalation of less than 1 microgram of *Dermatophagoides pteronyssinus* material produced asthmatic attacks in a highly mite sensitive patient by inhalation (*McAllen et al.* 52).

CLIMATIC AERO-ALLERGENS

Aerobiology has helped greatly in the understanding of different influences of climatic factors on the atopic patient. Pollen sensitive patients badly affected by ragweed in America are often much better during the hayfever season in the ragweed-free countries of Europe.

The relation between climates with high relative humidity and bronchial asthma due to house dust had impressed *Storm van Leeuwen* (72, 73, 74). He postulated the existence of climate allergens and suspected a main influence of fungal spores. He took three asthma patients to Switzerland, and provoked an attack of asthma in one, who had become symp-

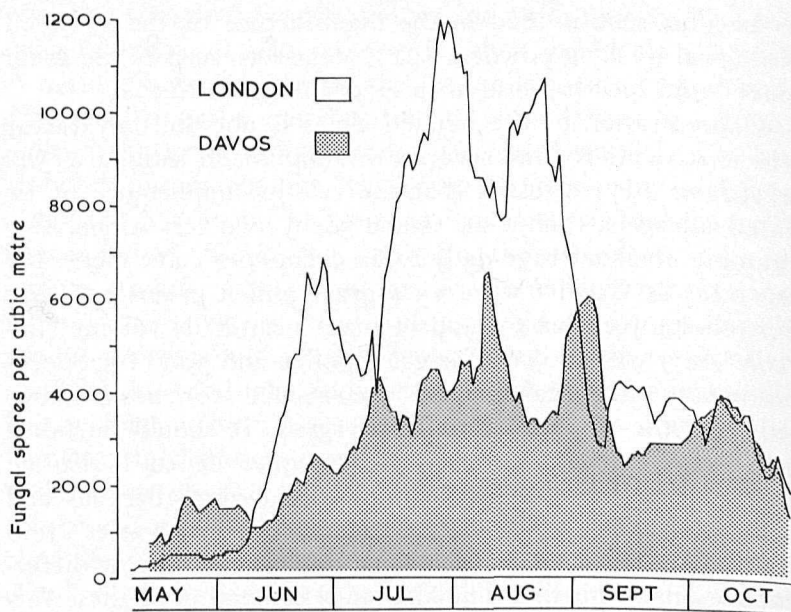


Fig. 4.

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

From R. R. Davies (10).

tom free in Switzerland, by exposing him to house dust from Holland. His observations were not forgotten and reports of the adverse influence of climates with high relative humidity came from all over the world (43, 30, 77, 47, 55, 2 and others). *Storm van Leeuwen's* observations in Holland and in the high mountains prompted *R. R. Davies* to check the extramural aero-allergens in Davos and London for comparison (11). Grass pollen concentrations tended to be lower in Davos than in London but clinically the difference was not great enough to produce freedom from pollen asthma. Most fungal spores were much lower in Davos than in London, for example *Cladosporium* spores were trapped only on eight days out of three months in comparison with sixty-seven days in London (Figure 4). *Basidiospores*, on the other hand, sur-

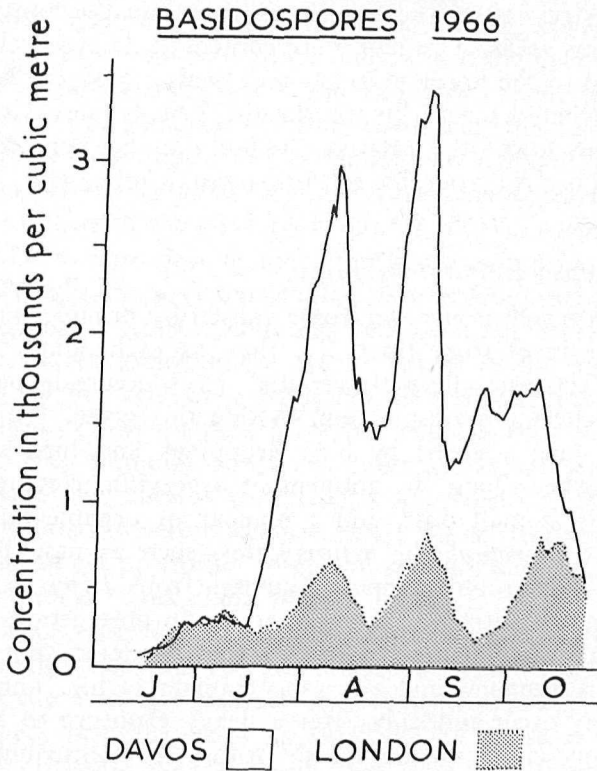


Fig. 5.

Basidiospores, total concentrations in 1966 plotted as a 10 day continuous mean.
From R. R. Davies (10).

prisingly were much greater in Davos than in London. The mean daily concentration was 1,000 per cubic metre of air on seventy-one days in Davos and only on seven days in London (Figure 5). These high concentrations of basidiospores were associated with local coniferous forests. These climatic investigations show that patients with sensitivities to fungal spores, other than those of basidiomycetes, should gain some relief from their symptoms at Davos.

The influence of the high altitude affects not only moulds but also the house dust mite and *Spieksma et al.* (69) believe

that this is related to the low humidity within the houses in mountainous areas. The high mite content in damp dwellings as opposed to the lower in dry homes is also apparent within a general humid macroclimate like the British Isles. Central heating can lower the relative humidity to 60 per cent or less which is not favourable to the growth of mites (49).

B. *The Lower Respiratory Tract*

The hypersensitivity syndrome caused by organic dusts in the lower respiratory tract, *i.e.* the gas exchanging tissues termed "extrinsic allergic alveolitis" (61) occurs in patients with no allergic predisposition. Within this group fall "bird fancier's lung" caused by bird droppings and bird serum, "malt worker's lung" by antigens of *aspergillus clavatus* and *fumigatus* in malt dust, and a number of organic dusts infested by *thermophyllic actinomycetes*, such as mouldy hay, bagasse, mushroom compost (quoted from Pepys 61). In these hypersensitivity diseases there is no obstruction of the bronchial tract but a restrictive ventilatory defect, not reversible by adrenaline, and a low gas transfer factor. The symptoms may occur suddenly after a heavy exposure to a cloud of organic dusts, or insidiously with less intense but more prolonged exposure. The syndrome consists of dyspnoea, cough, expectoration, malaise and fever, less commonly of tightness and wheezing.

Farmer's lung disease occurs in farm-workers after exposure of varying duration to mouldy hay. The study of this disease is a good example to show how aerobiology paved the way for the elucidation of the aetiology. In view of the increase of this disease, Gregory & Lacey (25, 26, 27) investigated at Rothamsted Experimental Station, Harpenden, the airborne flora from many batches of good hay, mouldy hay and hay from damp farms which were connected with the occurrence of this disease. The latter batches had heated spontaneously during maturation and a heat resisting organism was suspected as the cause of this disease. Because the re-

sponsible agent appeared to reach the alveoli, it was believed to be of very small size. With improved techniques of sampling, using an artificial wind-tunnel to blow off the spores and other microbes, and by sampling them with the Cascade Impactor, abundant spores of thermophillic actinomycetes were found (*Micropolyspora faeni*, *Thermoactinomyces vulgaris*). With extracts of these isolated cultures, *Pepys et al.* (64, 63) were able to reproduce by inhalation the symptoms in affected farmers, whereas previous inhalation tests using extracts from moulds grown from mouldy hay had been negative. Precipitins to the causative factor were present in the serum but also in 18 per cent of persons not suffering from farmer's lung disease. Occupations involving contact with birds and contact with pituitary snuff can cause inflammatory lesions with symptoms similar to farmer's lung disease, but more closely related to serum sickness (29). Again, healthy pigeon fanciers can get precipitins to pigeon serum, indicating contact but not disease (*Fink et al.*, 16).

The degree of severity of the symptoms of extrinsic allergic alveolitis depends on the concentration of the inhaled cloud at the time of exposure. Once hypersensitivity has been established, brief exposure is sufficient to cause a serious flare-up. Because clearance of this type of allergy is a slow process, the deposits of dust accumulate unless exposure is discontinued. This is quite different from the clearance of allergens in the upper respiratory tract where, as has been discussed, a highly effective clearance mechanism is working. Two thousand million spores of actinomycetes, equalling 840 micrograms of spores per cubic metre of air (the amount inhaled at an average per hundred minutes) were sampled in a place where mouldy hay was kept and stirred up. This is a much higher volume than the weight of particles involved in atopic disease. Even dry rot spores sampled close to the source, their fruiting body, equalled only 16 microgram, and grass pollen in the general air during the height of the season as little as 0.23 microgram per cubic metre of air (*P. H. Gregory*, 23).

One observation which is common to all allergic diseases is the beneficial effect on the patient if he can be removed from the source—or the source removed from him—before irreversible changes like fibrosis in alveolitis have occurred. In nearly all allergic diseases it is common that re-exposure may be followed by recurrent attacks. On this observation the first approach of *therapy* should be based, if possible, and in many instances aerobiology can give a guide. A knowledge of climatic aero-allergens and their seasons is helpful in the selection of holiday areas. If symptomatic treatment is required, the dosage should be increased and decreased in accordance with the rise and fall of the specific air spora. This is especially important in the control of the dosage of corticosteroids. Moreover, for hyposensitisation aero-allergens should be chosen which give not only positive skin reactions but are present in the air of the patient's environment, either perennially or seasonally.

Storm van Leeuwen's observations laid the foundation of modern aero-allergy. He had the deep-rooted instinct of noticing essential factors in the strange reactions of asthmatic patients, and with his scientific mind he collected observations over many years to see whether his belief in climatic allergens was justified. His judgment and intuitions are admired by allergists who read his carefully written papers, the results of labour of a short lifetime. I believed that he cared not for praise, but only for the truth, and it is tragic that he did not live to see the confirmation of his views. He knew much and his passion was to know more.

ACKNOWLEDGEMENTS

I wish to thank Dr. P. H. Gregory for his help and comments, and Dr. R. R. Davies for comments and for allowing me to use some of his drawings and graphs.

REFERENCES

1. *Adams, K. F.*: Year to year variations in the fungus spore content of the atmosphere. *Acta Allergol.* 29, 11, 1964.
2. *Alemaný-Vall, D. R.*: Climatología en las enfermedades alérgicas. Editorial Científico-Médica, Tratado de Alergia, 1960.
3. *Andersen, A. A.*: New sampler for the collection, sizing and enumeration of visible airborne particles. *J. Bact.* 76, 471, 1958.
4. *Blackley, C.*: Experimental researches on the cause and nature of Catarrhus Aestivus. Bailliere, Tindall and Cox, London. 1873. Reprinted Dawson, London, 1959.
5. *Bourdillon, R. R., Lidswell, O. M. & Thomas, J. C.*: A slit sampler for collecting and counting air-borne bacteria. *J. Hyg., Camb.* 41, 197, 1941.
6. *Cunnington, A. M. & Gregory, P. H.*: Mites in bedroom air. *Nature, Lond.* 217, 1271, 1968.
7. *Davies, C. N.*: Inhalation risk and particle size in dust and mist. *Brit. J. Industr. Med.* 61, 245, 1949.
8. *Davies, C. N.*: Dust sampling and lung disease. *Brit. J. Industr. Med.* 9, 120, 1952.
9. *Davies, R. R.*: Detachment of conidia by cloud droplets. *Nature, Lond.* 183, 1695, 1959.
10. *Davies, R. R.*: Aerobiology and relief of asthma in an Alpine valley. *Acta Allergol.* 24, 377, 1969.
11. *Davies, R. R.*: Climate and topography in relation to aero-allergens at Davos and London. *Acta Allergol.* 24, 396, 1969.
12. *Davies, R. R.*: Air sampling for fungi, pollen and bacteria. Chapter 13, 367-401 in *Methods in Microbiology*, Vol. 4, Edited by C. Booth. Academic Press, London and New York, 1971.
13. *Davies, R. R. & Noble, W. C.*: Dispersal of bacteria on desquamated skin. *Lancet*, Dec. 22, 1962.
14. *Durham, O. C.*: Incidence of airborne fungus spores. *J. Allergy* 8, 480, 1937.
15. *Durham, O. C.*: Incidence of airborne fungus spores. *J. Allergy* 10, 40, 1938.
16. *Fink, J. N., Sosman, A. J., Barboriak, J. J., Schleuter, D. P. & Holmes, R. A.*: Pigeon breeders disease. *Ann. int. Med.* 68, 1205, 1968.
17. *Floyer, J.*: A treatise on the asthma. London. 1st edition 1968.
18. *Frankland, A. W.*: Locust sensitivity. *Ann. Allergy* 2, 445, 1953.
19. *Frankland, A. W. & Lunn, J. A.*: Asthma caused by the grain weevil. *Brit. J. Industr. Med.* 22, 157, 1965.
20. *Ganderton, M. A.*: Phoma in the treatment of seasonal allergy due to *Leptosphaeria*. Synopsis. *Acta Allergol.* 23, 173, 1968.
21. *Gregory, P. H.*: The microbiology of the atmosphere. *Plant Science Monograph*. L. Hill, London, New York, p. 159, 1961.
22. *Gregory, P. H.*: The Leeuwenhoek Lecture 1970, Airborne microbes, their significance and distribution. *Proc. Roy. Soc. Lond.* 177, 469, 1971.

23. *Gregory, P. H.*: Personal communication, 1971.
24. *Gregory, P. H., Hirst, J. M. & Last, F. T.*: Concentrations of basidiospores of the dry rot fungus (*Merulius lacrymans*) in the air of buildings. *Acta allergol.* 6, 168, 1953.
25. *Gregory, P. H. & Lacey, M. E.*: The isolation of thermophillic Actinomycetes. *Nature, London* 195, 95, 1962.
26. *Gregory, P. H. & Lacey, M. E.*: Mycological examination of dust from mouldy hay associated with Farmer's Lung Disease. *J. gen. Microbiol.* 30, 75, 1963.
27. *Gregory, P. H., Festenstein, G. M., Lacey, M. E., Skinner, F. A., Pepys, J. & Jenkins, F. A.*: Farmer's Lung Disease. The development of antigens in moulding hay. *J. gen. Microbiol.* 36, 429, 1964.
28. *Hamilton, E. D.*: Studies on the air-spora *Acta Allergol.* 13, 143, 1959.
29. *Hargreave, F. E., Pepys, J., Longbottom, J. L. & Wraith, D. G.*: Bird breeder's (fancier's) lung. *Lancet* 1, 44, 1966.
30. *Harsh, G. F.*: The correlation between humidity and house dust sensitivity. *Proc. 1st. Int. Congress Allergy, 1951, Karger, Basle, New York.*
31. *Hatch, T. E. & Gross, P.*: Pulmonary deposition and retention of inhaled aerosols. Academic Press, New York, London, 1964.
32. *Helmont, Johann Baptiste van*: *Opuscula Medica Inundita*, Koln. (1644) Quoted from by Van der Werff, P. J. Mould fungi and bronchial asthma. Vol. 1, H. E. Stenfert Kroese N. V., Leiden, 1958.
33. *Herxheimer, H., Hyde, H. A. & Williams, D. A.*: Allergic asthma caused by basidiospores. *Lancet* 2, 131, 1969.
34. *Hesse, W.*: Uber quantitative Bestimmung der in der Luft enthaltenen Mikroorganismen. *Mitteilungen Kaiserl. Gesundheitsamt* 2, 1894.
35. *Hinson, K. F. W., Moon, A. J. & Plummer, N. S.*: Pulmonary Aspergillosis. *Thorax* 7, 317, 1952.
36. *Hirst, J. M.*: An automatic volumetric spore trap. *Ann. appl. Biol.* 39, 157, 1952.
37. *Hyde, H. A. & Adams, K. F.*: Airborne allergens at Cardiff 1942-59. *Acta Allergol.* 7, 139, 1960.
38. *Hyde, H. A. & Williams, D. A.*: Studies in atmospheric pollen. II. Diurnal variations in the incidence of grass pollen. *New Phytol.* 44, 83, 1945.
39. *Hyde, H. A. & Williams, D. A.*: A daily census of *Altenaria* spores caught from the atmosphere at Cardiff in 1942 and 1943. *Trans. Brit. Mycol. Soc.* 29, 78, 1946.
40. *Hyde, A. H. & Williams, D. A.*: A census of mould spores in the atmosphere. *Nature, Lond.* 164, 664, 1949.
41. *Hyde, H. A. & Williams, D. A.*: Studies in atmospheric pollen. IV. Pollen deposition in Great Britain in 1943. I. The influence of situation and weather. *New Phytol.* 49, 398, 1950.
42. *Ishizaka, K. & Ishizaka, T.*: Identification of gamma E antibodies as a carrier of reaginic activity. *J. Immunol.* 99, 187, 1967.

43. *Jummenetz-Díaz, C., Sanchez-Cuena, B. & Ping, J.*: J. Allergy 3, 396, 1932.
44. *Leeuwenhoek, Anthony van*: Letter to Henry Oldenburg, Royal Society Meeting February 1676. Quoted from "The Leeuwenhoek Lecture, Airborne Microbes" P. H. Gregory. Proc. Roy. Soc. London, 177, 469, 1971.
45. *Lidwell, O. M.*: Micro-organismer. Termisk og atmosfærisk Indeklima 524 (Editor: Niels Jonassen, Lyngby, Danmark. Polyteknisk Forlag), 1970.
46. *Maunsell, K.*: The seasonal variations of bronchial asthma in relation to the concentration of pollen and fungal spores in the air in 1955 and 1956. Acta Allergol. 12, 257, 1958.
47. *Maunsell, K.*: Quantitative aspects of allergy to house dust with special reference to the environment. Proc. 1st. Int. Congr. Allergy, 306, Karger, Basle, New York. 1951.
48. *Maunsell, K.*: Airborne fungal spores before and after raising dust. Int. Arch. Allergy 3, 93, 1952.
49. *Maunsell, K., Hughes, A. M. & Wraith, D. C.*: Mite asthma. Cause and management Practitioner 205, 779, 1970.
50. *May, F. G.*: The Cascade Impactor. J. Scient. Instrument 22, 187, 1945.
51. *May, K. R. & Druett, H. A.*: The pre-impinger; a selective aerosol sampler. Brit. J. Industr. Med. 10, 142, 1953.
52. *McAllen, M., Assem, E. S. K. & Maunsell, K.*: Housedust mite asthma. Results of challenge tests. Brit. med. J. 2, 50, 1970.
53. *Meisner, P. & Hugh Jones, P.*: Pulmonary function in bronchial asthma. Brit. med. J. 1, 470, 1968.
54. *Miquel, P.*: Les organismes vivantes de l'atmosphère. Annual Obs. Montsouris, 198, 1883.
55. *Ordman, D.*: The climate factor in perennial respiratory allergy and its relation to house dust sensitivity. Int. Arch. Allergy appl. Immunol. 9, 129, 1956.
56. *Pasteur, Louis*: Experiences relatives and generations sites spontanees. C. R. Acad. Sci. 50, 303, 1860.
57. *Pasteur, Louis*: Memoires sur les corpuscles organisés qui existent dans l'atmosphère. Ann. Sc. Nation. (Zool.) 4, series 16, 5, 1861.
58. *Patterson, P. M. & Gay, L. N.*: The pollen content of the air and its relation to hayfever in Baltimore, Maryland, during 1930. J. Allergy 3, 282, 1932.
59. *Pepys, J.*: Pulmonary hypersensitivity disease due to inhaled organic antigens. Postgrad. med. J. 42, 698, 1965.
60. *Pepys, J.*: Hypersensitivity to inhaled organic antigens. J. R. Coll. Physicians. 2, 42, 1967.
61. *Pepys, J.*: Hypersensitivity diseases of the lungs due to fungi and organic dusts. Monographs in Allergy 4, S. Karger, Basle, New York, 1970.
62. *Pepys, J., Hargreave, F., Longbottom, J. & Faux, J.*: Allergic reactions of the lungs to enzyme of *Bacillus subtilis*. Lancet 1, 1181, 1969.

63. *Pepys, J. & Jenkins, P. A.*: Precipitins test in Farmer's lung. *Thorax* 20, 21, 1965.
64. *Pepys, J., Jenkins, P. A., Festenstein, G. N., Gregory, P. H., Lacey, M. E. & Skinner, F. A.*: Farmer's lung thermophilic Actinomycetes as a source of "Farmer's lung hay" antigen. *Lancet*, Sept. 21st, 607, 1963.
65. *Rackemann, F. M., Randolph, T. G. & Guba, E. F.*: The specificity of fungus allergy. *J. Allergy* 9, 447, 1938.
66. *Richards, M.*: Seasonal periodicity in atmospheric mould spore concentrations. *Acta Allergol.* 7, 357, 1954.
67. *Scheppegrell, W.*: Analysis of six annual seasons of hayfever in New Orleans. *Publ. Hlth. Rep., Washington*, 38, 1565, 1923.
68. *Spiekma, F. T. M.*: The house-dust mite, *Dermatophagoides pteronyssinus*, producer of the house-dust allergen. Thesis, Leiden, 1967.
69. *Spiekma, F. T. M., Zuidema, P. & Leupen, M. J.*: High altitude and house-dust mites. *Brit. med. J.* 1, 82, 1971.
70. *Sreeramulu, T.*: Effects of mowing grass on the concentrations of certain constituents of the air spora. *Cur. Sci.* 27, 61, 1958.
71. *Storm van Leeuwen, W.*: Allergic Diseases. J. B. Lippencott and Company, Philadelphia, London, Montreal. 1924.
72. *Storm van Leeuwen, W.*: Weitere Untersuchungen ueber asthma und Klima. *Klin. Wschr.* 4, 1294, 1925.
73. *Storm van Leeuwen, W.*: Allergic disease in relation to climate. *Intern. Clin.* 2, 37, 1927.
74. *Storm van Leeuwen, W., Varekamp, H. & Biez, Z.*: Asthma bronchiale und Klima. *Klin. Wschr.* 3, 520, 1924.
75. *Sudlow, M. F.*: Inhalation of particles into the lung. *Proc. Brit. Allergy Soc.* 28th Feb. 1970. *Clin. Allergy* 1, 141, 1971.
76. *Tyndall, J.*: Essays on the floating matter of the air in relation to putrefaction. Longmans, London, 1881.
77. *Ufford, W. J. Q. van:* Quelques recherches preliminaires sur des questions socio-géographiques concernant l'asthme bronchique dans les Pays-Bas. *Proc. 1st Int. Congr. Allergy*, 111, Karger, Basle, New York, 1951.
78. *Varekamp, H.*: De exogens oorzaken van asthma bronchiale. Thesis, Leiden, 1925.
79. *Voorhorst, R., Spiekma-Boezeman, M. T. E. & Spiekma, F. T. M.*: Is a mite (*Dermatophagoides pteronyssinus*) the producer of the house-dust allergen? *Allergie und Asthma*, 10, (6) 329, 1964.
80. *Werff, P. J. van der:* Moulds, fungi and bronchial asthma. Vol. 1. Stenfert Kroese, Leiden, 1958.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.