
ANTONI VAN LEEUWENHOEK,
LID VAN DE KONINGHLYKE SOCIETEIT IN LONDON.
GEBOREN TOT DELFT. A. 1632.

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Airborne microbes: their significance and distribution

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[Plate 14]

Introduction

Under the date 9 October 1676 Antony van Leeuwenhoek wrote a letter to Henry Oldenburg about the Protozoa. It was read to this Society in February of the following year, and contained reference to claims that someone in Rome had seen extraordinarily small living creatures hovering in the air.

'For my part', wrote van Leeuwenhoek, 'notwithstanding the manifold observations made by me on the subject, I have up till now seen no smaller animalcules moving in the air than those which are so big that we can easily discern them with our naked eye...'

The very small particles which he had always found moving about in the air consisted of dusty debris of larger bodies. He continued: 'But I will not deny that there may be living creatures in the air, which are so small as to escape our sight; I only say that I have not seen them. Nor do I believe that they would be able to remain alive in the air about our horizon, but rather that they would be found in the clouds and could remain alive in the continual dampness, and so would be conveyed to us, alive in mist and rain.' (*Collected Letters*, **2**, 155, 1941.)

A quarter of a century later, in a letter about rotifers adressed to Hendrik van Bleyswyk, he wrote: '...in all kinds of water, standing in the open air, animalcules can turn up. For these animalcules can be carried over by the wind, along with the bits of dust floating in the air; and on the other hand, animalcules which are a hundred million times and more smaller than a coarse grain of sand, can be borne aloft, along with the water particles, albeit not as high as the clouds, but at least a little way up; and then when the sun goes down, they fall to earth in what we call dew; and they may well be taken up too [again?] and carried along by the wind.' (Cited from Dobell 1932, p. 267.)

To account for the ubiquitous occurrence of microbes van Leeuwenhoek was forced to the conclusion that they could be airborne. And he raised four of the essential questions that have been asked repeatedly during the microbial exploration of the atmosphere.

(1) How can we overcome the technical difficulty of observing such organisms in a mobile medium like air?

(2) Can such tender microbes remain alive in the apparently uncongenial medium of the atmosphere?

(3) Is the atmosphere inhabited by an enduring aerial plankton, a population of microbes passing their lives drifting in the atmosphere, comparable with the populations he had discovered in fresh water?

(4) Were his little organisms wafted about by wind from place to place in air-borne dust? Clearly his answer to this question was: 'Yes'.

Development of aerobiology

The study of airborne microbes has nearly always been pursued for ulterior motives, usually to throw light on some aspect of disease, or more recently in relation to palynology; seldom, unfortunately, since van Leeuwenhoek's time, out of sheer curiosity. Now, after 300 years, it is time to report back on the status of airborne microbes.

'Microbes': this rather unfashionable term is deliberately chosen here because it has a wider scope than the word 'micro-organisms'. 'Microbes' can include not only bacteria, moulds, yeasts and protozoa, but also viruses on the one hand and pollen grains on the other. Both viruses and pollens play an important role when airborne, but for different reasons neither quite qualify to be called micro-organisms.

Serious examination of the air for its microscopic life began just over a century ago with the rise of bacteriology. Surely, it seemed, the air must be the source from which people became infected with the fevers that then plagued city life. Pasteur (1861) concentrated airborne city dust by aspirating the air through a soluble filter and found moulds, yeasts and bacteria. Cunningham (1873) trapped airborne dust in a Calcutta jail and tried to correlate his catch with outbreaks of cholera and other diseases among prisoners for whom he had good clinical records.

Interest waned when it became clear that the main agents spreading major epidemics were contaminated water, milk, food, vector insects and lice, rather than airborne microbes. One achievement remained from that period. C. H. Blackley (1873), a Manchester physician, demonstrated a correlation between the incidence of hay fever and hay asthma in his patients and his measurements of the pollen concentration of the air. Interest increased again when the treatment of hay fever by injecting small doses of pollen extracts to desensitize the patient was developed early in this century. Interest is still growing with the discovery of new and unsuspected respiratory allergens.

A fresh impetus to the study came from aeronautics. Once man had made machines to fly, he could study the properties of the atmosphere, including its microbial content. Some balloonists sampled the atmosphere for microbes. Harz (1904), for instance, found quantities of moulds and bacteria at 2000 m altitude, using a horse's stomach pump to aspirate air through a filter. Plant pathologists

soon used the aeroplane, pioneered by Stakman, Henry, Curran & Christopher (1923) who found pollen grains and fungus spores (including viable uredospores of cereal rusts) at up to 3300 m over the Mississippi Valley.

The study of airborne microbes slowly evolved into 'Aerobiology', a term made current by Fred C. Meier of the United States Department of Agriculture. Unfortunately his ambitious programme for exploring the upper air by aircraft ended tragically when he perished on a flight across the Pacific Ocean in 1938 (Haskell & Barss 1939).

'Aerobiology' is now taken to include the study of airborne viruses, bacteria, protozoan cysts, spores of fungi and lichens, small algae, plant spores and pollen grains, all of which I have referred to as the 'air spora' (a term analogous to flora and fauna). Aerobiology was firmly established with the publication of a symposium under that title by the American Association for the Advancement of Science (Moulton 1942). At this stage the discipline showed signs of bifurcating into extramural and intramural aerobiology.

There is now sign of another bifurcation, into study of the large spore types that we expect to be hardy and to survive the rigours of at least a short flight if not passage into the upper atmosphere; contrasted with aerobiology of the smaller, tender organisms whose viability and infectivity is in question (Gregory 1961; Dimmick & Akers 1969).

Intramural aerobiology was stimulated by the need to study overcrowded buildings during the two World Wars. This application led to the development of the slit sampler for airborne bacteria by Bourdillon, Lidwell & Thomas (1941), and to the evolution of a type of sampler known as the liquid impinger.

Another stimulus to aerobiology developed when the use of toxic gases in World War I conjured up the possibility of using microbial agents against military and civilians in future wars. It is known that in several countries research has been done on the hazards of attack and defence. A United Nations Report (1969) warns us that: 'Infection through the respiratory tract by means of aerosols is by far the most likely route to be used in warfare'. Paradoxically, now that the results of some of these investigations are published, this disturbing prospect has greatly benefitted aerobiology.

Commenting with backsight on van Leeuwenhoek's letters, we realize that he failed to find microbes in the air because examining air through a high-power light microscope reveals nothing. This troublesome fact beset would-be aerobiologists up to World War II. It had to be overcome on an *ad hoc* basis by depositing what was floating. The procedure adopted by most investigators was simple. Think of a method; then use it. We lacked principles for air sampling.

The commonest device was a horizontal sticky microscope slide or a Petri dish of culture medium, exposed to the air, and then examined for deposited microbes. The horizontal slide is not inappropriate for studying microbes deposited on a surface; sometimes a plant pathologist needs just this, but an allergist must know the airborne concentration. I once incurred wrath by describing the horizontal slide

method as a 'logarithmic lie': logarithmic because it is so heavily biased in favour of large particles. Our topic is not about dusty surfaces, but about microbes suspended in the air.

A short publication by May (1945) marked a turning point in aerobiology. It not only described a valuable sampling device, the Cascade Impactor, but also firmly delineated for the first time the principles of efficient air sampling. Henceforward we knew that we must aim at 'isokinetic' sampling (Druett 1942), even if we could not quite attain it in practice.

May's Cascade Impactor heralded a new generation of equipment for microbial air sampling. The Hirst (1952) automatic volumetric spore trap, and the Andersen (1958) sampler, were both based directly on the Cascade Impactor. Electrostatic high volume samplers, and irrigated cyclone separators recently described by Errington & Powell (1969), designed to meet the need for sampling large volumes of air, have different origins. All these devices put into our hands powerful tools for investigating the air spora.

Results of air sampling

Traditionally air sampling has usually been intermittent. For particles in the 5 to 50 μm range, continuous sampling with the Hirst trap is more revealing (Hirst 1953). Outdoors, in nature, much microbial activity goes on out of normal working hours. Introduction of the Hirst trap gave a new and vivid picture of the ambient fungal air spora. An account of this will illustrate what may be expected when similar studies become possible with other kinds of microbe.

The Hirst trap samples air continuously, drawing air by means of a vacuum pump, through a narrow slit. Particles down to about 5 μm equivalent diameter are deposited on a sticky microscope slide moving close behind the slit at 2 mm per hour, where they are laid out in a band for identification (so far as is morphologically possible) under the high-power light microscope.

The apparatus gives no information on bacteria or viruses. It is valuable in recording a wide range of plant pathogens and saprophytic moulds. It catches common airborne respiratory allergens, a bonus additional to the original purpose of the trap as an aid to plant pathology. The Hirst trap does not distinguish between living and dead spores. In some contexts of plant pathology viability is not in question; applied to allergy it is irrelevant.

Let us now consider sampling at 2 m above ground level in a rural area during summer weather: soon after dawn the atmosphere near the ground becomes dominated by spores of a dark coloured mould, *Cladosporium*, at a concentration of perhaps 10^4 per cubic metre of air. (In the context of allergy remember that one cubic metre is only a little more than the volume of air inhaled each hour by a man at rest.) During the hay fever season, this *Cladosporium* concentration is supplemented by a hundred or two grass pollens per cubic metre. At various periods during the summer it is added to by spores of plant pathogenic fungi or potential

fungal allergens, such as those of rusts, smuts, mildews and moulds including *Alternaria* and *Epicoccum*.

At dusk this air spora characteristic of the summer day diminishes rapidly, and the air may be somewhat cleaner during the early part of the night. After midnight on dewy summer nights the air spora is often dominated by the minute colourless spores of the mirror yeasts, such as *Sporobolomyces* and *Tilletiopsis*, that live saprophytically on nearly all leaf surfaces. These may number 10^6 per cubic metre of air. Spores of mushrooms and toadstools, especially from species of *Coprinus*, are present all through summer and reach large concentrations during the autumn, especially at night. Also on dewy nights ascospores from the cup fungi and their allies abound; they are especially abundant during late summer and autumn. When dawn comes, this characteristic night air spora vanishes and is soon replaced by the usual *Cladosporium* and pollen concentrations characteristic of fine days.

Rain completely alters this summer fine weather air spora. When rain starts to fall, *Cladosporium* and some other fine weather spore types often show a sharp but transient increase. But soon the dry air spora, just described, is thoroughly washed out of the air (it can be found if we collect the rain water), and is replaced by a different microbial population, rich in the spores of ascomycetes.

For example, within an hour of the fall of about a millimetre of rain, in a field of wheat stubble at Rothamsted, we recorded over 3000 spores per cubic metre of air of the take-all fungus (*Ophiobolus graminis*) which had been absent from the air, so far as we could detect, during the previous 20 dry days (Gregory & Stedman 1958).

The circadian periodicity during dry weather, and its changes with rain, have been confirmed with traps operated in various parts of the world. Yet each place has its local variations on the theme, and in detail each day differs from the last.

With the Hirst trap, the time of catching a spore can be determined within approximately $\pm$ half an hour. The daily sequence of any identifiable dry-weather spore type shows on average a characteristic daily rhythm of concentration (Hirst 1953; Hamilton 1959). These circadian changes sometimes correspond to changes in rhythm with which spores are launched from their substratum, and sometimes associated with meteorological factors.

I have described the fungus and pollen component of the air spora in some detail for various reasons. They are the components I know best. They are important in plant pathology and allergy. They are the easiest to observe because they can be handled with the light microscope, whereas viruses and bacteria are not so amenable. With the light microscope pollen grains can often be identified to species level; fungus spores can be categorized microscopically, or even identified more precisely, given circumstantial evidence on neighbouring sources. Under most conditions fungus spores and pollens dominate the air spora, evidently because some species have adapted to aerial dispersal during evolution. The final reason for dwelling on this aspect of aerobiology is that it is the group best explored by systematic, *continuous* sampling. This distinction between continuous and discontinuous sampling must be stressed.

Pierre Miquel, of the Observatoire Montsouris, sampled Paris air regularly at short intervals each day (but not continuously) for most of the last quarter of the nineteenth century. As far back as 90 years ago he experimented with a 15 min sampling period, and drew the important conclusion that his hourly values are merely a smoothing of still more rapid fluctuations. From this fact arises the need for continuous sampling to ensure that transient, but possibly dense, concentrations are not overlooked.

Despite the need for continuous sampling, it is nevertheless true that discontinuous but systematic sampling of air has served to establish that circadian fluctuations occurred in bacterial concentrations in Paris air during the last century (Miquel 1886), and in Savannah, Georgia, during this century (in: Wolf *et al.* 1959).

Miquel's long-term sampling in Paris showed average concentrations of 290 bacteria/m^3 of air in a park, and more than 7500 bacteria/m^3 in a busy street. In the air of South Kensington, Hamilton (1959) found that spores of fungi averaged 6500/m^3 during the six warmer months, and pollens about 100/m^3. During the same period in an arable field at Rothamsted, 25 miles to the north, the concentrations of fungus spores were more than double the London concentrations.

Such values are averages, based on long-term sampling: the instantaneous concentrations fluctuate violently. A peak concentration of 10^7 pollen grains/m^3 was recorded by Durham (1947) near a stand of *Ambrosia* in the United States, where ragweed is the most important cause of hay fever. Instantaneous concentrations of 10^6 fungus spores/m^3 and 10^5 pollen grains/m^3 are probably not uncommon near ground level.

Occasional or 'spot' sampling has also demonstrated beyond doubt that protozoa, mosses, algae and other micro-organisms occur regularly in the air; but none of the other groups, even the bacteria, have been followed in the revealing detail of the fungi and pollens. Much valuable information, and many surprises, doubtless await the investigations of other organisms by continuous sampling.

The fungi contain many groups specialized for launching into the air. Most bacteria do not, except for the Streptomycetes. As a result, outdoors, fungus spores outnumber airborne bacteria, commonly by about a hundred to one.

Clearly, the abundant microbes in the air are those with abundant sources and good launching mechanisms. This is because air at the surface of solids or liquids forms a still or slowly moving layer, the surface boundary layer, usually of the order of a millimetre or two thick. This layer tends to impede microbial dispersal. Microbes growing or sporulating on surfaces must somehow cross this boundary layer, if they are to enter the mobile air layers overhead and be dispersed by the energy of the winds.

Ways in which wind-pollinated flowering plants achieve this are described in the classical literature of botany. Launching mechanisms in fungi are well known from the researches of Buller and of Ingold. They display a wide range of passive devices facilitating dispersal, in addition to some active, explosive mechanisms.

In trying to control any airborne microbe, one essential fact we need to know is

its predominant launching mechanism, as a possible vulnerable point for attack. This is an important principle to which I shall refer later.

The work on air sampling, fragmentary as it is, nevertheless builds up to an impressive picture of the air spora as a significant but relatively unexplored natural phenomenon. All our lives, like other vertebrates, we inhale a microbial suspension. This suspension flows in the wind over the surfaces of crop plants and elsewhere. It is one of the given factors of our environment, inescapable unless we filter it out. Its concentration can be increased by human activities such as harvesting and haymaking, or indoors by bed-making.

So far as our examination goes, the air spora seems to have its origin at the earth's surface. Part comes from the soil, fresh water or oceans, but most from vegetation and living or dead organisms growing at the air/earth interface. There is nothing to indicate the existence of a true aerial plankton. The idea of a population of microbes, passing their lives in suspension in the atmosphere (life teeming in the clouds as some have imagined), now seems improbable.

Rather the atmosphere is thronged with travellers: microbes using the wind, speaking teleologically, as a convenient transport from one place to another. Travellers mostly performing quite short journeys.

But not all the journeys are short. Wind transport of wheat rust uredospores from the Southern United States, north into the Canadian prairies in spring, and a return in autumn, has been familiar since the pioneer work of Stakman and his colleagues in the 1920s and 1930s. The story is repeated with variations, in India, the U.S.S.R. and in Europe.

Although experimental evidence shows that much of the airborne material liberated near ground level is deposited within 100 m or so of its source, a proportion is lifted by mechanical turbulence or convection into the upper air, and is then in play for long-distance dispersal. This 'escape fraction' is estimated at around 10 % of the day-time production, but it may well be less at night or more for very large spores. The land must therefore be thought of as an area-source, contributing to the air spora of the upper atmosphere. Over the oceans, downwind of a land mass, the spore cloud can be detected by sampling from aircraft.

Fulton (1966) and his colleagues used a highly porous, soluble gelatine-foam filter, mounted in the nose of an aircraft to sample microbial populations of air masses at several altitudes during flights from land to seaward. Starting from Houston, Texas, samples were taken at distances up to 640 km into the Gulf of Mexico. After exposure, the filters were dissolved in saline, and microbial numbers estimated by culturing aliquots of the suspension on culture media. At the lowest level, 152 m above the water, concentrations of microbes decreased steadily the further from land, as might be expected. At higher altitudes concentrations varied irregularly, and failed to decrease steadily with increasing distance from land. No satisfactory explanation was forthcoming for this irregularity in spatial distribution of concentrations at higher altitudes.

Explanation of the irregularity might perhaps be possible if the full records are

re-examined in the light of work by Hirst and associates (Hirst & Hurst 1967), based on aircraft sampling over the North Sea. By contrast with Fulton's technique, which revealed microbes viable and cultivable on the media employed, Hirst used a microscopic method for counting fungi and pollens, living and dead alike, after collecting in isokinetic suction impactors.

One flight took samples between Yorkshire and the Skagerrak, around mid-day with the wind between south and west in July 1964. The flight first encountered the expected day-time *Cladosporium* and pollen cloud coming off the land; this, again as expected, decreased in concentration soon after leaving the coast. But, rather unexpectedly, concentrations increased again to a maximum at 400 to 500 km out. Furthermore, in between, at 100 to 200 km, and again further out still at 500 to 600 km, they found maxima of the moist air spore types, such as *Sporobolomyces* and the hyaline ascospores, which had almost certainly been liberated into the air at night.

Evidently the aircraft, starting in that day's spore cloud, flew next into the previous night's cloud, on into the previous day's cloud and finally, near the Danish coast, into that of its preceding night! J. M. Hirst & G. W. Hurst (1967) remark: 'We were perhaps fortunate in studying dispersal from the British Isles, which has a length of wind run over source areas of only a few hundred miles so that steady winds of 10 to 20 knots blow across it in half a day, and carry out to sea discrete and detectable spore clouds'. Spore clouds, massive enough it was calculated, to deposit 1000 spores/cm^2 of the ground surface if washed out by rain.

But are even the hardy microbes of the types studied alive or dead after 48 h passage at 1 km altitude above the surface of the North Sea?

Viability

Study of microbial survival in the atmosphere is technically difficult. Much of the older work is of doubtful relevance as it concerns organisms dried down on glass in the laboratory, with at best only half the microbial surface exposed to the air, and even that half deeply embedded in the surface boundary layer of air covering the glass surface. How unlike the condition of a microbe suspended naked in the free atmosphere!

The problem is how to hold an aerial suspension of microbes in place so that it can be sampled repeatedly to measure decline in percentage viability as the suspension ages. Goldberg and his colleagues achieved this by placing the suspension in a drum, and then rotating the drum on a horizontal axis at such a speed that the enclosed air is continually inverted before the suspended particles have time to fall to the wall of the container (see Dimmick & Akers 1969).

The rotating drum has yielded much information, especially about the causes of death of the smaller microbes, but for larger spores with long survival periods its use is limited. At best the rotating drum is a laboratory tool, giving good reproducibility, but during the test the microbe is imprisoned in the same parcel of air.

The microthread technique (Druett & Packman 1969; May & Druett 1968) overcame some of these difficulties. The method uses the ability of small, web-forming spiders to manufacture strong, inert silk threads, fine in guage (about 0.5 μm in diameter), which are moreover bacteriologically sterile. The threads are wound on a frame, which is then placed in an air current bearing an artificially generated aerosol, so that the threads become loaded with microbial particles. The threads are then exposed in a test environment for periodical viability measurements during ageing.

When tested *in the laboratory* with *Escherichia coli* the microthread method gave survival curves substantially similar to the rotating drum method. However, unexpectedly, when *E. coli* was suspended on microthreads and exposed, not to laboratory air, but to outdoor night air, on some occasions viability decreased much faster than in the laboratory. Years of laboratory experiments had failed to detect the existence of a hitherto unknown toxic 'open air factor', which is shown to be destroyed by sunlight, and is thought by May & Druett (1968) to arise from a form of atmospheric pollution. Microbiology *in vitro* cannot yet entirely replace microbiology alfresco.

Testing viability in aerial suspension is bound to be difficult with the smaller microbes. For the spores of fungi we may hope one day to combine the two recent methods of sampling the upper air over the sea. Sequential sampling from aircraft in an identifiable spore cloud in a single air mass is needed, using for example the microscopic method of Hirst and his associates to give the total fungus spore count, and simultaneously using the soluble gelatine-foam filter method of Fulton and his associates to give the viable count. To determine the decay of viability of *Cladosporium* and *Sporobolomyces* in a natural spore cloud during 48 h travel downwind of the British Isles seems a reasonable and worthwhile objective.

Significance of airborne microbes

I have stressed the rich and kaleidoscopic air spora as a phenomenon of nature. Very interesting! But does it matter? Surely most of this activity in the air concerns the wanderings of harmless saprophytes. A few examples will show this is not so.

Indoor air is usually exchanged fairly rapidly by ventilation with outside air. Because of this exchange, the microbial content of air indoors tends to change in unison with that outside, but indoor concentrations may be only about half as great as those outside. But added to microbes coming in through the window, indoor air commonly has others derived from domestic sources. For example, it may have contaminated droplet nuclei—the minute residues left by evaporation of droplets expelled in sneezing or coughing.

Bacteria often travel on minute rafts. Indoor air is often loaded with rafts in the form of minute plates of human stratum corneum. Davies & Noble (1962) stress the importance of these scales as carriers of bacteria, and as potential sources of cross-infection.

House dust has long been known as a common cause of asthma. Its main allergen has now been shown to lie in its content of the house dust mite (*Dermatophagoides pteronyssinus*) which is allergically very potent. This mite eats the desquamated human stratum corneum scales, and, as pointed out by Maunsell, Wraith & Cunnington (1968), it flourishes on the surfaces of mattresses. Inhalation of mite fragments, or of its faecal pellets, can cause asthma.

Other airborne allergens associated with asthma in sensitive individuals include the dark-spored moulds *Cladosporium*, *Alternaria* and *Epicoccum*. Some people are allergic to inhalation of spores of toadstools, or of the mirror yeasts (*Sporobolomyces*); others, as pointed out by Ganderton (1968), to the *Leptosphaeria*-type ascospores liberated when the ground is wet by dew or rain.

A disease known as 'farmer's lung' is a major hazard of farm workers whose job it is to shake out hay in cowsheds, perhaps twice a day, for feeding livestock. In cool, moist climates hay is often baled before it is well dried and it may then heat spontaneously and become mouldy. When this happens the air of the cowshed may be fogged with microbial spores. Pepys *et al.* (1963) showed that farmer's lung disease is caused by inhaling spores of one or two species of thermophilic actinomycetes, especially one now known as *Micropolyspora faeni*. In the air of barns in the West of England where hay was shaken, Lacey & Lacey (1964) found up to 10^9 spores of these thermophilic actinomycetes per cubic metre of air. The disease is extremely disabling, and often involves a slow recovery followed by a change of job.

Few of the outdoor moulds are true human pathogens, but Emmons (1962) photographed visible clouds of *Aspergillus fumigatus*, a fungus with considerable power of invading lung tissue, arising from fermenting vegetation outdoors.

In various regions of the world three moulds are both widespread and pathogenic to man: *Coccidioides immitis*, *Cryptococcus neoformans* and *Histoplasma capsulatum*. All these invade by the respiratory route. They usually produce at most transient disease, except in rare cases when infection progresses to a systemic mycosis. Yet in many areas 60 to 90 % of the population react to intradermally injected antigen from one of these fungi—evidence of widespread subclinical infection from inhalation of outdoor air.

In plant pathology many fungi pathogenic to crops are regularly spread by wind. The process goes on season after season. It becomes spectacular when a pathogen reaches an area formerly free from it.

The well-known microbiological doctrine that all kinds of microbes are universally distributed and are merely selected by the substrate is not universally true, and when applied to pathogens it is dangerous.

Puccinia polysora is one of the rust fungi attacking maize. It was first recorded in Alabama in 1879. It probably originated in the New World; and American maize varieties sustain little damage, as though a balance had been established by selection. Records of its first occurrence in different parts of the Americas are probably examples of the increasing occurrence of trained observers.

However, in 1949 the rust suddenly appeared as a severe disease of maize in Sierra Leone. In contrast with American maize, the African varieties were susceptible and the attack was crippling. From Sierra Leone the pathogen spread rapidly across Africa, evidently by wind. It reached all other parts of West Africa by 1951, Congo and East Africa by 1952, Rhodesia and Madagascar by 1953, and the islands of the Indian Ocean by 1955. While this was happening another focus was developing, starting in Malaya in 1948 and spreading to Siam and the Philippines, reaching Queensland in 1959 and Fiji in 1961.

Cammack (1959) discussed possible ways in which *Puccinia polysora* could have reached Africa from America, and rejected the possibility of wind transport. He concluded that the pathogen must have been carried by aircraft, either with seed corn for experimental purposes, or on corn-on-the-cob for feeding troops. There seems no reason to doubt this conclusion.

Evidently, until 1949, the fungus lived on tolerant varieties of maize in America, doing little damage, separated from large areas of highly susceptible maize in Africa by the doldrums of the Atlantic Ocean, which formed an impassable barrier to the natural spread by wind of its not very hardy spores. But, once established in Africa, natural barriers were insufficient to prevent its spread east and south over the rest of the continent by windborne spores. No doubt it travelled in a series of leaps (maize is extensively grown in Africa) at the average rate of about 750 miles a year. Presumably a similar series of events occurred around the other focus in the Far East, where a separate introduction probably occurred from America across the Pacific.

The geographical spread of coffee rust has been less regular, partly because coffee cultivation is localized (in contrast with maize in Africa), and partly because transport by man and transport by winds have probably operated together. *Hemileia vastatrix* is thought to have originated in Ethiopia on wild coffee. In 1868 it somehow reached Ceylon, wiped out the cultivation of *Coffea arabica* there, and spread rapidly eastwards, reaching Samoa by 1894. Its irregular occurrence down the eastern side of Africa may reflect both the absence of trained observers and its milder attack on *C. robusta*. Its spread to West Africa was comparatively slow, and it reached the Ivory Coast only in 1952.

The important New World coffee growing areas remained free. However, this year it was reported in Brazil, attacking coffee in a wide area in the State of Bahia. The extent of wind transfer of coffee rust spores is controversial. But the answer to this controversy must affect measures designed to prevent spread within Brazil, and to the other American coffee areas farther north.

The disease could have been introduced into Brazil by human agency. Like maize rust, coffee rust seems to have been unable to cross the doldrums of the Atlantic near the equator in 20 years since it reached the west coast of Africa. However, it may be significant that the disease was recorded in Bahia only four years after its first record in Angola, from where, as pointed out by Johnson & Bowden (1971), it could easily be injected by convection into the South-East trade winds to make the crossing in 4 or 5 days.

The spread of virus diseases to man by wind was first indicated by circumstantial evidence with occasional cases of smallpox and *Q*-fever, and with an outbreak of psittacosis downwind of a poultry processing works. C. V. Smith (1964) gave evidence that fowl pest virus may be airborne in dust arising from intensive poultry houses. It now seems that our ignorance of virus transfer by wind has led us to neglect some of the appropriate precautions for preventing the spread of foot and mouth disease.

Foot-and-mouth virus was long assumed to be spread only between animals in close contact, and by fomites. The Report of the Committee of Inquiry (1969) into the 1967–8 epidemic in Britain indicated otherwise. So, too, do earlier outbreaks when records were examined retrospectively by Hurst (1968).

The initial outbreak in 1967, near Oswestry, is considered to have come from imported bones fed to pigs. Spread from this focus eventually reached 2300 farms, on which a total of nearly half a million animals were slaughtered. Spread of the disease from the Oswestry focus was strikingly confined to an arc downwind, while herds on other sectors at first remained free. Smith & Hugh-Jones (1969) showed that, in addition to wind direction, spread was correlated with the occurrence of rain at night. But whether the rain acts at the source, or during wind transport, or at the receiving end, is not clear. Just how animals become infected after wind transfer of inoculum is still in dispute. If the animal becomes infected through eating contaminated fodder, rain could act by scavenging the virus particles (perhaps already attached to larger carrier particles that would be more easily picked up by raindrops), and concentrating them on herbage, whence they would reach the alimentary canal, as suggested by Smith & Hugh-Jones (1969), and Chamberlain (1970).

However, as Norris & Harper (1970) point out, massive doses are needed to infect through the alimentary route, and infection by inhalation seems more probable. It was assumed that the virus aerosol is emitted by infected animals. If inhalation is the route, then the role played by rain in infection might be in maintaining an atmospheric relative humidity favourable for the virus to survive in small droplets during transit.

Reflexion suggests some inadequacies in our current explanations. Analogy with better-known components of the air spora suggests the existence of a massive source with an adequate launching mechanism. Can the production of an aerosol from infected animals account for the widespread infections observed, reaching a distance of 60 miles from a source—even with aid from lee waves as suggested by Tinline (1970)?

I would like to see a balance sheet showing the total quantities of virus an infected animal liberates into its environment in various ways. Sellers & Parker (1969) measured the concentrations of foot-and-mouth disease virus aerosols associated with *housed* animals. We now need a measure of the aerosol liberated by the total environment of animals in the field. Urine and faeces might well prove to be the most significant sources in transfer by wind between farms, not because

virus is highly concentrated in urine and faeces, but because of their relatively large volumes and ability to spread over a large area of ground where rainsplash could make the virus an aerosol.

A paper by Gregory, Guthrie & Bunce (1959) showed that impact of a single raindrop with a liquid surface film throws into the air a thousand or two small splash droplets coming from the rays of the splash cup. Further, we showed that most of these droplets consist of a mixture of liquid from the incident drop, plus liquid from the surface film with its contained microbes.

In the Oswestry outbreak the pigs were in a yard and in the surrounding field, and could have been excreting virus in urine and faeces over the field for several days before foot-and-mouth-disease was apparent. The fall of one centimetre of rain could easily launch 10^{12} virus-containing droplets from each acre of contaminated ground. Moreover, much of the virus-containing aerosol would be in the form of droplets or droplet nuclei, too small to be removed from the air readily by rain, but of the size readily inhaled and retained in the respiratory tract.

The purpose of these reflexions is to direct attention to contaminated ground as an aerosol source of great potential danger in foot and mouth disease. The source is vast enough to account for distant spread, and if it is eventually incriminated, it will be impossible to disregard ground-contamination in control measures.

Monitoring atmospheric microbes

The results obtained from continuous sampling for spores and pollen illustrate the kinds of phenomena to be expected when the atmosphere is searched systematically for other groups of microbes.

The Allergy Department at the Wright–Fleming Institute has monitored fungus spores and pollen almost continuously for nearly 15 years on the roof of St Mary's Hospital, Paddington. A comparable series is needed in rural areas. Aphids are now monitored by continuous sampling during the crop-growing season by a chain of trapping stations across the United Kingdom and extending to the Continent (Taylor & French 1968). It seems relevant that over level country in Britain the radius of validity of a single trap at 12 m (40 ft) above ground level is about 100 km. But catches from two traps 1000 km apart are not correlated.

International co-operation in microbial monitoring of the atmosphere is encouraged by the International Biological Programme through an International Working Group under the Chairmanship of Dr W. S. Benninghoff of the University of Michigan.

We need to determine the limits of concentrations of various microbial types, and set up norms for detecting long-term changes in the human environment (cf. Hyde 1959). Also to detect short-term changes, such as result from the practice of spraying effluent from farm animals over grass fields. We need to be able to predict unusual concentrations so as to take avoiding action, and to predict amounts of deposition and the diffusion of microbial clouds, and again, to recognize

in advance the onset of conditions for crop epidemics, perhaps by correlated changes in some indicator saprophyte. In all this, closest links with meteorologists are essential.

As yet we know little about the microbial life of our atmosphere. What little information exists is chaotic and uncorrelated: the air spora has never been systematically explored simultaneously in different parts of the world by comparable methods.

Conclusion

Now, Mynheer van Leeuwenhoek, watching from your portrait,† we can report back. Probably an aerial plankton living in the clouds does not exist; at any rate, like you, we have no evidence of it. But, as you surmised, there is in the atmosphere a rich, varied and constantly changing transient throng of minute organisms, not only carried by the dust, but often constituting that very dust itself, as it did the fiery red powder you examined that covered your shoes when you walked through grassy meadows in September 1678.

You under-estimated the ability of these microbes to reach great heights in the atmosphere.We can report that some are hardy and survive easily during wind transport, though others are tender and have viability problems.

In addition to being the direct origin of the teeming life you observed in your rainwater, some of these airborne microbes have a potency for disease in man, his herds, and his crops, that would have astonished you and your contemporaries, and still continually take us by surprise.

† By the terms of the bequest of George Gabb who founded the Leeuwenhoek Lecture, the framed copy of the engraved portrait of Leeuwenhoek, which he presented to the Society, must be exhibited to the audience on the occasion of each lecture. (Plate 14.)

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