The Microbiology of the Atmosphere

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THE MICROBIOLOGY OF THE ATMOSPHERE

By

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DEDICATED

to

my wife

MARGARET FEARN GREGORY

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PREFACE

AEROBIOLOGY is usually understood to be the study of passively airborne micro-organisms—of their identity, behaviour, movements, and survival. One characteristic, which it shares with many other population studies in biology, is that the ultimate relevant unit consists of the individual cell or small group of cells. Analysis at the molecular or sub-atomic level is irrelevant to our present purpose. Like geography, aerobiology is an agglutinative study, drawing information from many kinds of scientific research. Although it already has its patron saint, Pierre Miquel, and its martyr, Fred C. Meier, aerobiology is best regarded as an activity whose material will in due course be incorporated into the main body of biological science—without, I hope, any necessity for splinter societies, journals, and international conferences.

This book amplifies and extends a course of Intercollegiate Lectures given to botanical students in the University of London in 1956. The theme, which has occupied me for over fifteen years, is as follows. Transport through the atmosphere is the main dispersal route for such organic particles as the spores of many micro-organisms. How do the properties of the atmosphere, and the properties of these particles themselves, affect their dispersal? How do the particles get into the air? How far, and in what numbers, are they dispersed? By what processes do they become grounded, so that they can continue growth? What is in the air, and how can we measure it? What are the practical consequences of this process for the micro-organisms themselves, and for man, other animals, vegetation, and crops?

Although there are one or two other books on airborne microbes, this is the first to treat the subject as a world-wide phenomenon. It is, perhaps, inevitable that it should be attempted by a mycologist. Few other biologists find their material so dominated by the atmosphere, and no other micro-organisms have so thoroughly exploited the possibilities of aerial dispersal as the fungi. One of the fascinations of the subject is the impact of facets of its knowledge on such apparently diverse topics as artificial rain-making, allergy, smoke screens, effluent of nuclear power-stations, crop protection, icing of aircraft, air hygiene, and many other topics. This book treats of the development and principles of aerobiology rather than applications; yet the stimulus to nearly all aerobiological work comes from applied science.

In this book the term 'microbe' is used freely when a general word is wanted; but, like the word 'spore', it has admittedly been stretched beyond its normal meaning. Airborne pollen of flowering plants must be included and is safely covered by the term 'spore' (botanically: 'microspore'); but are pollen grains and mushroom spores microbes? There is no other commonly accepted word that covers quite what is meant by the word 'spore' as used here: 'propagule', 'disseminule', 'biota', 'diaspore'? We have isolated part of the continuum for study but find we are not well-equipped verbally for the task of dealing with it. The microbial population of the atmosphere is referred to here as the 'air-spora', using 'spora' as a word analagous to 'flora' and 'fauna'.

Botanical nomenclature has presented some difficulties: authorities have not been given for specific names, and the names used by other authors have usually been quoted as given in the original papers—without necessarily attempting to guess what was meant, or following the nomenclature fashionable in 1960. I have converted other workers' numerical data to the metric system, and temperature to the Centigrade scale, to aid comparison, and have moreover assessed spore concentrations on the uniform basis of number per cubic metre.

Frequently, in making general statements, I have omitted safeguarding, but tedious, escape clauses: this has been done to spare the reader who will understand that biological generalizations abound in exceptions and complexities.

Interpretations in this book are mostly my own responsibility, but I am grateful for help received from many people during its preparation. In particular I offer my thanks to the following: G. Samuel and W. Buddin for introducing me to dispersal problems in the field; F. C. Bawden for encouragement in the study of aerobiology and for reading this book in manuscript; E. C. Large for advice on planning the book; D. A. Boalch (and many other librarians) for continual help with the literature; members of the British Mycological Society for named specimens of fungi, and H. L. K. Whitehouse for mosses; A. Horne, V. Stansfield, and F. D. Cowland for photography; R. Adams, G. C. Ainsworth, J. R. D. Francis, E. J. Guthrie, Elizabeth D. Hamilton, J. M. Hirst, C. T. Ingold, C. G. Johnson, F. T. Last, Kate Maunsell, T. Sreeramulu, and O. J. Stedman for discussion and help with aerobiological problems and applications; Audrey Baker, Beatrice E. Allard, and Marie T. Seabrook for clerical assistance; and Maureen E. Bunce for experimental help, revision of the manuscript, and preparation of many of the illustrations —especially the paintings for Plates 1, 5, 6, and 7. I also wish to thank authors, editors, and publishers for permission to copy illustrations which are acknowledged in the text. Finally, for the calculations involved in Figs. 24 to 27, and for those chapters needing the help of a mathematician, I have been fortunate in having the constant advice and willing help of my wife, Margaret F. Gregory, to whom I am most deeply grateful.

Rothamsted Experimental Station PHILIP H. GREGORY
Harpenden, Herts., England.
September, 1960.

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I

HISTORICAL INTRODUCTION

THE air we breathe, like our food and drink, varies in quality from time to time and from place to place. This fact was recognized many centuries before industrialized man assumed the right to pollute the atmosphere with poisonous chemicals and radioactive isotopes.

In Britain we hold that, 'when the wind is in the East 'tis neither good for man nor beast'. Some places are noted for invigorating air, and some for relaxing air; but it is not yet clear whether these properties are associated merely with differences in temperature, humidity, and movement of a gaseous mixture consisting mainly of 78 per cent nitrogen, 21 per cent oxygen, and 0·03 per cent carbon dioxide with traces of the inert gases, or whether some other factor or factors are involved.

SPECULATIONS ON THE ORIGIN OF DISEASE

Classical writers believed that the wind sometimes brought sickness to man, animals, and crops. Hippocrates, the father of medical science, held that men were attacked by epidemic fevers when they inhaled air infected 'with such pollutions as are hostile to the human race'. A rival, though perhaps not entirely incompatible, view held that epidemics were the result of supernatural agencies, and were to be warded off or cured by taking appropriate action.

Lucretius in about 55 B.C. held quite modern views. He observed the scintillation of motes on a sunbeam in a darkened room and concluded that their movement must result from bombardment by innumerable, invisible, moving atoms in the air. This brilliant intuition enabled him to account for many interesting phenomena, including the origin of pestilences. We now know that bodies which transmit human diseases through the air are larger than those which Lucretius thought of as atoms—the mosquitoes carrying malaria, for instance, or the droplets which spread the common cold and influenza viruses indoors. But in his concept of baleful particles carried in clouds by the wind, settling on the wheat or inhaled from the polluted atmosphere, Lucretius touched on some of the main problems existing in plant pathology and allergy today.

EARLY MICROSCOPISTS AND THE DISCOVERY OF SPORES

After Lucretius, more than 1,500 years passed before men even began to be aware that the air teems with microscopic living organisms.

The discovery had to wait almost until the invention of the microscope.

For a long time after Aristotle and Theophrastus, the lower plants lacking obvious seeds were believed to be generated spontaneously in decaying animal or vegetable matter. The same view was held of the origin of many of the lower animals. However, the minute 'seeds' or spores of several kinds of plants were observed in the mass long before the invention of the microscope allowed them to be identified and observed individually. What was more natural than to suppose that these minute particles were wafted about by the winds?

The discovery of reproduction of ferns is attributed to Valerius Cordus (*b.* 1515, *d.* 1564), and spores of the fungi seem to have been observed soon after this by a Neapolitan botanist, J. B. Porta, although the rusty-coloured spore deposits under bracket-fungi on beech trees must always have been familiar to the countryman.

It was P. A. Micheli (*b.* 1679, *d.* 1737), botanist to the public gardens at Florence, who first illustrated the 'seeds' of many fungi, including mushrooms, cup-fungi, truffles, moulds, and slime-moulds. Further, by sowing spores on fresh-cut pieces of melon, quince, and pear, and reproducing the parent mould for several generations, he showed that the spores of some common moulds were, indeed, 'seeds' of the fungi. He noted, however, that some of his control slices also became contaminated, and he concluded that the spores of moulds are distributed through the air (*see* Buller, 1915).

The hand-made lenses of Anton van Leeuwenhoek rendered visible the world of minute organisms whose existence had only been guessed at before, and whose significance in nature had scarcely even been imagined. He could just see bacteria, and in his letters to the Royal Society in 1680 he described some yeasts, infusoria, and a mould. From his experiments he came to doubt the current belief in spontaneous generation; it seemed more plausible to him to suppose that his 'animalcules can be carried over by the wind, along with the bits of dust floating in the air' (Dobell, 1932). The controversy over spontaneous generation was to last for a couple of centuries; but, in the second half of the eighteenth century, ideas were developed by Nehemiah Grew and E. F. Geoffrey on the function of the pollen of flowering plants. J. G. Koelreuter, in 1766, was perhaps the first to recognize the importance of wind-pollination for some plants and of insect-pollination for others. C. K. Sprengel in 1793 developed these views and concluded that flowers lacking a corolla are usually pollinated in a mechanical fashion by wind. Such flowers have to produce large quantities of light and easily-transported pollen, much of which misses its target or is washed out of the air by rain. Thomas A. Knight in 1799 reported that wind could transport pollen to great distances.

By the beginning of the nineteenth century, therefore, it was recognized that pollen of many, but by no means all, species of flowering plants, and the microscopic spores of ferns, mosses, and fungi—as well as protozoa—were commonly liberated into the air and transported by the wind. The potential sources of the air-spora had been discovered and identified in the main before the year 1800, but their role remained obscure.

Controversy on Spontaneous Generation *

Leeuwenhoek had come to doubt the belief, dating from Aristotle, that flies, mites, and moulds were generated spontaneously by decaying animal and vegetable matter. To him it seemed likely that animalcules could be carried by the air, and this provided an alternative explanation to spontaneous generation. J. T. Needham (*b.* 1713, *d.* 1781) had claimed that minute organisms would appear in heated infusions; but L. Spallanzani (*b.* 1729, *d.* 1799) showed, by a series of experiments, that when organic materials were subjected to sufficient heat-treatment (with various precautions against contamination) they would neither putrify nor breed animalcules *unless exposed to air*. From this Spallanzani concluded that the microbes were present in the air admitted experimentally to his sterilized vessels. A rearguard action was fought to explain away these results. J. Priestley (*b.* 1733, *d.* 1804) and L. J. Gay-Lussac (*b.* 1778, *d.* 1850) claimed that heating the vessels drove out the air and that it was *shortage of oxygen*, not lack of 'seeds', which prevented heat-sterilized materials from generating a microbial population.

Meanwhile, Appert (1810) put heat sterilization on a commercial basis by applying it to food preservation; but the controversy lingered on, even into the present century, although the experiments and polemics of Louis Pasteur were decisive. Pasteur showed that food could be conserved *in the presence of oxygen* and that preservation depends on the destruction by heat of something contained in the air. In 1859 F. A. Pouchet, of Rouen, had raised the objection that a very minute quantity of air sufficed to allow the development of numerous microbes in heated infusions, and that the air would have to be a thick soup of microbial germs.

In reply, Pasteur (1861) sterilized a series of evacuated flasks containing nutrient medium. So long as the flasks remained unopened they all remained sterile; but, even when they were opened and air was admitted, he found that one or two out of each batch would remain sterile on incubation. Pasteur replied to Pouchet, denying that only a minute quantity of air needs to gain access for a microbe population to develop and for putrefaction to take place. On the contrary, the cause of the phenomenon was discontinuous and a sample of 250 cc. of air might *or might not* contain germs.

Pasteur then showed, by opening batches of about forty such flasks in various sites, that the quantity of airborne germs differed in different places. In the open air in Paris he obtained bacteria, yeasts, and moulds;

* *See also* Bulloch (1938) and Oparin (1957).

but some flasks remained sterile. In cellars of the Observatoire, where the temperature was constant and the air still and dust-free, many more flasks remained sterile.

On 5 November 1860, Pasteur deposited at the office of the Academy no fewer than seventy-three quarter-litre flasks, some of which he had opened to the air in batches of twenty at various heights ranging from the foothills of the Jura to high up on Mont Blanc, as follows:

		Number of flasks	
Altitude	Locality where air sampled	Contaminated	Sterile
	Country air, far from dwelling houses, on the first plateau of the Jura	8	12
850 metres	Jura mountains	5	15
2,000 metres	Montanvert, near Mer de Glace on Mt. Blanc	1	19

The cause of this supposed 'spontaneous generation' was not only discontinuous but, moreover, its concentration decreased with height.

F. A. Pouchet had admitted that among dust particles of vegetable origin there were some spores of cryptogams, but he held that these were too few to account for the phenomena of putrefaction.

Pasteur decided that he would abandon Pouchet's method, which relied on examining spontaneous deposits of dust on the surface of objects, in favour of a new method of studying the particles by collecting from actual suspension in the air. Pouchet had drawn invalid conclusions from surface deposits because, according to Pasteur, the light air-movements which constantly play over surface deposits would pick up and remove the extremely minute and light spores of microbes more readily than they would any coarser particles. (It now appears, however, that the small numbers of the lighter bodies in surface deposits is due to the extreme slowness with which they are deposited, rather than to their preferential removal after deposition.)

Pasteur's apparatus for extracting the suspended dust in the air, for microscopic examination, was quite simple (Fig. 1). A tube of ½ cm. diameter was extruded into the open air through a hole drilled in a window frame several metres above the ground. The rear part of the tube was packed with a plug of gun-cotton to catch particles. Air was drawn through the apparatus by means of a filter pump, and the volume of air was measured by displacement of water. Tests were made on air drawn from beside the Rue d'Ulm, and from the garden of the École Normale in Paris. During aspiration, solid particles were trapped on the fibres of the gun-cotton plug. After use, the gun-cotton was dissolved in an alcohol-ether mixture, the particles were allowed to settle, the liquid was decanted, and the deposit was mounted for microscopical examination.

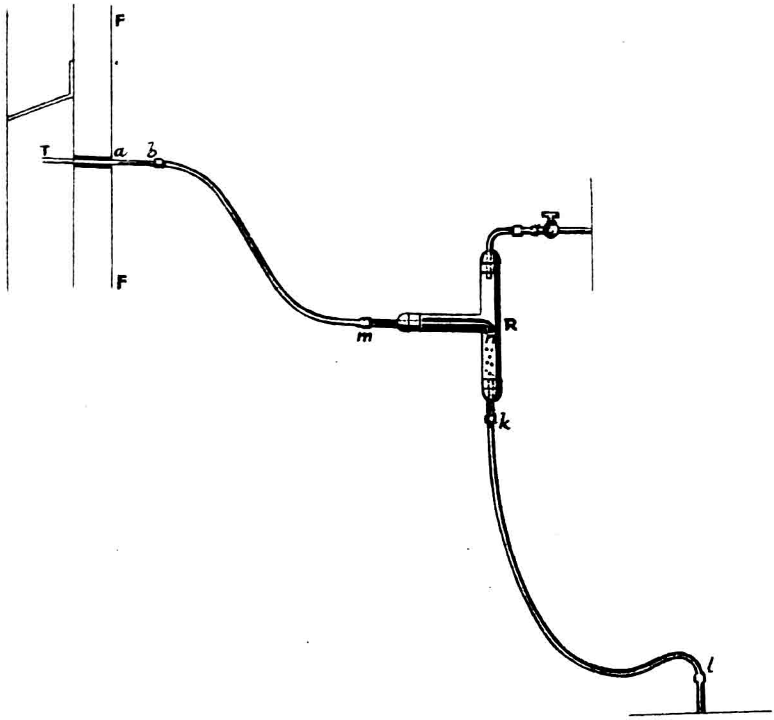

FIG. 1.—Pasteur's gun-cotton filter for airborne microbes.

a = gun-cotton plug, 1 cm. long, held in position by:
b = spiral platinum wire.
FF = window frame drilled to allow passage of:
T = tube to exterior for sampling outdoor air.
R (*m.k.l.*) = aspirator.

Pasteur, as usual, had little interest in the specific identity of his organisms; he was no taxonomist. The particles exactly resembled the 'germs' of lower organisms. They differed in volume and structure so much among themselves that they clearly belonged to very many species or even groups, including bacteria, moulds and yeasts. Their numbers contradicted the general conclusion that the smallest bubble of air admitted to a heat-sterilized medium is sufficient to give rise to all the species of infusoria and cryptogams normal to an infusion. This view was shown to be highly exaggerated, and Pasteur indicated clearly that it is sometimes possible to bring a considerable volume of ordinary air into contact with an infusion before living organisms develop in the latter.

Pasteur had demonstrated visually the existence of an air-spora, he had pointed out that it should be measured while in suspension and not after deposition on surfaces, and he had made the first rough visual measurements of its concentration in the atmosphere of the City of Paris: a few metres above the ground in the Rue d'Ulm, after a succession of fine days in summer, several thousands of micro-organisms were carried in suspension per cubic metre of air. He then abandoned the method—remarking, however, that it could doubtless be improved and used more extensively to study the effects of seasons and localities, and especially during outbreaks of infectious diseases.

The Germ Theory of Disease

We must now look back and trace the growth of the microbial theory of disease, that had been developing for more than a century.

The minute growths of fungus noticed for centuries on mildewed or 'rusted' plants were believed to be a consequence of the diseases; the dusty powder on rusted wheat was regarded as a curiously congealed exudation of the diseased plant itself. But might this not be putting the cart before the horse? Could the rust possibly be the cause of the disease instead of an effect? Perhaps the first to give reasonably affirmative evidence was Fontana (1767), who examined wheat rust with his microscope and described what he saw as a grove of parasitic plants nourishing themselves at the expense of the grain.

As further crop diseases were studied it became clear that, in some, infection is acquired by planting in contaminated soil, while others are carried on seed and still others are spread in the wind by airborne fungus spores (*see* Large, 1940).

The discovery that microbes can cause disease in man and animals came somewhat later, and the first animal pathogens to be recognized were again fungi—no doubt because they were easier to find than bacteria. In 1835, Agostini Bassi showed conclusively, by inoculation experiments, that a specific mould is the cause of the 'muscardine' disease of silkworms which was then threatening the silk industry of Piedmont. Next, historically, came the recognition of the fungi causing favus, ringworm, and 'thrush' in man, as a result of the work of David Gruby and Charles Robin.

Pasteur had demonstrated that microbes are normally abundant in the air. Many of them can cause fermentation or putrefaction when introduced into sterile organic substrates; and it was natural to speculate that others might be the causes of epidemics of some of the so-called 'zymotic' diseases whose etiology was then unknown. Medical workers soon began a systematic search among airborne microbes for the unknown causes of infectious diseases.

The search was long, and on the whole unfruitful because most epidemic diseases that attacked man were gradually traced to sources other than the outdoor air. However, in the course of the search, most of the important characteristics of the air-spora were discovered—and then forgotten. The search occupied the last thirty years of the nineteenth century and coincided with the golden age of bacteriology. Listing the dates of contemporary salient advances in bacteriology will help to give the background to this phase of aerobiology (*see* Bulloch, 1938).