Characterization of Biological Particulate Loads in Metropolitan Air

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ABSTRACT.—The atmospheric particulate load includes a wide range of naturally occurring particles of biological origin that serve as a reservoir of allergenic agents in respiratory disease. Improved knowledge of potential aeroallergens is needed by medical clinicians. Aims are to better characterize air spora, qualitatively and quantitatively, and determine daily (by hour) periodicities of occurrence and prevalence of different kinds of spores, and seasonal variations. Main emphasis is on fungus spores about which least is known though they occur in considerable numbers. Efficient volumetric samplers provide time-related deposits of air spora at three sites in Pennsylvania.

INCLUDED IN THE ATMOSPHERIC load of particulates is a wide range of naturally occurring particles of biological origin, such as microorganisms, pollens and other plant spores, fragments of plants and insect parts, etc. This diverse air spora of natural origin also serves as a reservoir of allergenic agents in respiratory disease. Improved knowledge of potential aeroallergens is much needed by medical clinicians.

Characterization of airborne biological particulates is the first phase of a study of aeroallergens being carried out at the Center for Air Environment Studies at The Pennsylvania State University. Aeroallergens are defined as substances encountered in the air which can incite allergic responses. Generally, these are inhalant allergens, and the major responses are allergic rhinitis, including seasonal hay fever, and bronchial asthma.

Primary aims of this phase of the project are to apply modern techniques of air spora study to: (1) characterize qualitatively the potential aeroallergens, mainly fungus spores and pollens, of major regions of Pennsylvania; (2) to provide quantitative volumetric data on air spora; and (3) to determine circadian (daily, time-related) and seasonal periodicity of occurrence and prevalence of different kinds of spores. We also are beginning to relate spore data to appropriate meteorological variables where possible.

The economic impact of aeroallergens can be assessed in only general terms. Of the United States population, about 15 percent may be sensitized easily to many allergenic materials and another 25-30 percent are considered somewhat sensitive *(Kern 1962)*. Approximately 15 million persons in the U.S. suffer effects of hay fever and/or asthma. More than $70 million are spent on prescription medications related to allergic response each year. About 25 million work days are lost per year due to hay fever. Further, lowered work efficiency due to symptoms and sedative effects of some medications is probable. Allergic rhinitis also often increases susceptibility to infections of the respiratory tract. Asthma incidence leads to an estimated $260 million a year in resource costs, including premature deaths, medical treatments and absenteeism *(Finkelstein 1969)*.

Almost any organic material, from plant or animal sources, may be a potential allergen. Among the more common known aeroallergens are pollens, fungus spores, danders (animal hairs, feathers, flakes of epidermis, etc.); house dust (heterogenous mixture); insect parts; algae; certain cosmetics; and products containing linseed oil, organic solvents, etc.

The two most important groups of naturally occurring aeroallergens are the pollens and fungus spores. Pollens are usually considered to cause the most problems, and ragweed is believed to account for some 90 percent of pollenosis in the U.S. *(Hewson 1967)*.

POLLENS IN THE ATMOSPHERE

Pollens are produced in great numbers throughout the world. Of the approximately 100,000 known species of flowering plants, about 10 percent are adapted to wind pollination *(Gregory 1973)*. Most pollen grains range in size from 15 to 50 μm, and most species have distinctive features and characteristic size. Gravitational fall rates range from about 1 to 10 cm/sec. In general in the temperature zone, the tree-pollen season is in the spring, the grass season from late spring through summer, and the weed season mostly in late summer and fall. There are of course variations and overlappings depending on species and climate as well as more local meteorological conditions.

The members of the ragweed group *(Ambrosia* sp.*)* are prolific pollen producers, and the pollen grains are exceptionally well suited to aerial dissemination. The plants may grow in abundance where soil and cover vegetation have been disturbed as in housing developments, along highways, railroads, etc., as well as on agricultural land.

Millions of acres in the U.S. are infested by ragweeds to some extent. Surveys have revealed as many as 170,000 plants/acre (about $42/m^2$) in some fields when no cover crops were grown after winter cereal grains were harvested. About 56,000 plants/acre ($11/m^2$) were found in a subdivision area under construction *(Hewson 1967)*. An estimated 50 pounds (22 kg) of pollen could be produced by an acre of giant ragweed plants in a season *(Finkelstein 1969)*; at 200 million grains/gram of pollen, this means 4.5 x 10^{12} pollen grains could be produced per acre over an 8-week pollen production period. About 5 to 10 percent of the pollen can be expected to become airborne.

The distance traveled depends on many factors, and it should be noted that airborne particles are being deposited continuously on surfaces near the ground. Despite deposition, the proportion escaping to be transported some distance from the source places considerable numbers in the air to be dispersed over large areas. Concentrations of several hundred ragweed pollen grains/m^3 of air are not uncommon over much of the central and eastern U.S. during the ragweed season each year *(Hewson 1967)*. Peak concentrations over cities some distance from major sources may reach several hundred per m^3 in some areas. Cities often have local sources of ragweed as well.

The relationship of concentration of pollen in the air to production of symptoms is not well known. Individuals may differ greatly in their sensitivity to dosage. However, it has been reported that as few as 100 pollen grains inhaled over a 1-hour period often caused symptoms, and in one individual as few as 30 incited hay fever *(Connell 1968)*. Since the volume of air inhaled approximates 1 m^3/hr for an adult engaged in light activity, it is apparent that exposure levels commonly encountered could readily incite allergic responses in susceptible individuals. It is also known that being sensitized to one pollen or substance can increase sensitivity to certain other allergens *(Connell 1969)*.

Ragweed pollen fall rate in air due to gravity is about 1cm/sec, thus convection currents can readily carry them aloft. Most ragweed pollen is released in the morning hours, but concentration peaks may occur also later in the day or at night due to pollen descending from high altitudes. In general there is a decrease in concentration with height, though greater numbers may occur at 1 or 2 km altitude than at ground level, due to various atmospheric factors and source locations *(Gregory 1973)*.

FUNGI IN THE ATMOSPHERE

Fungus spores, which are usually more prevalent in air than pollens (often >75 to 1) also can act as potent allergens *(Hamilton 1959)*. Fungi grow on organic matter and some parasitize living plants, insects, and animals. Most spores occurring commonly in outside air originate from fungi growing on plant debris or on surfaces of living plants, trees, etc., rather than from soil as sometimes inferred. Air in buildings may in addition to spores from outside air also contain spores from moldy surfaces and mildewed materials within the building.

Fungus spores are usually smaller than pollens. Most range in size from 3 to 30 μm (the size range of water droplets in clouds). Gravitational settling rates are 0.05-2 cm/sec, and thus most fungus spores become airborne and remain suspended longer than pollens. Viable fungi have been recovered at altitudes of 17 miles (27 km), and over oceans thousands of miles from land as well as over the North Pole *(Ingold 1971)*.

Many thousands of species of fungi produce spores that may become airborne. (Fortunately only a few hundred types are found in air samples that we have studied!) A few examples of prolific spore production are as follows: a single large puffball may contain 7×10^{12} spores; a large specimen of bracket fungus may produce 3×10^{10} spores/day for a 5-month season; an average field mushroom might produce 10^8 spores per day; a single smut-diseased wheat grain may contain 12×10^6 spores; and a single colony of blue mold *(Penicillium* sp.*)* 2.5 cm (1 in) diameter may bear about 4×10^8 spores *(Ingold 1971)*.

Concentrations of about 10,000 fungus spores/m^3 of air may be typical on a summer afternoon *(Hirst and Hurst 1967)*. Numbers may vary greatly, depending on species prevalent, previous conditions for growth and spore production, location, climate, geography, season, weather (temperature, humidity, wind speed, turbulence, etc.), time of day, biotic factors, etc. Exposure depends on an individual's activity and location at any particular time.

Spores of the genus *Cladosporium (Hormodendrum)* seem to be the most numerous found in samples from several places in the world, often comprising about 50 percent of the total spores recorded. This genus is also considered one of the more allergenic encountered.

SAMPLING SYSTEMS

The practice of allergic medicine in the United States has, to some extent, been dependent upon an incomplete knowledge of the kinds and amounts of spores in the atmosphere, particularly fungal spores. This project aims at providing improved characterization, qualitative and quantitative, of air spora occurrence. The main emphasis is on fungus spores, since pollens are better known *(Wodehouse 1971, Jones 1952, Hyde 1959, Hyde and Adams 1958)*, and the modern techniques of air-spora study employed are well suited to improved sampling of the smaller fungus spores. Also, excellent studies of ragweed pollen production and dispersal have been undertaken *(Sheldon and Hewson 1962, Ogdon et al. 1966 and 1969, Harrington 1965, Raynor et al. 1973 and 1974)*.

In 1946, the American Academy of Allergy standardized the methods of assessing pollen in the air *(Durham 1946, Durham et al. 1947)*. This "gravity settling" method was extended to estimates of fungus spores in 1948 *(Merksamer and Sherman 1958)*. In prior surveys, culture-plate studies were made. Much useful information has been derived from these methods, and they are still in common use.

After the development and testing of efficient volumetric suction samplers *(May 1945, Hirst 1952, Andersen 1958)* it became quite clear that the simple gravity samplers can give very distorted estimates of pollen and fungus spore concentrations and may result in serious underestimation of the number of different types actually present in the air. Problems are more serious for fungus spores than for most pollens, and this is particularly true for species producing smaller spores *(Gregory 1952, Gregory and Hirst 1957, Ogden and Raynor 1960, Solomon 1970, Ingold 1971)*.

In the last 20 years a great deal has been learned about circadian and seasonal periodicities of several fungus spores and pollens, especially in England, and in some studies in the U.S. The techniques of volumetric sampling, coupled with time discrimination of deposits, showed clearly that a considerable variation often occurs from hour to hour, between night and day, as well as seasonally *(Hirst 1953; Pady et al. 1962, 1967, 1969; Gregory 1952* and *1973; Ingold 1971; Haard and Kramer 1970)*.

RATIONALE

There is little definitive quantitative information concerning fungal aeroallergens for most of North America and essentially none for Pennsylvania. It is to be expected that several other fungi will be shown to be quite prevalent in most areas when systematic volumetric sampling is done *(Gregory and Hirst 1957, Hamilton 1959, Adams 1964, Sayer et al. 1969)*. Further, we do not have qualitative or quantitative comparisons of fungal air spora for different regions as affected by climate, seasons, vegetation sources, etc.

A result of the general state of knowledge is that allergists are in a poor position to relate observed symptoms to known botanical events, and must rely mostly on sensitivity testing with prepared extracts of a limited number of empirically determined allergenic materials. Improved methods of characterizing the air spora will reveal more organisms that should be tested as potential allergens, and may elucidate the causes of some of the seasonal and perennial allergies whose etiologies are still unknown *(Greenburg et al. 1966, Girsh et al. 1967, Paulus and Smith 1967)*.

PROCEDURE

Volumetric samplers are operated at such heights above ground (about 30 m) to determine the general concentrations of air spora. Many if not most of the spores moving over a city have exogenous origins some distance away *(Harrington 1965, Ogden et al. 1966, Freedman 1967, Davies 1969b)*. By situating samplers well above ground level (to avoid obstructions to free air movement, such as nearby buildings, tall trees, etc.), local site effects are minimized. Bias due to very localized sources of spora from within a city is reduced by allowing vertical and horizontal mixing time before reaching the sampler. Thus each sampling station becomes representative of a considerably larger area *(Davies 1969a)*.

The Hirst *(1952)* spore trap is a volumetric suction-impaction sampler that gives continuous and time-related particle deposits from known volumes of air. The Casella model (C. F. Casella & Co., Ltd., London, England) is a 24-hour sampler utilizing a standard microscope slide. The Burkard model (Burkard Manufacturing Co., Ltd., Herts, England) operates continuously for 7 days, using a plastic tape on a revolving drum as the collection surface. Both models draw air at 10 liters/

minute through a 2x14-mm orifice. Particles impact on the silicone-greased collection surface, which moves continuously at 2 mm/hr.

Counts and identification are made by microscope at about 1,000 magnifications at positions corresponding to 2-hour intervals. Data derived show the kinds and number of air spora present (per m^3 of air) and the times of occurrence throughout each day.

Weather data are being obtained from stations near the sampling sites for correlation with air spora data. Meteorological information includes temperature, precipitation, wind speed and direction, and relative humidity. Information on large-scale weather systems or other meteorological data available may also be useful in characterizing patterns of occurrence of air spora as related to changing environmental conditions.

The three sampling stations, started in mid-1972 and currently operating are at: (1) Pittsburgh, atop the 11-story Veterans Administration Hospital in Oakland; (2) Philadelphia, on the roof of a 12-story building in Jenkintown; and (3) State College, at the Pennsylvania State University campus on the top of the press box at the football stadium. They are located to provide data representative of three different regions and climatological zones *(Dailey 1971, Dethier and Vittum 1963)* and should provide information typical of large portions of the state, as well as comparisons of two urban settings and a rural setting. Additional sites may be sampled in the future for more direct comparisons of air spora and clinical observations in close cooperation with allerists.

SIGNIFICANCE

Improved knowledge of air spora is needed by medical clinicians. Prospects are good for correlating clinical observations with types, concentrations, and variations in air spora. A better understanding of what is in the air, and when, will give allergists important leads for selecting allergenic materials and extracts for testing and treatment. Fungal spores are ubiquitous and the most common biological component of the atmosphere in most areas. Their importance may well be underestimated in general, particularly those that occur in great numbers but have seldom been revealed by methods commonly in use. Knowledge of circadian periodicities of air spora can be important in determining times of day and peak concentrations of aeroallergens to which patients may be exposed. It seems likely that large populations can be included within the zones applicable to the selected sampling sites and representative of the areas, particularly the regions surrounding the large urban centers.

RESULTS

A great number of different kinds of spores are observed in most samples. Many can be identified readily, and separate counts are made of several generally considered allergenic, such as *Cladosporium, Alternaria, Helminthosporium,* and *Epicoccum.* Special note is made of others when they occur in relatively greater numbers than usual.

Counts are routinely made by using a microscope at about 1,000 magnification to ensure seeing the smallest spores, which can be missed at lower powers. This high magnification, though more time-consuming, allows less chance of misidentifications, especially of the smaller and colorless types, which often are seen in great numbers. To set some reasonable limit on the time involved in reading samples, other spores are placed in categories as to type and appearance. These groups are basidiospores, either colored or hyaline, ascospores (two or three types), Penicillium-Aspergillus types, etc. Separate counts are kept of ragweed pollens, which occur for approximately 2 months in the fall, and a total count for other pollens. Total insect parts and other plant parts are also routinely counted. Special notice is made of unusual occurrences of types not usually seen, or normally of low frequency, for possible correlations with clinical data at a later time. The sample slides are essentially permanent and can be reexamined when desired for more detailed or special studies.

Cladosporium

The most consistent and generally numerous type of fungus spores found are of the genus *Cladosporium*. This fungus, in the medical literature often referred to as *Hormodendrum*, has long been recognized as allergenic. The spores are usually released during drying conditions, that is, during the period of lowering relative humidity and increasing air turbulence such as found in the mornings of sunny days. High counts of this group may also occur at other times, even at night, as a result of gravitational settling or the subsidence of air from higher altitudes containing heavy spore loads derived from air that rose earlier in the day, or even on previous days, many miles upwind. Figure 1 illustrates typical occurrences of *Cladosporium* to be expected.

Basidiospores

Most of these spores come from the more commonly seen fungi, due to their larger size, such as mushrooms, bracket fungi, and puffballs. Some basidiospores are released throughout the day as long as sufficient moisture is available to the fruiting structures. Others apparently are released mainly at night when humidity is usually the highest. As a result, peaks of basidiospores in the atmosphere are usually observed between midnight and 0600 hours. It should be noted that because of their small size (mostly 10 μm or less), basidiospores have not often been seen or counted by using the old standard gravity slide techniques. Only with volumetric sampling are we able to provide data as shown in figure 1. The common occurrence of basidiospores in considerable numbers, at times the most prevalent spore group in the atmosphere, has not been generally known to allergists. A need for more clinical testing for sensitivity to members of this group of fungi seems warranted.

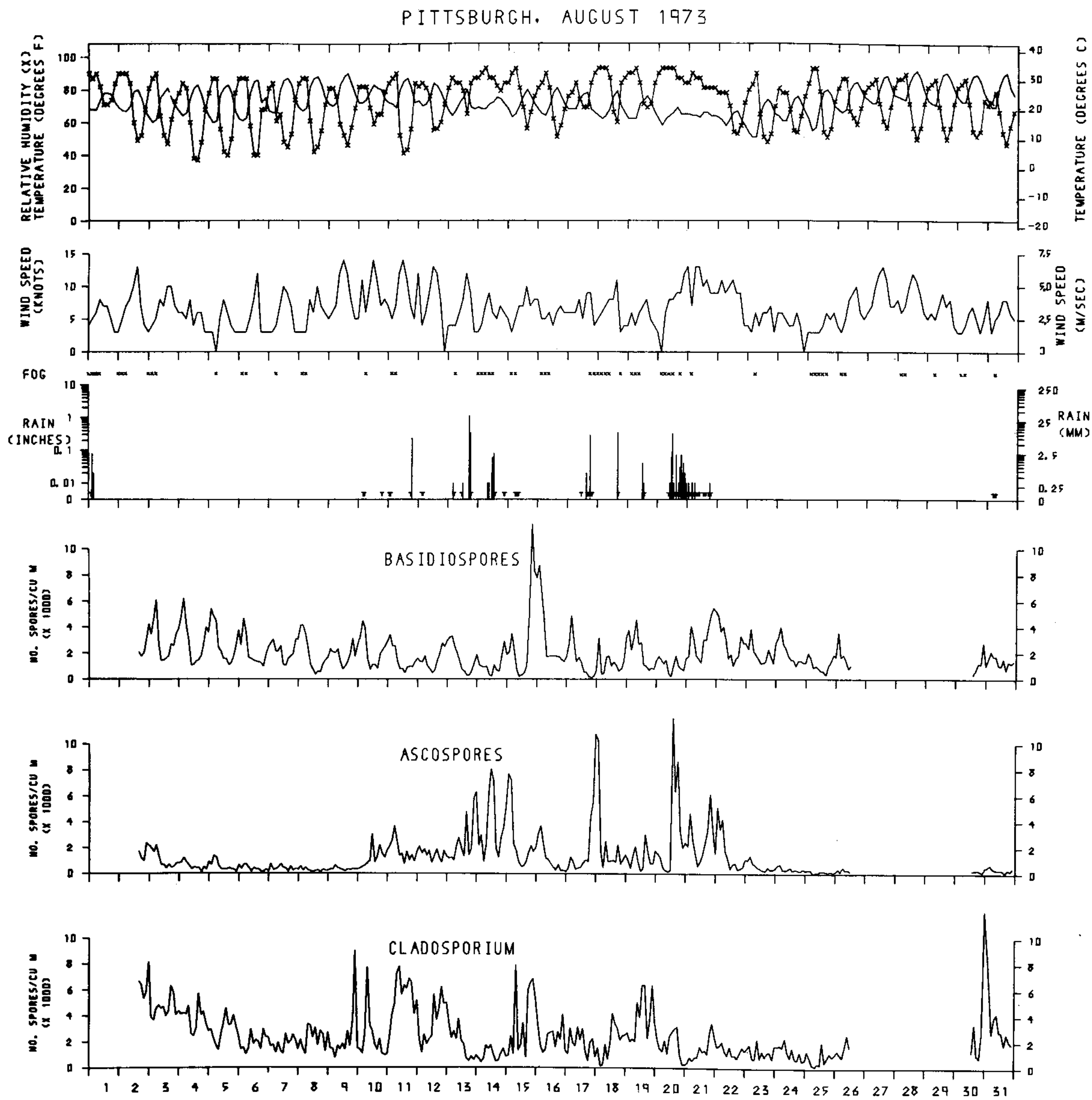
Ascospores

These are the spores from another major group of the fungi, the Ascomycetes. Characteristically, spore release in this large group is dependent upon moisture, and therefore counts are usually highest at night. Following periods of dry weather there may be tremendous increases in spore release shortly after rain. This is well illustrated by our data for 10 and 11 August (fig. 1), where immediately after very small amounts of rain as recorded by the Pittsburgh weather station, the number of ascospores collected increased immensely. Ascospores are considerably smaller than most pollens and often have not been found in such numbers in earlier studies. Because of the high numbers of ascospores sometimes encountered, it seems likely that allergies may be incited by at least some of these. It may be that certain cases of known sensitivity to other spore types, such as *Phoma or Fusarium*, may also be incited by the ascospore stages of these fungi that may more readily enter the atmosphere in outdoor air.

Other Fungi

Many other fungi occur regularly, or occasionally, in variable numbers. Some that have long been known as allergens, such as *Alternaria* and *Helminthosporium* types, occur fairly regularly but in much lower concentrations compared to the above-mentioned types. This does not necessarily mean they are less important than assumed as aeroallergens, since they may have potent antigens and thus require few spores to produce allergies. It does, however, seem to em-

Figure 1.—Fungus spore concentrations at 2-hour intervals, from Pittsburgh air samples, August 1973.



phasize the potential importance of spore types usually occurring in much greater numbers but which have not been known or tested clinically.

Miscellaneous Biological Particulates

Several other biological particles are seen in samples, usually in relatively low numbers, such as insect scales, algae, plant hairs, lichen fragments, and moss and fern spores. Their importance as potential aeroallergens has not been fully assessed.

Pollens

Even though the data presented were collected during the usual ragweed season, the numbers of pollens were low compared to the fungus spores present. During August (fig. 1) the daily average concentration of ragweed pollen was 53/m^3, ranging from 2/m^3 on 3 August to 250/m^3 on 30 August; and in general increasing throughout the month. Average concentrations for the first half of the month were 22/m^3, and for the second half, 85/m^3. Peak hourly concentrations ranged from about 25/m^3, occurring on 3 to 7 August, to about 600/m^3 at 1100 hours on 31 August. That was also the peak recorded for the season in Pittsburgh, although ragweed pollens persisted in low numbers well into October.

Few other pollens were seen during August. One type, probably from the nettle plant, reached about 200/m^3 on 11 August. This type is small for pollen (15 μm) and has probably been underestimated on gravity samplers. It has been reported as allergenic.

General

As would be expected, the air spora are most prevalent during the warmer months. Fewer occur in winter, but concentrations of several hundred fungus spores per m^3 of air are not uncommon.

It is clear from the data thus far studied that there occurs a tremendous variation in types and numbers of pollens and fungus spores in the atmosphere, from day to day and hour to hour, as well as seasonally at all sites sampled. The circadian periodicities are evident (fig. 1) as the cyclic variations that occur approximately every 24 hours much of the time.

DISCUSSION

Most previous studies of aeroallergens have depended upon sampling methods, the result of which correlate very imperfectly with the true spore content of the atmosphere, both qualitatively and quantitatively. More reliable assessments are needed. In addition, most previous methods could not provide data on variations in air spora occurring throughout a 24-hour period. Such information could have an important impact on determination and control of allergic diseases.

Improved characterization of the biological particulates in the atmosphere should be of considerable use to allergists. Prospects are good for correlating clinical observations with types, concentrations, and variations of air spora. It is expected that new associations will be evident in the etiology of asthmas and epidemiology of seasonal allergic rhinitis when certain spores are found in previously unrealized high concentrations. Knowledge of circadian periodicities can be important in determining times of day and peak concentrations of aeroallergens to which patients might be exposed. Data accumulated will also be tested for possible correlations with climatic and meteorological parameters. These and other studies in cooperation with allergists will constitute the second phase of this study of aeroallergens and their impact on the susceptible population.

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