United States
Environmental Protection
Agency

Office of Health and
Environmental Assessment
Washington, DC 20460

EPA 600/8-91/202
January 1992

IAQ5111

Research And Development

EPA

Indoor Air - Assessment

Indoor Biological Pollutants

EPA 600/8-91/202
January 1992

Indoor Air - Assessment
Indoor Biological Pollutants

Environmental Criteria and Assessment Office
Office of Health and Environmental Assessment
U.S. Enviromental Protection Agency
Research Triangle Park, NC 27711

ECAO-R-0315
JANUARY 1992

INDOOR BIOLOGICAL POLLUTANTS

Prepared by: Dr. Harriet Burge and
Dr. Thomas Platts-Mills

Environmental Criteria and Assessment Office
Office of Health and Environmental Assessment
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

DISCLAIMER

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

FOREWORD

There are significant problems associated with exposure to airborne substances of biological origin in the indoor environment. It now seems clear that a significant percentage of the diseases associated with indoor air pollution are related to bioaerosols, and that the diseases can be more serious, and cause more distress in terms of mortality and morbidity than those diseases attributed to the common outdoor air pollutants.

This document is intended to represent a few of the known diseases associated with inhalation exposure to biological aerosols in the indoor environment. This review summarizes the data available on the nature of bioaerosols and their health effects, methods of measurement, standards for exposure, approaches toward developing such standards, and remedial actions. The document is not intended to cover all of the available information on biological aerosols but instead is designed to be an introduction to a more complete and comprehensive evaluation of biological aerosols in the indoor environment to follow. An additional objective is to focus attention on areas of particular importance where little or no research is being conducted.

PREFACE

In October 1986 Congress passed the Superfund Amendments and Reauthorization Act (SARA, PL 99-499) that includes Title IV—The Radon Gas and Indoor Air Quality Research Act. The Act directs that EPA undertake a comprehensive indoor air research program.

Research program requirements under Superfund Title IV are specific. They include identifying, characterizing, and monitoring (measuring) the sources and levels of indoor air pollution; developing instruments for indoor air quality data collection; and studying high-risk building types. The statute also requires research directed at identifying effects of indoor air pollution on human health. In the area of mitigation and control the following are required: development of measures to prevent or abate indoor air pollution; development of methods to assess the potential for contamination of new construction from soil gas, and examination of design measures for preventing indoor air pollution. EPA's indoor air research program is designed to be responsive in every way to the legislation.

In responding to the requirements of Title IV of the Superfund Amendments, EPA-ORD has organized the Indoor Air Research Program around the following categories of research: (A) Sources of Indoor Air Pollution; (B) Building Diagnosis and Measurement Methods; (C) Health Effects; (D) Exposure and Risk (Health Impact) Assessment; and (E) Building Systems and Indoor Air Quality Control Options.

EPA is directed to undertake this comprehensive research and development effort not only through in-house work but also in coordination with other Federal agencies, state and local governments, and private sector organizations having an interest in indoor air pollution.

The ultimate goal of SARA Title IV is the dissemination of information to the public. This activity includes the publication of scientific and technical reports in the areas described above. To support these research activities and other interests as well, EPA publishes its results in the INDOOR AIR report series. This series consists of five subject categories: Sources, Measurement, Health, Assessment, and Control. Each report is printed in a limited quantity. Copies may be ordered while supplies last from:

U.S. Environmental Protection Agency
Center for Environmental Research Information
26 West Martin Luther King Drive
Cincinnati, OH 45268

When EPA supplies are depleted, copies may be ordered from:

National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Road
Springfield, VA 22161

CONTENTS

CONTENTS (cont'd)

CONTENTS (cont'd)

TABLES

ABSTRACT

Biological aerosols have been recognized as indoor hazards for several hundred years. Pasteur demonstrated that infectious diseases are transmitted through indoor air. Dust has been a recognized allergen since the mid-19th century. Recently, however, the role of indoor air in transmission of infectious disease has been de-emphasized, and the problems associated with other kinds of indoor bioaerosols have received only minimal public health attention. This is in spite of the fact that we spend an average of 22 hours/day indoors. Influenza causes 10,000 deaths per year. The house dust mite is probably the single most important cause of asthma among children and young adults. Indoor allergens are thought to be responsible for as much as 50% of the incidence of acute asthma in adults under 50 years old. Microbial toxins are among the most toxic substances known to man with effects that include acute toxicity symptoms, birth defects, cancer, and, in some cases, death. The concentrations and health effects of these toxins are completely unknown for the vast majority of indoor environments. Volatile organic compounds are produced by all microorganisms and accumulate in confined spaces, causing odors and possibly unknown health effects. The nature of these substances, their health effects, and concentrations in indoor environments is unknown.

AUTHORS, CONTRIBUTORS, AND REVIEWERS

The principal authors of this document are Dr. Harriet Burge, University of Michigan, and Dr. Thomas Platts-Mills, University of Virginia. This document was prepared under contract with the Environmental Protection Agency's Office of Health and Environmental Assessment (OHEA), Environmental Criteria and Assessment Office (ECAO). The ECAO, Indoor Air Research Program, had the overall responsibility for coordination and preparation of this document (Beverly M. Comfort, Project Manager).

The following individuals provided peer reviews of this document:

Michael A. Berry
U.S. Environmental Protection Agency
Environmental Criteria and Assessment Office
Research Triangle Park, NC

Arthur Chiu
U.S. Environmental Protection Agency
Human Health Assessment Group
Washington, DC

Sheila Rosenthal
U.S. Environmental Protection Agency
Human Health Assessment Group
Washington, DC

Chin S. Yang
Federal Employee Occupational Health
3585 Market Street
Philadelphia, PA

William Ewald
U.S. Environmental Protection Agency
Environmental Criteria and Assessment Office
Research Triangle Park, NC

Harriet Ammann
Washington State Department of Health
Office of Toxic Substances
Olympia, WA

Steve Bayard
U.S. Environmental Protection Agency
Human Health Assessment Group
Washington, DC

Robert Dyer
U.S. Environmental Protection Agency
Health Effects Research Laboratory
Research Triangle Park, NC

1. SUMMARY AND CONCLUSIONS

1.1 BIOLOGICAL POLLUTANTS

Biological pollutants are agents that are derived from or are living organisms. These agents include viruses, bacteria, fungi, protozoa, and their toxins and arthropod, mammal, and bird antigens.

1.1.1 Viruses

Viruses are the smallest of all life forms and contain either RNA or DNA. They are heterogeneous and vary in size, morphology, chemical composition, host range, and host effects. Viruses have no physiology of their own, but rather are obligatory intracellular parasites that mobilize host cell processes and lack the ability to reproduce on their own. Viruses enter the host through the skin via animal bites or open wounds, through the respiratory tract, the alimentary tract, and the urogenital tract. Those entering through the respiratory tract must be able to survive in air.

Airborne viruses almost always require the presence of someone with an active infection in a state that includes coughing or sneezing and less commonly, infected animals housed indoors. Factors affecting viral survival in air include temperature, relative or absolute humidity, the presence of ultraviolet light, the nature and size of the particle to which the virus is attached, and possibly the presence of other pollutants. Some of the most common diseases produced by viruses are influenza, some common colds, measles (rubeola), chicken pox, and rubella (German measles).

1.1.2 Bacteria

Bacteria are prokaryotic (have no true nucleus) microorganisms characterized by a rigid, polysaccharide-rich cell wall; a single chromosome unbounded by a nuclear membrane; and no mitochondria. Bacteria are classified by cell shape (spherical, rod shaped, filamentous) and arrangement (single, chains, clumps, pairs, tetrads), by reaction to the Gram stain, and by biochemical and physiological reactions. Almost all bacteria require a source of

carbohydrate. Most bacteria utilize nonliving organic matter and are called saprophytic or saprophytes. Bacteria utilizing living tissue are known as parasites or are parasitic. Bacteria able to utilize both living and nonliving matter as an energy supply are facultative parasites and those bacteria that survive only on living tissue are known as obligate parasites.

To transmit infectious disease, bacterial cells must remain viable in air. The factors affecting their survival are similar to those for viruses. Some bacteria produce spores that are highly resistant to environmental damage and are difficult to kill even with biocidal materials. Aerosol-transmitted diseases caused by bacteria include infectious diseases, hypersensitivity diseases, and toxicoses.

1.1.3 Fungi

Fungi are eukaryotic microorganisms with cells containing one or more organized nuclei as well as other membrane-bound organelles. Fungi may be unicellular or multicellular and reproduce mainly by spores. Formally, fungi are classified into two groups based on sexual reproduction: Zygomycetes (characterized by a resting zygospore) and Dikaryomycetes (characterized by a binucleate multicellular stage preceding nuclear fusion). Most of the important fungi that are associated with indoor air quality are in the Dikaryomycetes group.

The primary source of indoor airborne fungal spores is the outdoor air. Water is the single most important factor in determining whether saprophytic fungi will be found and survive in a given indoor environment.

Most fungi are saprophytic. There are, however, some facultative parasitic fungi and a few obligate parasites. Some fungi and their metabolic by-products have had a major impact on humans. Antibiotics and mycotoxins are fungal by-products and fungi are used in the production of some food items. Airborne fungi are responsible for some infectious diseases, hypersensitivity diseases, and toxicoses.

1.1.4 Protozoa

Protozoa are primarily unicellular organisms that can live wherever water and nutrients are of sufficient quantity to support life. Many protozoa are parasitic. Some protozoa (amoebae) are capable of ingesting gram-negative bacteria, which remain alive within the

organism protected from environmental stresses. Two genera of the amoebae (*Naegleria* and *Acanthamoeba*) have been implicated in indoor air-related hypersensitivity disease.

1.1.5 Arthropods

Arthropods include mites, cockroaches, crickets, house flies, moths, and a variety of beetles. Many different species of mites are found in the home. Live house dust mites are found deep in carpets, furnishings, and bedding. The mite population of a home is closely related to the relative humidity in the house; the higher the humidity, the greater the mite population. The major source of food for mites is human skin scales; however, mites also depend on fungi for growth.

Cockroaches are present in many homes and can increase to overwhelming numbers if not controlled. Mites and cockroach allergens are suspected of causing hypersensitivity reactions in asthmatics.

1.1.6 Mammals and Birds

Many different mammals and birds are kept in the home and work environment. It is estimated that approximately 100 million domestic animals reside in homes in the United States today. The most common of the domestic animals is the cat. All of these animals shed proteins and occasionally bacteria or viruses into the environment. Animal effluent can cause respiratory allergies and, in rare cases, infectious disease.

In most parts of the world, dogs are of much less importance than cats as the cause of asthmatic attacks. Possibly 10% of all acute asthma in young adults is related to cat allergen exposure. Laboratory-animal allergy has become a serious occupational problem. Also, individual cases of sensitization to pet rodents, producing rhinitis or contact urticaria, have been documented. Sources of allergens include skin scales, saliva, urinary proteins, serum, and feathers.

1.2 BIOAEROSOL-RELATED DISEASE

1.2.1 Infectious Disease

The potential for transmittal of an infectious microorganism in air is first dependent upon the presence of the organism in the environment. The microorganism must also be able to survive and multiply in the environment, become airborne in sufficient concentration, and remain viable long enough to cause disease. For infection to occur the microorganism must be virulent. Once airborne, the microorganism must come in contact with a susceptible human host. The susceptibility of the human host is related to the immune status of the host. Factors that damage the immune system will increase the risk of infection in the exposed person.

1.2.1.1 Human-Source Infections and Animal-Source Infections

Human-source infections or diseases usually rely on the human host to function as a reservoir, amplifier, and/or disseminator. These aerosol-transmitted diseases are generally respiratory infections whose symptoms include coughing and sneezing. Airborne human-source diseases rarely occur outdoors because of the large mass of air available to dilute the aerosol and because of environmental factors hostile to microorganisms.

The human-source infections that are currently considered important with respect to indoor air quality are influenza, common colds associated with some viruses, and tuberculosis. Other aerosol-transmitted diseases include measles, rubella, and chicken pox. Chicken pox is probably the most contagious of these aerosol-transmitted diseases. However, the measles virus is so virulent that only 4 infectious units/minute released from an infected host can initiate an epidemic.

Under certain circumstances some microorganisms usually restricted to animal species may infect humans. The best known of these diseases are Q-fever, anthrax, and brucellosis; however, the incidence of these diseases is probably low.

1.2.1.2 Environmental-Source Infections

Environmental-source infections result from exposure to inanimate reservoirs contaminated primarily with saprophytic organisms.

The primary fungal pathogens (*Histoplasma*, *Coccidioides*, and *Blastomyces*) grow and reproduce in nature as soil saprophytes. These saprophytes produce mycelium and spores as fungi do. When the spores gain access to the human respiratory tract they can produce disease. The opportunistic saprophytic pathogens normally occupy natural environments and cause infectious disease only when they penetrate susceptible human hosts. Susceptibility requires some lack of immune system function. Some of the most common opportunistic pathogens that cause air-borne disease are the bacteria *Legionella pneumophila*, *Pseudomonas*, and *Acinetobacter*. The best known opportunistic fungal pathogen is *Aspergillus fumigatus*. This organism produces toxicoses and allergies and occupies both natural and manmade environments.

1.3 HYPERSENSITIVITY DISEASE

The hypersensitivity diseases are caused by individual immunologic sensitization to specific antigens. Antigens are able to stimulate production of antibody or antigen reactive cells and serve as specific targets for the antibody or sensitized cell. Proteins, lipoproteins, glycoproteins, polysaccharides, lipopolysaccharides, larger polypeptides, and nucleic acids are all potential antigens. There are three forms of immune response to indoor air biological contaminants (antigens): immunoglobulin E (IgE) antibody response (immediate allergic response); immunoglobulin G (IgG) antibody response (only detected through serum immunoassays); and T cell response (delayed allergic response).

The hypersensitivity diseases most clearly associated with indoor air quality are asthma, rhinitis, and hypersensitivity pneumonitis. Asthma and rhinitis produce an IgE antibody response (immediate) and a T cell response (delayed). It is estimated that 30 to 45% of acute asthma in children over 7 years old and in adults under 50 years of age can be attributed to indoor allergen exposure. For some of the indoor inhaled allergens, the relationship to a disease is obvious to the patients (e.g., cat allergens where the onset of rhinitis, asthma, or conjunctivitis follows within 15 minutes of entering a house with a cat). Other allergen-related hypersensitivity diseases are not as obvious, requiring challenge studies with specific allergens and or epidemiological studies on random populations. Biological agents known to

produce antigens that cause allergic rhinitis and asthma include fungi; algae; higher plant spores (pollen) and other parts used as food; and arthropod, avian, and mammalian effluent.

Hypersensitivity pneumonitis is an IgG antibody response and T cell response. It is one of the most serious of the building-related illnesses. This form of T cell response differs from that of asthma and rhinitis, and is believed to be caused by some bacteria, fungi, protozoa, and bird and mammalian serum proteins. Because the disease is not expected to occur in "so-called" clean environments (offices, homes) and in early stages misdiagnosis is probably common, the actual incidence of hypersensitivity is unknown. Until the antigen source is removed, sensitized individuals cannot return to that environment. Progressive, irreversible lung damage may occur with continued exposure to the antigen.

1.4 BIOLOGICAL TOXINS

Biological toxins may enter the mammalian system by ingestion; absorption through the skin; inhalation; and subcutaneous, intraperitoneal, or intravenous injections. Toxic effects can be acute and/or chronic. The biological toxins are mainly cytotoxic; however, many are also teratogenic, mutagenic, and/or carcinogenic. The biological toxins important in indoor air are bacterial toxins, mycotoxins, and fungal volatile organic compounds.

Bacteria produce both exotoxins and endotoxins. The exotoxin produced by *Clostridium botulinum* is responsible for the potentially lethal form of food poisoning known as botulism. The role of airborne exotoxins have not been studied to any great extent. Endotoxins, known as "fever inducers", are a component of the outer membrane of gram-negative bacteria that cause acute pulmonary changes and local inflammatory responses in exposed individuals.

Mycotoxins (fungal metabolites) have a range of toxic effects from mild acute toxicity to potent carcinogenicity. Mycotoxins enter the body through the skin, gastrointestinal tract, or respiratory tract. Generally, the mycotoxins are cytotoxic. Some mycotoxins are toxic without metabolic conversion, whereas others require metabolic conversion to exert their toxic effects. Those requiring metabolic conversion usually affect the organs where the metabolism takes place, such as the liver.

All organisms produce and emit volatile organic compounds (VOCs) during growth. The effects of these volatile organics are generally restricted to annoying odors (smelly socks,

body odor); however, some compounds may produce significant adverse health effects. It has been suggested that fungal VOCs may contribute to some cases of "sick building syndrome" or produce symptoms which mimic that condition.

1.5 AIR SAMPLE COLLECTION AND METHODS OF ANALYSIS

Air sampling can be done with the use of personal samplers or by using ambient monitoring equipment (centrifugal force, inertial impaction, filtration, and electrostatic impaction devices). The most commonly used types of equipment are the inertial impactor and filtration devices.

When choosing a sampler for collection of a viable aerosol, the size of the particles, the relative fragility of the organisms, and the expected concentrations must be considered. Some organisms must remain viable during sampling and analysis for identification. Therefore, the media used for collection should not adversely affect viability. Culture plate impactors, liquid impingers, membrane filter cassettes, and high-volume electrostatic devices have been used to collect viable microorganisms. Viruses and infectious bacteria, fungi, and protozoa are usually sampled by methods that allow their culture both in vitro and in vivo. For very small particles (small fungus spores, thermophilic actinomycetes), suction devices and isokinetic samplers should be used. Once the sample has been collected, it may be examined by direct microscopy without prior staining (fungal spores) or with prior staining (bacteria).

Commonly used methods for sampling aerosols for microscopic identification are by suction slit impactors, membrane filter cassette samplers, and rotating arm impactors. Suction slit impactors overestimate small aerosolized particles in still air and underestimate them in moving air. However, samples can be collected over long periods of time for analysis using these devices. The most commonly used samplers in the United States for outdoor bioaerosol collections are the rotating arm impactors.

Many of the allergens, antigens, and toxins that accumulate in the indoor environment are carried on particles that do not grow in culture and are not readily identifiable microscopically. Collection of these particles from air must enable the particles themselves or the soluble adhering material to be eluted for assay or assayed directly from the sampling

medium. For water-soluble materials that are not hydrophobic, liquid impingement may be used. For endotoxins, smooth surface polycarbonate filters minimize the risk of permanent adherence of the toxins to filter surfaces. *Limulus* (LAL) bioassays are necessary to determine the biological activity of the endotoxin. High-volume filtration devices have been used for mite, cat, and small mammal antigen collection. Proteins derived from these sources can only be measured by immunoassay.

1.6 RESEARCH NEEDS

The quest for knowledge related to indoor bioaerosols is essentially a quest for a means to control the diseases they cause. Although we have made some progress in collecting the existing knowledge that applies to this quest, very little research has been specifically directed toward indoor biological aerosols, and gaps remain that prevent an accurate assessment of the risk to human health imposed by these aerosols. The following are needed to more adequately understand the role of biological aerosols in the indoor environment and health.

- Field tests of available bioaerosol instruments for variability, sensitivity, and reliability.
- Experimental determinations of statistically appropriate sampling strategies.
- Development of integrated sampling devices for viable aerosols.
- Development of single instrument sample collection devices susceptible to multiple analysis approaches.
- An in-depth evaluation of the role of ventilation in the spread of influenza and other airborne respiratory infections and the effects of dilution ventilation on the spread of such diseases. A relatively straightforward epidemiological approach may be appropriate.
- Dose-response relationships for environmental exposures. Until these relationships have been established at least for the most common and important bioaerosols, guidelines for determining safe levels cannot be set. These studies will require sophisticated chamber research, as well as collection of baseline air prevalence data, and carefully designed investigations that include air sampling as well as epidemiology of specific epidemics of bioaerosol-related disease.

- Baseline data on airborne prevalence for common and important bioaerosols. These data are essential for establishing rational guidelines and for the development of dose-response relationships. Adequate equipment is currently available to begin this effort on a nationwide basis.
- Real-time sampling methods that will allow analysis of multiple types of bioaerosols from a single sample.
- Immunological and biochemical assays for a wide range of the most common bioaerosols. For many, basic research on the nature of antigens and/or toxins, and development of specific monoclonal antibodies, will be necessary.
- Time-discriminating methods for sampling viable microorganisms. At present, only short term (minutes) grab samples are possible, making mapping of changing viable aerosol concentrations cumbersome. Immunological assays do not substitute for these cultural sampling methods. Immunological assays do not assess viability, information required for pathogen exposures.
- Evaluation of complaint environments for multiple bioaerosols as well as other pollutants. It is becoming clear that bioaerosols interact with each other and with other air pollutants to cause health effects. In particular, endotoxin appears to be important in connection with exposure to sensitizing agents, and environmental tobacco smoke may exacerbate health effects from a variety of infectious, antigenic, and toxic bioaerosols. In addition, carefully designed chamber studies using animal models as well as people will be necessary.
- Examination of antigenic cross-reactivity patterns need to be examined for the most common environmental fungi and identification of widely prevalent (common) fungal antigens that can be used to make monoclonal antibodies for clinical assessment and in assays of air samples. These studies require the interaction of mycologists experienced in "aeromycology" and immunologists experienced in developing immunoassays and monoclonal antibodies.
- Characterization of microbial volatile organic compounds with respect to sources, factors controlling production, prevalence in the environment, and health effects. Characterization will require choosing appropriate strains of common environmental microorganisms and

designing studies to evaluate conditions controlling VOC production as well as identification of VOCs produced.

- An assay system, possibly based on monoclonal antibodies, that will quickly detect specific toxins from both dust and air samples. In addition, the many fungi common in indoor environments should be studied for toxin production, concentrations of specific toxins estimated with respect to spore levels, assays designed to measure specific new toxins, and assessment of health effects of these compounds.

2. BACKGROUND INFORMATION

2.1 INTRODUCTION

Most Americans spend the majority of their lives in the indoor environment. For many, the time spent indoors can be as long as 22 hours/day (Spengler and Sexton, 1983). It is, therefore, not surprising that the quality of indoor air is at least as important to health as the quality of outdoor air. Considerable effort over the last decade has focused on outdoor air pollution. Research on outdoor air pollution has focused on establishing risk levels or exposure limits for many of the known hazardous nonbiological pollutants (Yocom et al., 1971). Most research on indoor air quality has focused on the same pollutants that are found in outdoor air (e.g., nitrogen oxides, carbon monoxide) and a few other nonbiological agents that are susceptible to easy measurement (e.g., asbestos, radon), or have stimulated major controversy (environmental tobacco smoke). It is important to note that research on nonbiological pollutants is often designed to demonstrate whether or not measurable health effects occur, whereas research on bioaerosols focuses on methods for measurement of pollutants that have been known to cause serious health effects for hundreds of years.

Biological aerosols have been the subject of few coordinated research efforts designed to measure risk or to establish standards for exposure. However, the role of indoor exposure in the spread of infectious diseases, including tuberculosis, influenza, measles, and whooping cough, has been known for years. Some information is also available on the dose required to cause these diseases (Knight, 1980).

Over the last 20 years, there has been a steady increase in knowledge of the biological sources that give rise to immunological sensitization. This information has not only identified specific health risks, but has established methods for measuring some of the agents in homes that are thought to cause hypersensitivity disease. For a few sensitizing agents, it has been possible to propose specific levels of exposure that represent a risk for sensitization and for symptom development (Platts-Mills and Chapman, 1987), but for most, insufficient data are available to propose risk or threshold levels. No research has been directed to identifying the health effects, prevalence, or relative risks of toxins and volatile irritants of biological origin in indoor air.

2.2 HISTORICAL PERSPECTIVE OVERVIEW

Throughout the early days of human existence, hygienic considerations were of low priority. Although attempts were made to control bacterial or fungal damage to food supplies (i.e., by drying or salting), little attention was applied to the condition of the house. Floor coverings often consisted of straw or dirt, and refuse (including human waste) was allowed to accumulate either within or immediately adjacent to indoor environments. Given our knowledge of mite and fungal growth, it seems inevitable that materials in these houses rotted and became infested with mites and vermin. In fact, rats, mice, and arthropod infestations were continuing problems that were addressed only when food supplies were threatened. Stately European houses of the 16th and 17th centuries became so foul after 6 months that it was common practice to move to allow cleaning. At least partly as a result of these indoor conditions, the average life expectancy remained near 25 years, and disease (e.g., whooping cough, smallpox, plague, tuberculosis) regularly decimated major population centers well into the 19th century.

2.2.1 Infectious Disease

Gregory (1961) presents a fascinating history of the discovery of germs and their connection with human disease. He points out that Hippocrates felt epidemic fevers were the result of inhalation of air infected with pollutants hostile to the human race. According to Gregory (1961), Lucretius hypothesized, from observations of the movement of dust motes in a sunbeam, the existence of what he termed "atoms" that carried disease. It was, however, not until the 17th century that Leeuwenhoek developed hand-made lenses that allowed bacteria to be seen for the first time. He described yeasts, infusoria, and a mold. That these microscopic creatures actually caused disease was not established until the mid 19th-century. The last 25 years of the 19th century were considered the golden age of bacteriology. Koch's postulates describing the steps necessary to establish the cause-effect relationship between an agent and a disease were published in 1878. Before 1900, the microbial agents responsible for cholera, tetanus, the black plague, leprosy, gonorrhea, and tuberculosis had been found and viruses had been discovered (Gregory, 1961).

In 1873, Cunningham attempted to collect the agent causing cholera from the air of jails. He found many fungus spores and pollen grains, but found no correlation between the

agents he saw and the incidence of cholera. In fact, most disease-producing bacteria are relatively fragile in the airborne state, and can rarely be isolated. This problem continues today. For example, *Legionella* has not been isolated from air in any quantitative fashion, and establishing cause-effect relationships between specific sources and disease is dependent on hypothetical dispersion from the source (Muder et al., 1986). Because of these sampling problems, demonstration of the role of air in the transmission of infectious disease has focused on elimination of any other form of contact between the infected and noninfected person. Unequivocal evidence now exists that, among others, influenza, some forms of the common cold, measles, chicken pox, tuberculosis, anthrax, Q-fever, brucellosis, and a variety of fungal infections are transmitted via the airborne route (Burge, 1989b).

2.2.2 Allergies

Episodes of disease and demise, now recognized as allergic reactions, have been recorded for over 5,000 years. For example, King Menes of Memphis died in about 3000 B.C. either of anaphylaxis from the sting of a hornet, or was trampled by a hippopotamus (hornet and hippo sharing the same ancient Egyptian word). During the 16th, 17th, and 18th centuries, reactions were noted, mostly from foods, that were surely allergic in nature and some surprisingly intuitive observations were made regarding cause and effect. During these centuries, cats, dogs, horses, feathers, and many foods were observed to cause asthma. In the early 19th century, it was recognized that pollen caused hay fever, and that dust from beaten carpets produced similar symptoms.

The first mold allergies were reported in 1924, and it was recognized that damp moldy homes were conducive to asthma. Mites were observed in house dust in the 17th century, and in the 18th century it was recognized that dust caused asthma. In the 1920s, mites began to be suspected as the cause of house dust asthma. The first concrete evidence that patients could be specifically sensitized to house dust came in the 1920s when wheal and flare responses produced by skin testing with extracts of dust from the homes of sensitized individuals were reported by Kern (1921). At that time, it was already known that cats, horse hair, and molds could give rise to this form of sensitization. Experiments to identify the house dust allergen continued until 1964 when Voorhorst and his colleagues in Holland

demonstrated the importance of dust mites of the genus *Dermatophagoides* (Voorhorst et al., 1967, 1969).

In the time since, much progress has been made toward recognizing specific causes of asthma and hay fever, and in defining the mechanisms by which symptoms are elicited. The wheal and flare skin test was developed for the diagnosis of allergies. In 1967 the Ishizakas established that wheal and flare skin responses are mediated exclusively by antibodies of the IgE isotype. Thus, it is now known that much of the acute sensitivity to allergens can be explained by the production of IgE antibodies specific for proteins and glycoproteins derived from living organisms.

2.2.3 Toxins/Volatiles

Ergotism, a devastating disease caused by ingestion of a mycotoxin, was described by the Spartans in 430 B.C. The disease results in loss of peripheral circulation, gangrene, and death. Epidemics during the Middle Ages were known as St. Anthony's Fire because sufferers prayed to St. Anthony for relief. During the last 30 years, more than 200 mycotoxins have been discovered in 150 different fungi and more are characterized each year. Nearly all mycotoxin research has centered on ingestion and diseases of animals (Kendrick, 1985).

The odor associated with fungal growth is caused by the release of volatile organic compounds. These compounds vary depending on the substrate being used by the fungus. A case of arsenic poisoning was described in 1891 that was ultimately connected to fungal volatiles. Arsenic compounds in wall-paper pigment were transformed to trimethyl arsine by the action of fungi (Foster, 1949). Remaining research has centered on the use of volatile compounds as an aid in fungal taxonomy (Halim et al., 1975) and the identification of fungal volatiles in foods (Kaminski et al., 1974).

2.2.4 Environmental Control

Attempts to isolate infected people and increase ventilation were made when it was discovered that diseases such as tuberculosis could be transmitted through the air. The very high ceilings and tall windows of public buildings and prosperous homes of the 18th and 19th century were an attempt to provide ventilation to reduce human-source aerosols (both

3.1 VIRUSES

3.1.1 Viral Morphology

Viruses are the smallest of all biological entities. They are a heterogeneous group of agents that vary in size, morphology, chemical composition, host range, and host effects (American Conference of Governmental and Industrial Hygienists, 1989b). All are submicroscopic (i.e., cannot be resolved with the light microscope). They range in size from 20 to 300 nm and have a nucleic acid core (either RNA or DNA) and a protein coat. Some viruses are enclosed in a lipoprotein capsule. The complete virus particle is known as the virion.

3.1.2 Viral Physiology

Viruses have no physiology of their own, but rather mobilize host cell processes. All are obligate intracellular parasites that lack the genetic information necessary for the synthesis of cellular systems. They use host cell metabolic pathways and ribosomes to power their reproductive cycle. Viruses interact with cells in several ways: They may invade the cell and produce no obvious effects; cause cell lysis and death; or become integrated in the host cell DNA, altering the cell's genetic makeup.

The actual life cycle of viruses involves several steps. First, the virus is absorbed or attaches to the cell surface of the host. The host cell is then penetrated by the entire virus particle or just the viral genetic material. Prior to replication, the viral nucleic acid separates from the coating materials. The genetic material then divides and new virus particles are formed and released.

Viruses can enter the host through the skin via animal bites or open wounds; the respiratory tract, with site of deposition depending on the size of the particle carrying the viruses; the alimentary tract; and the urogenital tract. Although many viruses primarily enter through a single pathway, most can probably use all portals of entry. Those that primarily enter by the respiratory tract via air must be able to survive in air. Many viruses that are not usually contracted from airborne exposure are fragile and do not live for long outside the protective host environment.

Viruses undergo evolutionary change, sometimes rather rapidly. Immunological methods designed to protect against specific diseases require recognition of specific viral

characters and become ineffective over time. Viruses for which this phenomenon is especially evident include influenza and human immunodeficiency virus (HIV) (the acquired immunodeficiency syndrome [AIDS] virus).

3.1.3 Viral Ecology

As stated above, all viruses are obligate intracellular parasites and, therefore, never grow or reproduce in environmental reservoirs. Thus, sources (humidifiers, cooling towers) that may harbor bacterial and fungal infectious agents are never sources for viral infections. Although some viruses are relatively hearty and survive for hours or days in air and dust, it is important to remember that transmission of airborne virus disease almost always requires the presence of someone with an active infection in a stage that includes coughing or sneezing.

Factors affecting viral survival in air include temperature, relative or absolute humidity, ultraviolet light, and possibly other factors such as the presence of other pollutants. In addition, the nature and size of particles on which viruses are carried are important factors in viral survival (Goodlow and Leonard, 1961; Buckland and Tyrrell, 1962; Gerone et al., 1966; Karim et al., 1985).

3.1.4 Diseases Caused by Airborne Viruses

Diseases caused by airborne viruses include influenza, the common cold, measles (rubeola), chicken pox, rubella (German measles), and other less common entities. Viruses are infectious agents and do not cause hypersensitivity disease or toxic syndromes. There are animal viruses, plant viruses, and bacterial viruses. Within each class, individual viruses are usually specific for one or a small group of hosts, recognizing specific sites on the appropriate host cell. Thus, most animal viruses do not infect humans. Exceptions, of course, do occur. Rabies, for example, attacks a wide range of mammals.

3.2 BACTERIA

3.2.1 Bacterial Morphology

Bacteria are prokaryotic microorganisms characterized by a rigid, polysaccharide-rich cell wall, a single chromosome unbounded by a nuclear membrane, and no mitochondria or other membrane-bound organelles. Individual bacterial cells range in size from ~1 to 5 μm. Bacteria are classified by cell shape (spherical, rod-shaped, filamentous) and arrangement (single, chains, clumps, pairs, tetrads), by reaction to the Gram stain, and by biochemical and physiological reactions. The Gram stain divides the bacteria into gram-positive (able to retain crystal violet stain) and gram-negative (unable to retain crystal violet stain). The cell walls of gram-negative bacteria contain a lipopolysaccharide called endotoxin. Table 3-2 categorizes some common environmental bacteria according to these characteristics.

TABLE 3-2. MORPHOLOGICAL CHARACTERISTICS OF SOME COMMON BACTERIA

Genus	Gram Rx	Shape	Arrangement
Staphylococcus	+	spherical	clumps
Streptococcus	+	spherical	chains
Pseudomonas	—	rods	single
Legionella	—	rods	single
Bacillus	+	rods	single, chains
Mycobacterium	+	rods	chains
Thermoactinomyces	+	filamentous	
Mycoplasma	—	spherical, filamentous	clumps

3.2.2 Bacterial Physiology

Almost all bacteria require an environmental source of carbohydrate, as opposed to plants, which can make their own carbohydrates from carbon dioxide (CO_2) and water. Most bacteria are saprophytic (saprophytes), that is, they utilize nonliving organic material. Bacteria that invade living tissues are parasitic (parasites). The parasitic bacteria are divided into two groups: (1) facultative, which can utilize both living and nonliving organic material as food sources and (2) obligate, which must have living tissues to supply growth

requirements. Saprophytes and facultative parasites can grow in environmental reservoirs, and can also be grown on culture media.

To transmit infectious disease, bacterial cells must remain viable in air (Kethley, 1957). Bacterial survival in air is dependent on temperature; relative humidity; presence, wavelength, and intensity of ultraviolet light; and the size and composition of the particle carrying the bacteria, all interacting with characteristics of the bacteria themselves. Some bacteria, including *Bacillus* species and some of the thermophilic actinomycetes, produce spores that are highly resistant to environmental damage, and are difficult to kill even with traditional biocidal materials and conditions.

3.2.3 Bacterial Ecology

Bacteria are ubiquitous, but the species composition of bacterial populations differs in different environments. In general, gram-negative bacteria are common in outdoor air, especially in rural areas where available leaf surface area is large (Nevalainen, 1989). The gram-negative rod-shaped bacteria form colonies on the surfaces of leaves and can live for long periods of time in this microenvironment. In the indoor environment, gram-negative rods may reside in water reservoirs of humidifiers, air conditioner drip pans, sumps, water pipes, and other moist areas (Nevalainen, 1989; Fraser, 1984) and are abundant wherever plant materials are handled (Clark et al., 1983a). Gram-positive cocci colonize human skin and mucous membranes (Spendlove and Fannin, 1983). In so-called clean indoor air, gram-positive cocci are shed into the air with human skin scales and respiratory droplet emission (May and Pomeroy, 1973). *Bacillus* species (gram-positive) are common in many environments. The spore-forming (endospore) *Bacillus* can survive long periods of dryness, low or elevated temperatures, and other environmental conditions that would be fatal to most other bacteria (Sussman and Halvorson, 1966). The spore-forming thermophilic actinomycetes also survive under adverse circumstances (Kalakoutskii and Agre, 1973). These thermophilic bacteria thrive in environmental reservoirs where the temperature is maintained above 50 °C. When thermophilic actinomycetes, gram-negative rods, and/or *Bacillus* species are observed to be dominant in an indoor environment, it is probable that environmental reservoirs in the environment are contaminated. This, of course, is an over-simplification. However, until more research has been done on the indoor bacterial

populations and their sources, these generalities are useful in interpreting data collected in indoor environments where complaints have prompted air quality investigations.

3.2.4 Diseases Caused by Airborne Bacteria

Aerosol-transmitted diseases caused by bacteria include infectious disease (e.g., legionellosis), hypersensitivity disease (e.g., hypersensitivity pneumonitis), and toxicoses (e.g., endotoxicosis) (see Chapter 4).

3.3 FUNGI

3.3.1 Fungal Morphology

Fungi are either unicellular or multicellular. The yeasts are a group of unicellular fungi that reproduce primarily by budding. Most fungi exist, however, as long chains of cells called hyphae. Hyphae are often massed into a mycelium. Some mycelia can differentiate into one or more fruiting bodies (e.g., mushrooms).

Most fungi reproduce by spores that are disseminated through the air. Spores can be either clones (asexual) of the original plant or can result from genetic recombination (sexual). Many fungi produce both kinds of spores during a single life cycle. However, most fungal spores resulting from growth in indoor environments are asexual.

Fungi are classified in many different ways. The layman description of fungus growth is usually limited to mold or mildew. More formally, fungi are classified into two groups based on their mode of sexual reproduction: Zygomycetes (characterized by a resting zygospore resulting from nuclear fusion) and the Dikaryomycetes (characterized by a binucleate multicellular stage preceding nuclear fusion). Most of the fungi that are of importance in air quality, especially those associated with disease, belong to the Dikaryomycetes. This large group is further divided into two groups based on the form that sexual spore production takes: the Ascomycetes, which include most of the common molds, form sexual spores within a sac; and the Basidiomycetes, which includes the plant rusts, smuts, and mushrooms, form asymmetrically shaped spores externally on pegs (Burge, 1985, 1989a).

3.3.2 Fungal Physiology

Unlike plant cell walls, which are made of a glucose polymer called cellulose, the fungal cell walls are made of polymers of acetyl glucosamine called chitin. Furthermore, fungi are heterotrophic. That is, unlike plants, they do not have chlorophyll and are unable to synthesize carbohydrates from CO_2 and water. Most fungi are saprophytic; however, some are facultative parasites. A very few of the fungi are obligate parasites, requiring living tissue to complete their life cycle (Ainsworth and Sussman, 1965).

As fungi grow, they produce metabolic by-products that may affect indoor air quality. The fungi and their by-products have had a major impact on human kind. Antibiotics and mycotoxins are fungal metabolic by-products. Fungi are also used in the production of some food items (e.g., cheese, soy sauce) and beverages (e.g., beer, wine) (Kendrick, 1985).

3.3.3 Fungal Ecology

The outdoor air is the primary source for indoor airborne fungus spores. Spores of the genus *Cladosporium* usually dominate the air spora during dry weather over most of the world. During wet weather, ascospores and basidiospores may be predominant (Burge, 1985, 1989a; Burge and Solomon, 1990). Agricultural activities provide a primary source for fungi in outdoor air. Crops that are harvested after the plant dies become well-colonized with saprophytes that, in turn, become airborne in high numbers during harvesting operations. The obligately parasitic plant pathogenic fungi are only found in environments where their host organisms are present, and also may become airborne when affected plants are handled (Christensen, 1975).

Water is the single most important factor that determines whether saprophytic fungi will be found in a given indoor environment. Almost any carbon-containing material can provide a substrate for fungal growth. However, this growth will not occur if water is not present. Some carbon-containing materials are hygroscopic and can absorb enough water from the air (at relative humidities above 60%) to support fungal growth. Condensation on surfaces will also provide sufficient water. Of course, standing water that contains a carbon source as well as flooded and/or water-soaked materials will support fungal growth. Any environment where organic material is stored or handled must be considered contaminated with respect to both bacteria and fungi.

Fungal aerosols, as with bacteria, are always mixed with respect to viability so that viable counts alone always underestimate total counts (Burge et al., 1977a). Factors controlling airborne survival of fungal spores include availability of water and intensity and wavelengths of ultraviolet light (Ingold, 1971). In general, thick-walled colored spores tend to survive longer than colorless and/or thin-walled spores (Pathak and Pady, 1965).

3.3.4 Diseases Caused by Airborne Fungi

Airborne fungi cause infectious diseases, hypersensitivity diseases, and toxicoses. Some fungal products may be irritants and contribute to sick building syndrome (see Chapter 4).

3.4 PROTOZOA

3.4.1 Protozoan Morphology

Protozoa are primarily unicellular organisms that live in water. Many are parasitic and cause some serious human diseases. Amoebae are protozoans that are amorphous and change shape by extruding pseudopods. Free-living amoebae are relatively small (8 to 20 μm), unicellular, eukaryotic organisms that usually contain a single nucleus. They divide by simple binary fission. Two genera of free-living amoebae have been implicated in indoor air-related illness: *Naegleria* and *Acanthamoeba*. *Naegleria* is generally slug-like; the anterior end is broader than the posterior end. *Acanthamoeba* is characterized by spike-like cytoplasmic projections. Both move by means of pseudopodia. In addition to its infectious, trophozoite form, *Naegleria* can also be transformed into flagellate and cyst forms. Transformation from the trophozoite to the flagellate form usually occurs when the supporting medium is diluted with water. The rapid motility of the flagellate form is by means of two to four anterior flagella. When conditions are unfavorable, that is, when food and/or water is unavailable, oxygen supply is inadequate, or the environment is otherwise unsuitable, *Naegleria* can encyst. The spherical cysts are 9 to 12 μm in diameter. A return to the trophozoite or flagellar state occurs when conditions are once again favorable.

3.4.2 Protozoan Physiology

The protozoa require environmental carbohydrates and are therefore heterotrophic. The carbohydrates can be in the form of dissolved organic material or living or dead cells. Some protozoa are capable of ingesting gram-negative bacteria, including the *Legionella* species. These bacteria may remain alive and virulent within the amoebae, protected from environmental stresses, including biocides.

3.4.3 Protozoan Ecology

The free-living protozoa can live wherever water and nutrients are present in sufficient quantity. Usually, bacteria are a necessary nutrient source. Protozoa can live at a relatively wide range of temperatures, and are found in cold water humidifiers, as well as hot tubs (Edwards, 1980).

3.4.4 Diseases Caused by Protozoa

Amoebae of the genera *Naegleria* and *Acanthamoeba* have been implicated in building-related hypersensitivity disease and possibly infection. If amoebae are present in a reservoir, that reservoir is contaminated. Given such contamination, there is a potential risk for sensitization and even infection. The number of amoebae required in a reservoir for significant risk depends on the dissemination mode.

3.5 ARTHROPODS

3.5.1 Mites

Mites are members of the class *Arachnida* and the order *Acarina*. Many different species of mites are found in homes. The most important species in temperate regions are *Dermatophagoides farinae*, *D. pteronyssinus*, and *Euroglyphus maynei*, although other species may become locally dominant. Mites are not visible in dust because they are only about 0.3 mm in length. Live house dust mites stay deep inside carpets, furnishings, and bedding. Originally these mites were often called bed mites. Now it is recognized that very high levels of mites can also be found in drapes, upholstered furniture, clothing, and carpets. The major food source for mites is human skin scales. However, they are also dependent on

fungi for growth. Optimal growth requirements for mites are very similar to those requirements for fungi. The levels of mites and mite allergens, primarily found in mite feces, in homes are closely related to humidity (Arlian, 1977). In humid areas, nearly all homes have mites and up to 90% have greater than the levels now considered to create a risk for sensitization and asthma (e.g., Florida; Memphis, Tennessee; New Orleans, Louisiana; southern England; coastal Australia; Sao Paulo, Brazil). Levels of mites in homes in these areas range from 100 to 18,000 per gram of dust. In drier climates (e.g., Denver, Colorado; the central part of northern California; inland Australia), levels of mites may be very low, with 90% of the houses having less than 100 mites/g of dust. Finally, there are areas where the climate is very humid in the summer and then becomes dry in the winter. This pattern effects much of the east coast and central United States. In these areas, mites often increase rapidly during the late summer and decrease steadily over the winter. Detailed studies on seasonal variations of mites and mite allergens in the United States have been reported from Cincinnati (Arlian et al., 1982) and Virginia (Platts-Mills et al., 1987). Within each of these geographic areas, mite prevalence varies between homes for reasons that are as yet unclear.

3.5.2 Cockroaches

Cockroaches are members of the class *Insecta* of the order *Blattaria*. Cockroaches are present in many homes and can increase to overwhelming numbers if not exterminated aggressively, and it is now clear that in many inner city areas a significant proportion of patients with asthma are sensitive to cockroach derived proteins (Bernton and Brown, 1967; Twarog et al., 1977; Kang et al., 1979; Hulett and Dockhorn, 1979). The German cockroach (*Blatella germanica*) is probably the most common sensitizer in the United States. *Periplaneta americana* (the American cockroach), *Blatta orientalis*, *Periplaneta australisiae*, and *Supella supelledium* can also become locally abundant and are probably sensitizing. All of five commercial house dust preparations studied by enzyme-linked immunosorbent assay (ELISA) inhibition were found to contain cockroach allergens (Mathews, 1989). In general, cockroaches are not thought to be vectors of infectious disease.

3.5.3 Other Arthropods

Many other insects live in houses and can become a source of allergens (e.g., crickets, house flies, moths, and a variety of beetles) (Mathews, 1989). Occupational exposure data have provided convincing evidence of the allergenicity of a wide variety of insects including locusts, crickets, grasshoppers, cockroaches, beetles, moths, blow flies, sewer flies, fruit flies, and the stinging insects. However, heavy infestation is rare and only occasional reports of disease association have been made. It is very difficult to prove for each of these cases that exposure to that source is contributing to the disease. At present, the only appropriate measure is to take careful medical histories and skin test symptomatic individuals with extracts of insects that are indicated by the history.

3.6 MAMMALS AND BIRDS

Microorganisms and arthropods are usually uninvited sources of indoor bioaerosols. However, in many homes, creatures of various sorts are kept as pets. It is estimated that approximately 100 million domestic animals reside in homes in the United States, the most common being cats and dogs (Knysak, 1989). Other animals that share the indoor domestic environment with humans include birds, small mammals (mice, hamsters, guinea pigs), and snakes. Rodents are also found in laboratory facilities. All of these animals shed proteins and occasionally bacteria or viruses into the environment. Animal effluents can cause respiratory allergies and, in rare cases, infectious disease (e.g., lymphocytic choriomeningitis from virus shed in mouse urine, Q-fever from sheep blood). As many as 30% of allergic people may be sensitive to domestic animals (Barbee et al., 1981; Fontana et al., 1963; Ohman et al., 1977; Ohman, 1978), and 57% of asthmatic children are sensitive to at least one animal species (Kjellman and Pettersson, 1983). It is estimated that 11 to 30% of those exposed regularly to laboratory animals experience allergic symptoms (Cockcroft et al., 1981b; Gross, 1980; Hook et al., 1984; Lutsky and Neuman, 1975). Sources of antigens include skin scales, saliva, urinary proteins, serum, and feathers. Factors affecting the abundance of these materials include numbers of animals, time spent indoors, ventilation rates, and furnishings that can act as reservoirs (see Chapter 4).

About 25% of the families in the United States have a cat, and it has been estimated that 2 million cat-allergic people live with cats despite their symptoms. Possibly 10% of all acute asthma in young adults is related to cat allergen exposure. Cat allergens accumulate in furnishings, and it may take as long as 16 weeks for cat allergen levels to fall after removal of the cat (Wood et al., 1989).

In most parts of the world, dogs are of much less importance than cats as a cause of biological pollution. This probably reflects the fact that most dogs live, at least in part, outside the house. Acute allergic reactions to dogs are certainly far less common than those to cats. This may be because cats are more commonly allowed in bedrooms, exposures are often more intimate, and cat allergens may be more immunogenic or shed more copiously than dog allergens (Knysak, 1989).

Rodents can be present in houses either as domestic pets or as pests, and are commonly used in the laboratory setting. Laboratory animal allergy has become a severe occupational problem. Rodents have a common property of leaking protein into their urine. This problem is particularly well-defined in male rats but is common to all species. These urinary proteins appear to give rise to sensitization. Individual cases of extreme sensitization to pet rodents are well recognized as causes of rhinitis or contact urticaria. In addition, rodent urinary proteins are thought to contribute to asthma, predominantly among children. In areas of this country where mice and rats are major pests, a significant proportion of allergic patients have positive skin test to rodent urinary proteins. To date, there have been no epidemiological surveys to confirm the importance of allergic reactions to rodent urine as a risk factor for asthma.

4. BIOAEROSOL-RELATED DISEASES

4.1 INFECTIOUS DISEASE

More than 100 years have passed since Pasteur demonstrated that airborne microorganisms can cause infectious disease (Gregory, 1961). Despite the availability of information from the research of Pasteur and others, emphasis on infectious disease prevention has focused on human-to-human, direct-contact transmission. This philosophy assumes that the principles of herd immunity apply, and relies on immunization and isolation as the primary means of prevention (Patriarca et al., 1986). Herd immunity assumes that the number of susceptible people and the nature and frequency of direct, nonaerosol contact among them determines the rate of spread of infectious disease (Fox et al., 1971). For some diseases, even though resulting from potentially airborne vectors, these methods are appropriate. For example, smallpox, a highly virulent, potentially airborne virus, has ostensibly been eradicated by diligent immunization programs. However, highly virulent airborne diseases, especially those caused by unstable viruses, are not likely to be controlled until the dynamics of transmission are understood.

The potential for transmittal of an infectious microorganism via air is dependent on several factors. First, the disease-causing microorganism must be present in the environment (reservoir). Second, the microorganism must be able to survive and multiply in that environment (amplification), and finally, the microorganisms must become airborne in sufficient concentration and remain viable long enough to produce disease (dissemination) (Feeley, 1985; Burge, 1989b).

For infection to occur, the organism must be virulent and at least one susceptible host must be present. Virulence is determined by both genetic and environmental factors. Some microorganisms are inherently more virulent than others. For example, it appears that a single *Mycobacterium tuberculosis* cell is adequate for infection (Houk, 1980; Riley, 1982), whereas several hundred *Legionella* organisms are probably necessary to cause disease (Meyer, 1983; Baskerville et al., 1981). Physiological factors such as life cycle stage are also important. Some organisms are more virulent during very rapid (log-phase) growth, whereas other organisms are most infective during the slower, stationary phase.

Environmental factors such as temperature, relative humidity, and radiation can affect both viability and virulence. Each organism is different with respect to the effects of all of these virulence factors (Kethley et al., 1957).

The susceptibility of the human host is related to the immune status of that person (Burge, 1990). Contact with most infectious agents usually results in an immunity to that organism for a given period of time, possibly for life. For these diseases, immunity can be induced by immunization or inoculation with parts of the responsible organism. For other diseases, especially those caused by organisms that change in virulence, immunization is effective only for short periods. A disease of this latter type is influenza (Selby, 1976).

Factors that damage the immune system will increase the risk of infection in the exposed person (Williams et al., 1976; Gardner, 1982). In particular, these factors lower the thresholds at which pathogens that are otherwise relatively innocuous can cause disease. Immune system damage can be caused by disease (e.g., HIV infection), immunosuppressive agents (e.g., cytotoxic chemicals, large doses of steroid hormones) (Hesse et al., 1986), and direct damage to cells that function as a part of the immune response within the respiratory tract (Kark et al., 1982; Petitti and Friedman, 1985; Storch et al., 1979).

4.1.1 Human-Source Infections

The majority of human-source infections are probably transmitted from person-to-person by direct contact. Such infections do not stem from a bioaerosol problem and will not be considered in this review. However, some very common human-source infectious diseases are transmitted by air. Logical modes of control of these airborne diseases may lie in the area of indoor air quality (see Chapter 6).

Human-source infections usually rely on the human host to function as a reservoir, amplifier, and/or disseminator. The virus or bacterium resides in the human (or animal) host, is amplified in the host during incubation of the disease, and is disseminated from the host in respiratory or other secretions. In general, aerosol-transmitted diseases are respiratory infections that include coughing and sneezing among their symptoms (Riley, 1982); however, the act of singing has been identified in the transmission of tuberculosis (Houk, 1980). Airborne human-source diseases rarely occur outdoors because of the large mass of air available to dilute the aerosol and hostile environmental factors (ultraviolet light,

temperature extremes, and humidity extremes). In the indoor setting, rates of infection depend on the number and virulence of organisms (infectious dose) required to initiate infection, the number of susceptible hosts in the indoor space, and the number of infectious doses in the air (Burge, 1990). Low ventilation rates in indoor environments allow for accumulation of infectious units, often in the presence of an accumulation of susceptible people.

The human-source infections that are currently considered important with respect to indoor air quality are influenza, common colds associated with some viruses, measles, rubella, chicken pox, and tuberculosis. Influenza is an aerosol-transmitted virus disease (Knight, 1980), although it is still being studied as a direct contact disease by most epidemiologists (Longini et al., 1982). It occurs in explosive epidemics, which is characteristic of airborne transmission, and coughing is a common symptom (allowing airborne spread). The virus is highly virulent so that only a small dose is necessary for infection, and the disease is reproduced in volunteers and animals by aerosol more easily than by nasal instillation (Knight, 1980; Schulman, 1968). A contained epidemic aboard a commercial airliner was unequivocally caused by air transmission resulting from a single active case of influenza and a period of inadequate ventilation (Moser et al., 1979).

At least 100,000 episodes and 13,000 excess deaths are attributable to influenza each year (Garibaldi, 1985; Schoenbaum, 1987). Although influenza-related mortality is highest in the elderly, morbidity is greatest in children. Some evidence exists for the hypothesis that influenza epidemics begin in the schools (Monto, 1987). Immunization programs for the elderly population may decrease mortality, but will not halt the spread of this serious and costly disease. Dilution ventilation in the school room is a control approach that deserves attention, but is not popular because it conflicts with energy conservation policies.

There is still controversy over the mode of transmission (direct contact vs. aerosol) of the common cold viruses. However, Couch (1981) was able to experimentally transmit the coxsackie virus between volunteers in a situation where direct contact was prevented. Similar disagreement exists for the rhinoviruses, with some investigators presenting data for or assuming direct contact (Longini et al., 1988) and others for the aerosol route of infection (Dick et al., 1987). Colds are caused by many different viruses, some of which do not survive well in aerosols, and are probably transmitted primarily by direct contact in most

situations. Under conditions that favor aerosol survival, however, airborne transmission can occur. Some human-source infections, such as influenza, are most readily infective by lower airway challenge, rather than nasal instillation, and are probably primarily airborne (Knight, 1980).

At least 90,000,000 episodes of the common cold occur each year in the United States, resulting in 200,000,000 days of restricted activity (Dixon, 1985). Attempts to control this common disease have relied on interruption of direct-contact transmission (biocidal handkerchiefs) or immunization. Neither of these methods have been effective. Brundage et al. (1988) has demonstrated that inadequate ventilation may facilitate the spread of adenovirus in army recruits. His study was not properly controlled, however, and should be repeated.

Measles, rubella, and chicken pox, the common childhood diseases, are all aerosol-transmitted viral diseases (Habel, 1945). The measles virus is so virulent that only 4 infectious units/minute released from an infected host can initiate an epidemic (Riley, 1980). Resistance to measles requires either previous infection or vaccination. Because of its virulence, measles usually infects all exposed susceptible hosts. Therefore, those remaining sensitive are mostly very young children and a few people born before active immunization programs were instituted (Davis et al., 1986). Measles virus has been documented to travel through ventilation system components to infect distant susceptible people (Bloch et al., 1985). In such a situation, all susceptible people usually become infected. Immunization alone is unlikely to control this disease. Potentially more effective measures have been proposed including:

- disinfecting air in high-risk enclosed spaces (such as schools) with ultraviolet light (Riley, 1980),
- more effective control of recirculation patterns in clinical spaces (Davis et al., 1986), and
- increasing fresh air ventilation rates (i.e., increasing percentages of outdoor air in recirculation systems).

Control by environmental intervention is only effective if the environment being treated is the only transmission site. It is not useful to treat air in a school if the disease is being transmitted on school buses. Careful analysis of suspected sources is essential to effectively control all environmentally transmitted diseases (Wells et al., 1942).

Rubella, a mild disease in children, presents a significant risk of birth defects when contracted by pregnant women. Most attempts at control of rubella have centered on immunization of school-age children based on the assumption that they spread the disease at school and bring it home to their pregnant mothers. It appears, however, that women are just as likely to get the disease directly from public contact in poorly ventilated spaces (e.g., public transportation) (Langmuir, 1980).

Chicken pox is probably the most contagious of infectious diseases and is transmitted by air whenever an infected person coughs (Couch, 1981). Epidemics have been recorded in hospitals (Gustafson et al., 1982; Leclair et al., 1980; Tsujino et al., 1984), and it has been suggested that the disease may be spread through ventilation systems (Wells and Holla, 1950). However, the overall role of indoor air in the transmission of chicken pox is unknown, and control efforts have not been seriously undertaken.

Tuberculosis, another highly contagious disease, is transmitted through coughing, sneezing, and by talking and singing (Houk, 1980; Riley, 1982). There have also been reported cases in which tuberculosis was transmitted through ventilation systems (Houk, 1980). The tuberculosis bacterium (*Mycobacterium tuberculosis*) is highly resistant to environmental stresses, probably survives for an extended time in the environment, and could be resuspended in an infective state from settled dust (Kent, 1967). Although it is unlikely that air quality control will prevent an epidemic, transmission away from the infected environment could be avoided by eliminating recirculation of ventilation air. It appears that everyone who converts to a positive tuberculosis skin test acts as a low level source of infection at least temporarily (Kent et al., 1967). Transmission from asymptomatic subjects (that is, subjects with low-level tuberculosis infections) could possibly be prevented by maintaining a high level of outdoor air ventilation in high-risk environments.

4.1.2 Environmental-Source Infections

Environmental-source infections result from exposure to reservoirs where saprophytic organisms are amplified and/or nonhuman (usually mammalian), living reservoirs. Any environment containing some kind of an organic carbon source, available nitrogen, and water can be home to one or more saprophytic organisms. Fortunately, most of these organisms cannot invade human tissue and do not cause infectious disease. Very few primarily saprophytic organisms can invade a normal healthy human who possesses an intact immune system. However, a few saprophytes (usually those that are adapted for growth in environments in which temperatures are maintained in the range of human body temperature) will attack minimally compromised individuals (e.g., those who are heavy smokers), and many will cause disease in severely compromised hosts (e.g., AIDS patients and patients who are on immunosuppressive medication to prevent transplant rejection). The environmental infectious agents can be divided into two general categories:

(1) the primary fungal pathogens (including *Histoplasma, Coccidioides*, and *Blastomyces*), and
(2) the opportunistic pathogens (including *Legionella*, and many organisms that are facultative parasites).

The primary fungal pathogens grow and reproduce in nature as soil saprophytes, producing mycelium and spores as ordinary fungi do. However, when spores of these fungi gain access to the human respiratory tract, they are able to adapt and grow in this unusual environment, and produce disease. Histoplasmosis, cryptococcosis, coccidioidomycosis, blastomycosis, and sometimes sporotrichosis are fungal diseases of this sort. In normal people, these diseases are usually self-limiting. However, when immune system defects are present, the diseases can be serious or fatal. Although these diseases all have primary foci that are outdoors, the outdoor aerosol can penetrate into interiors, and, especially where debilitated people reside, they can present significant problems.

Histoplasma is very common in the Americas, and resides in soil enriched with bird droppings. Primary focus of exposure is outdoor air during disturbance of contaminated soil. Indoor air exposure may occur when such soil is disturbed adjacent to open windows or air-

intakes. In most cases, the disease, histoplasmosis, is subclinical (does not produce noticeable symptoms) and self-limiting. However, it can be severe or fatal in immunocompromised individuals. It is estimated that 40,000,000 people in the United States alone have had histoplasmosis, and that there are 200,000 new infections each year (Ajello, 1971; Furcolow, 1958).

Cryptococcus neoformans is almost exclusively associated with pigeon droppings and may be the predominant organism in old, dry droppings in roosting areas (Emmons, 1955). It does not compete well with other organisms and is rapidly overcome when soil is mixed with infected debris. Indoor exposure may occur when old pigeon roosting areas in attics are disturbed. As with histoplasmosis, the disease is usually subclinical and self-limiting in normal people, but can become severe and fatal in immunocompromised patients. Because a sensitive antibody assay is as yet unavailable, accurate estimates of the incidence of this disease are also not available. One estimate suggests that 15,000 cases occur each year in New York City alone (Kaufman and Blumer, 1978).

Coccidioides grows in dry soils (in the semi-arid southwest United States and Mexico) with high concentrations of carbonized organic material and high salt concentrations. Spores become airborne when contaminated soil is disturbed. Epidemics often occur during sandstorms. Most exposure occurs outdoors, but spores may enter the indoor environment. *Coccidioides* may be the most virulent of the fungal pathogens. A few spores are sufficient to cause disease in a host with a normal immune system, whereas a massive exposure will cause serious disease (Larsen et al., 1985). Risk factors for the development of serious or fatal coccidioidomycosis have yet to be clearly established, although an immune defect is suspected (Kirkland and Fierer, 1985). Probably more than 100,000 cases of coccidioidomycosis occur per year in the United States, most of them concentrated in the arid southwest.

Blastomyces is endemic in the eastern United States. The organism inhabits wet soil enriched with animal manure, cannot withstand drying, and does not compete well with other soil microorganisms (Klein et al., 1986). Epidemics of the disease (blastomycosis) are usually associated with soil disturbance, including construction activities (Kitchen et al., 1977). Whether or not blastomycosis exists as a subclinical disease is not known. The

disease does not produce lung or serological changes in recovered individuals, and no accurate test is available to assess rates of occurrence.

The opportunistic pathogens are saprophytes that normally occupy natural environments, but cause infectious disease when they penetrate susceptible human hosts. In these cases, susceptibility implies some lowering of defenses rather than absence of specific protective antibodies. Any factor (i.e., disease, smoking, alcohol or drug abuse, chemotherapy) that damages the human immune system can render a person more susceptible to these disease agents. In some sense, the primary fungal pathogens are opportunistic in that they are serious diseases only in those with some immune dysfunction. The true opportunists, however, do not apparently cause disease at all in people with normal immune systems. Some common opportunistic pathogens that cause airborne disease are the bacteria *Legionella pneumophila*, *Pseudomonas*, and *Acinetobacter*; many fungi, especially those able to grow at elevated temperatures; and a few protozoa.

Legionella is the most notorious of the opportunistic pathogens and has been extensively reviewed (Meyer, 1983; Winn, 1985; Davis and Winn, 1987). *Legionella* is a common environmental saprophyte, and it has been isolated from soil, water, and other outdoor environmental reservoirs. In addition, it can contaminate air conditioning equipment, potable water, humidifiers/nebulizers and other respiratory therapy equipment, whirlpools/spas, sprinkler systems, and industrial coolants (Winn, 1985; Davis and Winn, 1987; Doebbeling and Wenzel, 1987; Burge, 1990). It causes two distinct clinical syndromes: a bacterial pneumonia that carries a low attack rate but high mortality (Legionnaires' disease) (Fraser et al., 1977), and a nonpneumonic disease with a high attack rate and rapid recovery (Pontiac fever) (Glick et al., 1978). Most *Legionella*-related epidemics have been traced to *Legionella pneumophila* serotype I, although other serotypes and other species have been implicated in isolated cases and unusual epidemics (Plouffe et al., 1983). Airborne transmission has been clearly demonstrated (Baskerville et al., 1981; Davis et al., 1982). Immune suppression is a risk factor for Legionnaires' disease, especially suppression of lung defenses. As with all opportunistic diseases, normal healthy people are rarely at risk, whereas patients with severe immunosuppressive disease and those on immunosuppressive medication become infected with apparently low dose exposure (Davis and Winn, 1987; Guiguet et al., 1987). In addition, cigarette smoking and excess alcohol consumption appear to be risk factors (Storch et al.,

1979). Legionnaires' disease is not rare. At least 4% of the American population has anti-*Legionella* antibodies (Winn, 1985) and more than 20,000 community-acquired cases probably occur each year, with an additional 200,000 acquired in hospitals (Meyer, 1983).

Bacteria other than *Legionella* have been shown to cause pneumonia in high-risk populations. *Pseudomonas* and *Acinetobacter* may inhabit respiratory therapy equipment in medical facilities and humidifiers in home and work environments (Grieble et al., 1970; Smith and Massanari, 1977; Kelsen and McGuckin, 1980; Spendlove and Fannin, 1983; Williams et al., 1976). Incidence of community-acquired disease related to these types of exposures are unknown. Nosocomial infections from these types of sources are probably relatively common.

Control of *Legionella* and other bacterial saprophytes depends on preventing accumulation of stagnant water in the indoor environment, preventing entrainment of cooling tower effluent into the indoor space, and maintaining adequate temperature and/or chlorination of hot water systems, especially in hospitals. Potential health effects from the use of these preventive measures (e.g., effects from exposure to biocides, risk of scalding from hot water) must be compared to the risks associated with bioaerosol exposure (Stanwick, 1986). It should be noted that the risk of opportunistic infections are low for normal people.

The best known opportunistic fungal pathogen is *Aspergillus fumigatus*. The organism produces toxicoses and allergies, grows in mucus secretions in the human respiratory tract, and can invade living tissue (Rippon, 1988). It is an ubiquitous fungus that occupies natural and man-made environments where significant heating occurs (30 to 45 °C) (Emmons, 1962). Although *A. fumigatus* is the most common fungal agent in cases of infectious disease, Rinaldi (1983) lists 22 species that have been implicated in human infectious disease (Table 4-1). According to Rippon et al. (1965) many *Aspergillus* and *Penicillium* species can become pathogenic. Pathogenesis appears to be related to temperature tolerance. Solomon et al. (1978) recovered 10 species of thermotolerant aspergilli from air in a midwestern hospital in addition to representatives of 10 other thermotolerant genera.

Human infection with the opportunistic fungi depends on immune dysfunction, and diagnosis of invasive aspergillosis or fungosis should be considered indicative of underlying disease. The number of spores required to initiate infection is unknown. Probably one viable spore is sufficient in a severely compromised host, whereas a healthy normal person

TABLE 4-1. *ASPERGILLUS* SPECIES IMPLICATED IN CASES OF INFECTIOUS DISEASE

A. amstelodami	*A. nidulans*
A. candidus	*A. niger*
A. carneus	*A. niveus*
A. conicus	*A. ochraceus*
A. deflectus	*A. oryzae*
A. fischeri	*A. parasiticus*
A. flavipes	*A. restrictus*
A. flavus	*A. sydowi*
A. fumigatus	*A. terreus*
A. fumigatus var ellipticus	*A. ustus*
A. glaucus group	*A. versicolor*

Source: Rinaldi, 1983; Rippon, 1988

can resist infection when exposed to millions of spores. Epidemics of aspergillosis in hospitals have been traced to environmental contamination of fire-proofing materials (Aisner et al., 1976), renovation activities (Arnow et al., 1978; Krasinski et al., 1985), road construction, and contaminated air conditioners (Lentino et al., 1982). The risks of disease resulting from the constant background of *Aspergillus* in air is not known (Solomon et al., 1978). The disease is of major concern in transplant facilities, for AIDS patients, and for patients on high-dose steroid therapy. Although *Aspergillus* is the most notorious, many other fungi can become invasive pathogens under similar circumstances. *Candida* is not known to be commonly airborne, but it leads the list of opportunistic fungal pathogens, followed by *Aspergillus*, *Cryptococcus*, and the zygomycetes (e.g., *Mucor, Rhizopus*), all of which are commonly transmitted by air. The incidence of opportunistic fungal infection is not known. The disease is often fatal. Control depends primarily on prevention of exposure. Unfortunately, *Aspergillus* and other fungi reside in the human nose, and can probably cause infection when inhaled into the lower airways from this source (Walsh and Pizzo, 1988).

4.1.3 Animal-Source Infections

Under some circumstances, some microorganisms that regularly inhabit other animal species can infect humans via the airborne route of exposure (Spendlove and Fannin, 1983). The best known of these are Q-fever, anthrax, and brucellosis; however, the incidences of these diseases are probably quite low.

Q-fever is a rickettsial disease that is endemic in sheep. Epidemics of this disease have occurred in medical facilities where sheep were being used for research (Bayer, 1982; Meiklejohn et al., 1981; Huebner, 1947; Schachter et al., 1971) and in meat-handling plants (Feldman et al., 1950; Sigel et al., 1950).

Anthrax is also known as wool sorters disease because the endospores produced by *Bacillus anthracis* (the causative agent) can survive for long periods of time on the wool of infected animals (Dahlgren et al., 1960; Young et al., 1970). Fortunately, the infective dose of this organism appears quite high (>1,300 units).

Brucellosis is generally a disease of domestic animals; however, it can be contracted by people in the meat-packing industry (Buchanan et al., 1974; Hendricks et al., 1962; Huddleson and Munger, 1940; Kaufmann et al., 1980). A less well-known animal disease is lymphocytic choriomeningitis, a viral disease that occurs in rodents. The causative agent can be isolated from rodent urine and may become airborne if the urine is disturbed. Epidemics have occurred in animal vivaria, and sporadic cases in homes may have resulted from exposure to urine from infected house mice (Couch, 1981).

4.2 HYPERSENSITIVITY DISEASE

The hypersensitivity diseases are caused by individual immunologic sensitization to specific antigens, substances that can trigger an allergic response. Antigens or immunogens are able to stimulate production of antibody or antigen reactive cells and serve as specific targets for the antibody or sensitized cell produced. Proteins, lipoproteins, glycoproteins, polysaccharides, lipopolysaccharides, larger polypeptides, and nucleic acids are all potential antigens. Most antigens are 5,000 to 50,000 daltons in molecular weight and are soluble, a necessary requirement for antigenicity. A number of smaller highly reactive molecules may also be antigenic, provided they are able to bind to larger carrier molecules. These small

molecules are known as haptens. Common haptens include metal salts, isonicotinic acid hydrazide, trimellitic anhydride, and other highly reactive chemicals. Haptens usually cause sensitization in occupational settings where exposure occurs over long periods of time. An important characteristic of antigens, with the possible exception of some haptens, is that they do not elicit toxic effects in the absence of an immune response. Thus, nonallergic individuals have no significant symptoms from exposure to dust, even in homes with high levels of dust mite, cockroach, cat, or fungal proteins.

Because antigens produce immunological changes in allergic individuals, it is possible to identify sensitization to specific antigens. Once an individual becomes sensitized to a particular antigen, subsequent exposure produces an allergic response. Forms of sensitization of concern for indoor air are specific immune responses involving either antibodies or T cells. Determining sensitization provides two kinds of information. First, it is an indication that the individual has been exposed to an antigen. Second, the form of sensitization may act as a guide to the immunopathology of the associated disease.

There are three forms of immunity that have to be considered in the discussion of indoor biological pollution: (1) IgE antibody response, (2) IgG antibody response, and (3) T cell response. The IgE antibodies produce immediate hypersensitivity when exposed to an immunogen. These antibodies can be detected by wheal and flare skin responses or by serum assays. IgG antibodies are an important part of protective immunity and are also associated with some forms of hypersensitivity. IgG responses to antigen are only detected through serum immunoassays. T cell responses produce delayed hypersensitivity and in clinical practice are detected by 24 or 48 hour indurated erythematous skin responses.

The hypersensitivity diseases most clearly associated with indoor air quality are asthma and allergic rhinitis and hypersensitivity pneumonitis. Asthma and rhinitis are associated with IgE antibody responses possibly, for some antigens; IgG response; and a specific form of T cell response. Hypersensitivity pneumonitis is also an IgG antibody and a T cell response. However, the form of T cell response differs from that of asthma and rhinitis.

4.2.1 Rhinitis, Asthma, and Allergic Bronchopulmonary Aspergillosis

Allergic rhinitis, allergic asthma, and allergic bronchopulmonary aspergillosis (ABPA) result from interactions between antigens or allergens and IgE antibodies. They affect people

who have the genetic tendency to develop IgE antibodies usually in response to inhaled or ingested allergens.

Allergic rhinitis (hay fever) affects up to 20% of the population and, in some instances, exposure to the appropriate allergen may have an incapacitating effect. Allergic rhinitis is characterized by nasal itching, congestion, runny nose, sneezing, and watery eyes.

An estimated 10 million people in the United States have asthma. In 1987, 4,360 people died from asthma compared to 2,891 people in 1980 (U.S. Department of Health and Human Services, 1991). Asthma is characterized by chest tightness, wheezing, cough, and shortness of breath. Symptoms may occur within an hour of exposure to the allergens or may be delayed in onset for 4 to 12 hours. For many years it was assumed that airborne allergens caused asthma entirely by the release of histamine from mast cells in the lung. This belief was held because inhaling allergen extracts produced a rapid asthmatic response similar to that produced by histamine. However, closer observation of asthmatic individuals revealed that provocation with allergen often produced a delayed or late airway response as well as the immediate response (Booij-Noord et al., 1972; Warner et al., 1978). This late response is not seen with histamine and is associated with increased bronchial irritability, which can last for days or weeks (Cartier et al., 1982). It is now believed that this late bronchial response is caused by inflammatory cells other than histamine, in addition to possible epithelial damage in the airway (U.S. Department of Health and Human Services, 1991). In keeping with this, it has been shown that prolonged avoidance of exposure to house dust can lead to marked reductions in nonspecific bronchial reactivity (Kerrebijn, 1970; Platts-Mills et al., 1982; Charpin et al., 1988). Chronic exposure to low levels of indoor allergens over days or weeks might be more important than a larger amount over a short period.

The size of antigen-containing particles does not appear to be critical for development of IgE-mediated disease. Approximately 5% of particles as large as 15 or 20 μm will enter the bronchial tree (Task Group on Lung Dynamics, 1966; Svartengren et al., 1987).

Allergic bronchopulmonary aspergillosis is a disease of asthmatics where a fungus (often *Aspergillus*) colonizes the mucus secretions in the lung. Symptoms are similar to pneumonia. Patients develop both IgE and IgG antibodies against the specific colonizing fungus. The

disease is apparently related to host factors rather than intensity of exposure to viable spores (Slavin, 1983).

4.2.1.1 Causative Agents

Biological agents known to produce antigens that cause allergic rhinitis and asthma include fungi, algae, pollen, plant parts used as food, arthropods, and avian and mammalian effluents. Probably any protein or glycoprotein derived from any living organism can be allergenic if a predisposed person is appropriately exposed. Some antigens that are considered of primary importance epidemiologically in indoor air have been identified and characterized. However, with respect to the different kinds of possible antigens in the indoor environments, most remain unknown.

It is estimated that 30 to 45% of attacks of acute asthma in children over 7-years old and in adults under 50 years of age can be attributed to indoor allergen exposure (Pollart et al., 1989a; Gelber et al., 1990). Of the 2 million estimated annual emergency room visits for asthma, as many as 400,000 of these cases can be attributed to exposure to indoor allergens.

For sensitizing agents (allergens) there are two separate groups of individuals potentially at risk:

(1) those individuals who are exposed to a level of antigen that is considered sufficient to give rise to sensitization, and
(2) those individuals who are sensitized and continue to be exposed and develop symptoms.

For some of the major sources of indoor biological pollution, reducing exposure is not simple and, therefore, it has been difficult to obtain clear results showing that avoidance improves the disease (Korsgaard, 1982; Burr et al., 1980). There are, however, at least three studies showing improvement in asthma in mite-allergic individuals when exposure was decreased (Murray and Ferguson, 1983; Mitchell et al., 1985; Walshaw and Evans, 1986).

For most allergens, little is known about the actual dose-response relationship. To determine the dose-response relationship, the allergen must first be identified and standard

assays must be developed. Epidemiological surveys, including field measurements and assessment of disease rates, may also assist in defining the dose-response relationship. It should be noted, however, that any fixed threshold is simply a statistical value for exposure above which a given proportion of the population would be expected to develop sensitization and, if exposure continues, a proportion of the sensitized individuals will develop symptoms.

All fungi that have been used in skin-testing surveys have been shown to elicit reactions in some fraction of the allergic (IgE producing) population. This probably means that all fungi are potentially antigenic, and patterns of exposure may control the importance of each in allergic disease (Burge, 1985). Research on the biochemical characteristics of antigenic material derived from the fungi is limited. Semipurified antigens have been produced from *Alternaria alternata* (Yunginger et al., 1980), *Cladosporium herbarum* (Aukrust and Borch, 1979), and *Aspergillus fumigatus* (Kim and Chaparas, 1978). Other fungi have been extracted and the antigenic materials have been concentrated (Horner et al., 1988; Horner et al., 1989; Davis et al., 1988). Some antigenic cross-reactivity may exist between different fungi (Agarwal et al., 1982; O'Neil et al., 1988). However, in many cases, batch and strain variability has not been considered. The overwhelming numbers of fungi to which people are commonly exposed in the indoor environment, the intrinsic variability of fungi in culture, and the fungal enzymes always present during extraction procedures make the task of identification of even the major allergens formidable (Burge, 1989a).

Several of the important allergens of dust mites have been purified, cloned, and sequenced (Platts-Mills and Chapman, 1987; Chua et al., 1988). Some mite allergens are predominantly found in mite feces and are now known to be digestive enzymes. Structurally, the main allergens are glycoproteins with a molecular weight of 15,000 to 30,000 (Platts-Mills and Chapman, 1987; Yasueda et al., 1989). Another important characteristic is that they are rapidly soluble in salt water.

There are multiple studies on the relationship between exposure to the dust mite and sensitization (Korsgaard, 1983; Peat et al., 1989; Sporik et al., 1990; Platts-Mills et al., 1986a; Wood et al., 1989). Based on this information, the following provisional standards have been proposed for exposure/allergic response by an international workshop:

- 2 μg Group I allergen/g of dust represents a risk for development of sensitization and bronchial reactivity, and
- 10 μg Group I allergen/g of dust present a risk for the development of acute asthma (Platts-Mills and de Weck, 1989).

From studies on large numbers of samples, it has been concluded that 100 mites/g of dust is equivalent to 2 μg of Group I allergen. It has been suggested that if all houses in an area contain >2 μg Group I mite allergen/g of dust, then the susceptible individuals, perhaps as many as 30% of the population, will become sensitized. It is estimated that 10% of the population of the United States is sensitized to mite antigen.

The occurrence of respiratory symptoms from the inhalation of other insect-derived material has been well documented for more than a half century and may be a more prevalent problem than is currently appreciated. Much of the data on insect allergies is occupational. Locusts, crickets, grasshoppers, and cockroaches are grown for a variety of purposes (usually laboratory) and have induced sensitization in workers. Beetles, moths, butterflies, and flies have also been implicated in occupational allergies resulting from inhalation (Mathews, 1989).

Domestically, cockroaches are probably the most potent insect source for airborne allergens. Occupants of the poorer sections of large cities are more at risk of this kind of exposure, and cockroach antigen probably replaces mite antigen as the most important inducer of childhood asthma in this environment (Bernton and Brown, 1967; Mendoza and Snyder, 1970; Schulaner, 1970). The German cockroach (*Blatella germanica*) appears to be the most prominent sensitizer in the United States, but others may produce immunologic responses as well. Several different proteins between 70,000 and 75,000 daltons have been identified as important cockroach allergens (Stankus and O'Neil, 1988). In contrast to mites, cockroach feces is apparently not the primary source of antigenic material. Cast skins and whole body extracts appear to be more potent sources (Richman et al., 1984); however, levels of antigen that induce sensitization or produce symptoms remain unknown.

Other insects that cause sensitization in homes are the cat flea (Rolfsen et al., 1987), mushroom flies (Truitt, 1951), silk worm secretion (silk) (Dewair et al., 1985), and many others (Mathews, 1989). People who maintain fish in tanks may develop sensitivity to

chironomids kept as fish food (Baur et al., 1982). In the case of chironomid sensitization, 10 different hemoglobins have been found to be allergenic. It may be that, as is the case for fungi, any insect to which people are appropriately exposed over a long period of time can induce sensitization.

Allergic reactions to animal-derived allergens (danders) are frequent and have been known for many years. The allergic reactions result from exposure to domestic animals or to laboratory animals, especially rodents. In Sweden, more than 5% of unselected children were found to be sensitive to animal allergens (Kjellman and Pettersson, 1983). As many as 30% of individuals frequently exposed to laboratory animals in vivaria experience symptoms (Cockcroft et al., 1981b; Gross, 1980; Lutsky and Neuman, 1975).

In 1971, the first purification of a cat allergen established that this protein was present in saliva. The protein, which is now designated *Felis domesticus I* (Fel d I), is a 37,000 dalton, freely soluble glycoprotein, and has been identifiable for 15 years by a conventional antiserum (Leitermann and Ohman, 1984; Ohman et al., 1987; Chapman et al., 1987). Possibly 10% of all acute asthma in young adults is related to cat allergen exposure of patients who have IgE antibodies for cat proteins (Pollart et al., 1989a; Gelber et al., 1990). It has been suggested that a threshold level of 8 μg cat allergen/g of dust may be sufficient to produce sensitization (Gelber et al., 1990). Approximately 2 million Americans who are allergic to cats live in a house with a cat.

Canis familliaris I (Can F I) has been proposed as the major dog allergen (Schou and Lowenstein, 1990; de Groot et al., 1990). Urine, serum, and saliva (which contains serum proteins) all contain potent allergens (Viander et al., 1983). Although breed-specific allergens have been hypothesized, it appears that variation in reactivity to different canine breeds results from concentration differences between shared allergens (Knysak, 1989).

Urine and possibly saliva are the primary allergen sources for rodents. Two major allergens have been purified from rat urine; one primarily confined to male rats (Longbottom, 1980). Two major mouse allergens have been isolated; one from urine (Lorusso et al., 1986) and one from dander (Price and Longbottom, 1987). Three antigens have been recovered from guinea pigs; two from urine and one from other sources (Walls and Longbottom, 1983; Walls et al., 1985). As with the fungi and insects, it appears that any mammalian effluent can act as an allergen.

4.2.1.2 Diagnosis of Immediate Hypersensitivity

Patients with allergic diseases, such as hay fever, perennial rhinitis, anaphylaxis, and many cases of asthma, produce skin responses with a wheal (or hive) and surrounding flare (redness) when an extract of the causative allergen is introduced into their skin. The reaction occurs usually within 10 minutes, hence the term "immediate hypersensitivity". Evaluation of the significance of the skin test response depends on

- the quality and strength of the extract,
- the technique of skin testing, and
- the criteria used for identifying a positive response.

There are well-established techniques for preparing and storing extracts and evaluating their strength in vivo and in vitro (Aas et al., 1978; Dreborg and Einarsson, 1990). Recently, monoclonal antibodies have allowed much simpler purification and assay of Fel d I, a cat allergen (Chapman et al., 1988). However, until specific allergens are characterized and assays are developed to accurately measure them in extracts, allergen standardization will remain a significant problem.

Two general types of skin tests are commonly used: the epicutaneous (scratch, prick) test and the intradermal test. Skin testing for immediate hypersensitivity carries the risk that patients may rapidly develop generalized hives, hypotension, and/or airway obstruction (i.e., anaphylaxis). However, the risk of fatal anaphylactic reactions following epicutaneous or prick tests is so low that it can reasonably be ignored. By contrast, intradermal tests, particularly if carried out without a screening prick test, carry a significant risk of fatality (though even this is less than 1:2,000,000) (Lockey et al., 1987). Thus, skin testing should only be carried out by individuals familiar with the test and in the presence of a physician. Resuscitation equipment should also be readily available. Intradermal testing should only be considered after negative epicutaneous tests.

The primary objective of skin testing is to identify whether the individual being tested has made an immune response. Criteria for reliability of skin testing include repeatability and correlation with other methods of testing (i.e., challenge tests or serum assays) (Platts-Mills et al., 1982).

For some of the indoor inhaled allergens, the relationship to a disease is obvious to the exposed individuals. This is particularly so for cat allergens where the onset of rhinitis, asthma, or conjunctivitis may follow within 5 to 15 minutes of entering a house with a cat. For other allergens, the relationship is less obvious. For these allergens, formally establishing the relationship can be achieved with challenge studies to determine whether exposure to a specific allergen will produce rhinitis or asthma. Epidemiological studies on random populations may assist in showing that sensitivity or exposure to a specific allergen is common among individuals with the disease. Documented cases showing an improvement or subsidence of symptoms once subjects have been removed from exposure may also strongly support the relationship of exposure and disease (Platts-Mills et al., 1982; Kerrebijn, 1970; Charpin et al., 1988).

4.2.2 Hypersensitivity Pneumonitis

Hypersensitivity pneumonitis (HP), also called allergic alveolitis, reflects both antibody-dependent mechanisms and cellular immune responses (cell mediated immunity). It is characterized by recurrent pneumonia with fever, cough, chest tightness, and lung infiltrates. Progressive, irreversible lung damage may occur with continued exposure to antigens. High levels of IgG antibodies directed against the causal antigen are produced and can be used as a diagnostic tool in attempting to make connections between the environmental exposure and a specific disease (Fink, 1983).

4.2.2.1 Causative Agents

Any organic dust capable of penetrating the lower airways and present in high concentrations can probably cause HP (Salvaggio, 1987; Pepys, 1969). In addition, many of the agents known to be important in HP are, in themselves, adjuvants (agents capable of stimulating the immune system). For example, the thermophilic actinomycetes, which are common causes of HP epidemics, have been shown to have adjuvant activity (Bice et al., 1977). Most antigen exposures are mixed with respect to organisms. It may be that exposure to, for example, fungal spores, is only likely to cause HP in the presence of a known adjuvant, such as endotoxin. Biological agents that produce antigens known to cause HP include bacteria, fungi, protozoa, birds, and mammals. In fact, all the biological agents,

with the exception of the arthropods and plant pollen, that have been shown to produce antigens stimulating IgE-mediated dise..., have also been implicated in HP.

The classic form of HP is called farmer's lung disease, and is caused by inhalation of the minute spores produced by the thermophilic actinomycetes (e.g., *Micropolyspora faeni, Thermoactinomyces vulgaris*) (Kobayashi et al., 1963; LaBerge and Stahmann, 1966; Gregory et al., 1963; Gregory and Lacey, 1963; Lacey and Lacey, 1964). These organisms are abundant in organic material during degradation by other microorganisms (other bacteria, fungi). Bagassosis (HP resulting from exposure to moldy sugarcane bagasse) (Buechner et al., 1958; Salvaggio et al., 1966) and mushroom worker's lung (HP from exposure to moldy composting material) (Bringhurst et al., 1959) are also mediated by the thermophilic actinomycetes. Thermophilic actinomycetes have also caused HP when present in water-spray cooling systems (Banaszak et al., 1970), water in ventilation ductwork (Hales and Rubin, 1979), home humidifiers (Fink et al., 1971; Sweet et al., 1971; Burke et al., 1977), car air conditioners (Kumar et al., 1981), dust in air ducts (Weiss and Soleymani, 1971), console humidifiers (Tourville et al., 1972), and evaporative air coolers (Marinkovich and Hill, 1975). Glycopeptide and protein antigens have been purified from the thermophilic actinomycetes, although purification and complete characterization has not been achieved (LaBerge and Stahmann, 1966; Pepys and Jenkins, 1965; Wenzel and Emanuel, 1965; Edwards, 1972; Fletcher et al., 1970).

Other kinds of bacteria have also been suspected to cause HP. Humidifiers contaminated with *Flavobacterium* (Rylander et al., 1978), *Bacillus sereus* (Kohler et al., 1976), and *Bacillus subtilis* (Parrott and Blyth, 1980) have been implicated in HP epidemics. *Bacillus subtilis* was also suspected as the causative agent in wood dust HP (Johnson et al., 1980).

Suberosis, Sequoiosis, and cheese worker's lung are forms of occupational HP outside the farming environment. *Penicillium* species have been involved in a number of HP cases and epidemics (Bernstein et al., 1983; Campbell et al., 1983; Fergusson et al., 1984; Solley and Hyatt 1980; van Assendelft et al., 1985). *Aspergillus* species have been less frequently implicated in the development of HP (Blyth, 1978; Patterson et al., 1974; Vincken and Roels, 1984; Yocum et al., 1976). Other fungi that have been reported to cause HP include *Cryptostroma corticale* (Emanuel et al., 1966), *Phoma violacea* (Green, 1972), *Merulius*

lacrymans (O'Brien et al., 1978), *Cephalosporium* (Patterson et al., 1981), *Alternaria* (Schlueter et al., 1972), and *Trichosporon cutaneum* (Shimazu et al., 1984). Most of these fungi have spores that are less than 5 μm in aerodynamic diameter.

In addition to other diseases, the free-living amoebae can produce HP by excreting antigens into water. In one report, *Naegleria* was implicated as the causative agent in a case of humidifier fever (Edwards et al., 1976; Baxter, 1982; Cockcroft et. al., 1981a).

Parakeets (Edwards and Luntz, 1974; Lee et al., 1983), chickens (Korn et al., 1968), turkeys (Boyer et al., 1974), and doves (Cunningham et al., 1976) have all been shown to precipitate "bird fancier's lung" or HP associated with bird serum proteins, although pigeon-breeder's disease is the best studied (Reed et al., 1965; Stiehm et al., 1966; Stiehm et al., 1967). Pigeon breeder's disease has been reviewed by Christensen et al. (1975) and Schmidt et al. (1988). As with other biological antigen-producers, any serum proteins from any bird could probably cause HP in an appropriate host with appropriate exposure. Multiple antigens appear to be responsible for pigeon breeder's disease. Some antigens appear primarily related to exposure, whereas others appear only in the sera of symptomatic individuals (Berrens and Guikers, 1972; Berrens and Maesen, 1972a; Berrens and Maesen, 1972b). At least five major antigens have been identified and described (Edwards et al., 1970; Edwards et al., 1969; Fredricks and Tebo, 1975; Tebo et al., 1977).

Mammals produce proteins that can cause HP if aerosolized appropriately in the presence of susceptible hosts. Most cases of HP associated with mammalian proteins occur in occupational environments. Laboratory animal handlers, especially those handling rats, are occasionally affected (Salvaggio, 1987). Hypersensitivity pneumonitis associated with household mammalian pets has not been reported.

Thermophilic actinomycetes, although common on natural substrates in the outdoor environment (soil, compost), are not common in air and are rarely recovered from indoor air unless a contaminated reservoir/disseminator is present. Based on analyses of situations known to cause HP, it appears that massive exposure to appropriately sized antigens is necessary for the development of HP. For example, dairy farmers that use moldy hay in closed barns late in the season are more likely to contract the disease than hog or cattle ranchers who do not handle contaminated organic material in enclosed spaces (Fink, 1983). Hypersensitivity pneumonitis, then, is probably a disease of the indoor environment.

Preliminary evidence indicates that coexposure to an antigen and endotoxin may be more sensitizing than exposure to an antigen alone. Certainly, gram-negative bacteria are always present where dead plant materials are handled. Although high levels of antigens are important for sensitization, it is probable that very low antigen levels will subsequently induce symptoms and present a risk of continuing lung damage.

Risk factors for development of HP are unknown, but may involve a defect in the cellular immune system. Pigeon breeders with active disease were shown in one study to have depressed T suppressor-cell activity when compared with comparably exposed, asymptomatic breeders (Keller et al., 1984). The attack rate can be low in spite of large populations receiving high levels of exposure to suitable antigens, further suggesting some specific host risk factor. The role of these factors may be different when exposure to antigens is coupled with exposure to endotoxins. Elevated attack rates in some situations may be explained by this phenomenon.

Because the disease is not expected to occur in so-called clean environments (offices, homes), and in early stages misdiagnosis is probably common, the actual incidence of HP is unknown. It is estimated that 7% of British farmers have farmer's lung (Boyd, 1971; Grant et al., 1972). Epidemiologic studies in other environments have not been undertaken. Among clearly exposed populations, attack rates can be as high as 15 to 21% in offices and among pigeon breeders, respectively (Banaszak et al., 1970; Caldwell et al., 1973; Emanuel et al., 1964).

4.2.2.2 Detection of Sensitization

Serum IgG antibodies, as detected by the precipitin test, are produced in nearly all people with symptoms and in possibly 50% of exposed people without symptoms (Fink et al., 1972). The antigen-specific IgG ELISA is a more sensitive and quantitative assay that may be used to determine differences in levels of antibody in exposed asymptomatic and symptomatic people. However, this use has not been reported. Of course, these studies are only possible where a specific causative antigen has been identified. Tests of cell-mediated immunity (e.g., pulmonary lymphocyte blastogenesis) define more clearly the differences between symptomatic and asymtomatic exposed populations, suggesting a primary role for cellular immune systems in the pathogenesis of the disease (Moore et al., 1980). Challenges

(inhalation of antigen aerosols) with known or suspected antigens can be used to reproduce symptoms and to relate disease to specific antigen sources.

4.3 BIOLOGICAL TOXINS

Toxins can enter the mammalian system by ingestion; absorption through the skin; inhalation; and subcutaneous, intraperitoneal, or intravenous injection. Toxic effects can be acute and/or chronic. The toxin may exert its effect on a cellular level at the primary site of entry (skin, lung, esophagus, stomach), on organs where nontoxic precursors are metabolized (kidney, liver, bladder), or systemically (neurotoxicity). Most of the biological toxins are cytotoxic at relatively low doses. Many are also teratogenic, mutagenic, and/or carcinogenic. The bacterial toxins have been considered primarily important as part of infectious disease syndromes (e.g., tetanus) or as ingested poisons (e.g., botulism). Mycotoxins (fungal toxins) have been primarily studied as ingested poisons (e.g., ergotism, aflatoxin carcinogenesis). However, it is becoming clear that, when inhaled, mycotoxins may be responsible, either directly or in conjunction with other agents, for some of the diseases associated with indoor air quality. Three major kinds of biological toxins will be considered here: the bacterial endotoxins, the mycotoxins, and fungal volatile organic compounds.

4.3.1 Bacterial Toxins

Bacterial toxins are of two types: exotoxins and endotoxins. Exotoxins are bacterial metabolites that are excreted into the environment. The toxin produced by *Clostridium botulinum*, responsible for the serious form of food poisoning known as botulism, is a bacterial exotoxin. Exotoxins have not been studied with respect to aerosol exposures or their presence in the environment.

Endotoxins have been the focus of intensive study for many years, especially with respect to their role in infectious disease (Westphal et al., 1977; Westphal et al., 1983). Endotoxins are a component of the outer membrane of gram-negative bacteria and are known, in pure form, as lipopolysaccharides (LPS). Lipopolysaccharides are composed of three regions:

- the O-specific polysaccharide chain that is specific for each type of bacterium;
- the R-specific core polysaccharide that is relatively similar among bacteria; and
- lipid A, which has both constant and variable regions.

The lipid A part of the molecule is responsible for the toxic effects, whereas the polysaccharide parts constitute the antigens. Lipopolysaccharides are stable and resist routine autoclaving. In the environment, endotoxins may be part of whole cells, large membrane fragments, or in macromolecular aggregates of about 1 million daltons (American Conference of Governmental and Industrial Hygienists, 1989b).

Endotoxins are primarily known as pyrogens (fever-inducers) and are highly toxic. In addition to fever, they cause acute pulmonary changes and local inflammatory responses (Ellakkani et al., 1984; Lantz et al., 1985). Endotoxins are also nonspecific immune system stimulants and may be anticarcinogenic (American Conference of Governmental and Industrial Hygienists, 1989b; Enterline et al., 1985). Environmental exposure to endotoxins occurs in occupational environments where organic materials contaminated with gram-negative bacteria are handled. Byssinosis (pulmonary disease related to exposure to cotton dust) has been extensively studied with respect to the role of endotoxin (Castellan et al., 1987; Rylander et al., 1985; Ellakkani et al., 1984; Cinkotai et al., 1977; Kennedy et al., 1987). Poultry and swine confinement buildings (Clark et al., 1983b), composting facilities (Clark et al., 1983a), grain elevators (DeLucca et al., 1984), and environments where gram-negative bacteria are used in manufacturing processes (Palchak et al., 1988) all present a significant risk for exposure to endotoxin aerosols. In addition, humidification equipment, including home humidifiers, are frequently contaminated with gram-negative bacteria and, hence, with endotoxins. Some evidence indicates that symptoms associated with the operation of contaminated humidifiers result from endotoxin exposure and direct activation of the inflammatory process via the alternate complement pathway rather than through the agency of an antigen (Rylander et al., 1978; Rylander and Haglind, 1984). Alternatively, the endotoxin might be acting as an adjuvant. These hypotheses might explain the frequently high attack rate that has been reported in some epidemics of humidifier fever as compared to the much lower rates that prevail for farmer's lung disease and the frequent lack of association of precipitating antibodies to suspected source material in exposed people. Endotoxin may be an

auxiliary factor in immunologic sensitization for asthma as well, although this hypothesis remains speculative (Michel et al., 1989).

Doses of endotoxin that have caused symptoms in the environment are unknown. Measured levels in the environment range from 0 to >100 $\mu g/m^3$, with 0.1 to 5 $\mu g/mL$ measured in contaminated humidifiers associated with disease (Rylander and Haglind, 1984). Quantitative endotoxin data must be interpreted with caution because values are strongly dependent on the method of assay. The assays for endotoxins that are currently available are comparative rather than absolute and do not allow comparisons between laboratories (Milton et al., 1990).

4.3.2 Mycotoxins

Most fungi produce metabolites that have a range of toxic effects ranging from mild acute toxicity to potent carcinogenicity (Shank, 1981; Rodricks et al., 1977). Taxa such as *Penicillium*, *Fusarium*, and *Aspergillus*, members of which produce toxins that have dramatic acute effects (e.g., aflatoxins, antibiotics, trichothecenes), have been extensively surveyed for toxin production, whereas other taxa have not been well studied (Burge, 1987). Some fungal toxins are toxic without metabolic activity (e.g., T-2 toxin), whereas others require metabolic conversion for toxicity (e.g., aflatoxin B_1). Fungal toxins that require conversion often exhibit their toxic potential in target organs able to effect the metabolic conversion (e.g., liver). Although little studied, lung tissue can apparently convert aflatoxin B_1, and probably other mycotoxins, to their toxic form. Most of the mycotoxins are cytotoxic as measured in cell culture in the range of 0.1 to 10 μg of toxin/mL of culture fluid.

Mycotoxins can enter the body through the skin (Riley et al., 1985), gastrointestinal tract (Shank, 1981), or respiratory tract (Wicklow and Shotwell, 1983). Mycotoxins become airborne or aerosolized on substrate material, adsorbed onto dust particles or spore/mycelial surfaces, or as an intrinsic part of spores or mycelial fragments (Wicklow and Shotwell, 1983). Therefore, the toxins are carried on a wide range of particle sizes with different degrees of penetrability into the respiratory tract. The smallest particles may reach alveoli in significant mass, larger particles may deposit in the conducting airways, and the largest are probably held in the nose or trachea and subsequently swallowed. Respirable dust particles that remain on lung surfaces for some-time present a greater dose directly to lung tissue with

a resultant increase in the risk of local tissue damage and possible neoplasm development (Baxter et al., 1981). In addition, inflammation has been shown to be a cofactor in carcinogenesis so that toxins borne on potentially inflammatory dusts (e.g., grain dusts and other particles containing endotoxin) may be carcinogenic in very low doses (doPico et al., 1977).

Acute toxic effects from airborne mycotoxins are rarely reported, but do occur. Severe acute toxic effects have been reported from exposure to a massively contaminated return air duct containing the trichothecene-producing fungus *Stachybotrys atra* (Croft et al., 1986). "Yellow rain," a biological warfare agent, used in southeast Asia was thought to be concentrated trichothecenes (Mirocha et al., 1983). Tremorgenic mycotoxins produced by *Aspergillus fumigatus* may have caused occupational symptoms in a sawmill environment (Land et al., 1987). Acute symptoms experienced by grain handlers may be, in part, due to the mycotoxins as well as lectins and other toxic agents in grain dust (doPico et al., 1977, 1982, 1983; Dashek et al., 1983; Palmgren et al., 1983; Enarson et al., 1985). It has been suggested that some of the symptoms mimicking sick building syndrome (headache, dizziness, nausea, fatigue) may be due to exposure to airborne trichothecenes. Mycotoxins may also be responsible for the pathogenesis of invasive fungus diseases such as aspergillosis, the toxin paving the way for fungus invasion (Eichner and Mullbacher, 1984).

Chronic exposure to airborne mycotoxins also represents a significant health risk. Cancer, probably associated with low-level mycotoxin exposure, has been reported in peanut handlers (Burg et al., 1981, 1982), mycotoxin researchers (Deger, 1976), and in several farm-related cases (Dvorackova and Pichova, 1986). Reports of "leukemia houses" are thought to be the result of aflatoxin exposure (Wray and O'Steen, 1975). Three cases of pulmonary interstitial fibrosis have been reported where aflatoxin B_1 was measured in lung tissue on autopsy (Dvorackova and Pichova, 1986).

Analysis of air samples for mycotoxins is usually restricted to the aflatoxins, or occasionally, the trichothecenes. Aflatoxins have been recovered from airborne grain dust in levels exceeding 1,800 parts per billion (Burg et al., 1981; Burg and Shotwell, 1984; Dashek et al., 1983; Palmgren et al., 1983; Sorenson et al., 1981). For aflatoxins, particles less than 5 μm appear to carry the preponderance of toxin (Sorenson et al., 1981). Sampling methods used for airborne toxins were not designed for particles less than 0.1 μm, and toxins

on these small particles have not been studied. More importantly, considering the high potential for synergism between toxins, most studies have examined only single toxins. It is clear that many fungi produce multiple toxins and that many different toxigenic fungi may inhabit specific environments. The potential for exposure to unknown toxins must be assessed. For example, farmers harvesting corn are not only exposed to aflatoxins and trichothecenes, but to more than 10^{10} fungus spores per cubic meter of air, including known toxin producers such as *Alternaria*, *Cladosporium*, *Penicillium*, and *Aspergillus*. Acute or chronic exposure to any mixed microbial aerosol presents some risk of toxin exposure.

4.3.3 Fungal Volatile Organic Compounds

In addition to higher molecular weight toxins, all organisms exude volatile organic compounds (VOCs) during growth. Although these VOCs are gases, due to their source, they are closely involved with bioaerosol-related problems. Some of these biological volatiles impart characteristic odors to the environment. Examples of such odors include body odor produced by human occupants of interior spaces; dirty sock odor from bacterial growth on damp, sweaty clothing and carpeting; and the moldy smell, characteristic of some basements.

Some of the VOCs that have been reported from fungi grown on "natural" substrate (wheat meal) include 3-methylbutanol, 3-octanone, 3-octanol, 1-octen-3-ol, 1-octanol, and *cis*-2-octen-1-ol (Kaminski et al., 1972; Kaminski et al., 1974). Penicillium species grown on potato dextrose agar produces thujopsene, 3-octanone, nerolidol, 1-octen-3-ol, phenylethyl alcohol, 3 octenol, 1,5-octadien-3-one, 1,5-octadien-3-ol, 2-methoxy-3-isopropyl-pyrazine, 2-methylisoborneol, 2-methyl-1-propanol, 2-methyl-2-pentenal,, 3-methyl-1-butanol, naphtalene, damascenone, and octanoic acid (Halim et al., 1975; Karahadian et al., 1985). *Penicillium chrysogenum* grown on malt extract agar produces many of these same compounds and, in addition, many compounds that have been reported from building materials off-gassing in the indoor environment (unpublished data). Some of these reported compounds (e.g., acetaldehyde, heptane, 2-heptanone, 2-hexanone, 2-pentanone, etc.) may produce significant adverse health effects at sufficiently high concentrations. Others produce strong odors (e.g., decanoic acid, hexanoic acid, methanethiol). In addition, fungi are able to metabolize toxic solids into a gaseous state. For example, some fungi can convert arsenic compounds into trimethyl arsine (Foster, 1949) and have resulted in arsenic poisoning. Many

fungal VOCs produce mucous membrane irritation and may be involved in or produce symptoms (headache, nausea, dizziness) that mimic the sick building syndrome. Unfortunately, very little research has been done to characterize microbial VOCs, assess their exposures, or evaluate their potential health effects.

5. BIOAEROSOL INVESTIGATIONS

Bioaerosol investigations, whether for research purposes or to solve specific problems, rely on a knowledge of the principles of aerobiology, air sampling technology and analysis, as well as epidemiology, and a thorough familiarity with the nature of bioaerosols. Unfortunately, this combination of knowledge cannot be obtained in any single discipline. Those who do bioaerosol research effectively operate in a team environment. Research investigators approach questions related to bioaerosols differently than field investigators. However the same factors must be considered:

- investigative strategy,
- sample collection techniques,
- sample analysis methods, and
- data analysis and interpretation approaches.

5.1 Investigative Strategies

Nonbiological pollutants in the work place are easy to assess, relative to biological pollutants. Methods for aerosols, and standards or guidelines are available that allow interpretation of collected data (American Conference of Governmental and Industrial Hygienists, 1984). For bioaerosols, this simplified situation is rarely the case. Because available sampling methods have intrinsic differences and require skilled and time-intensive analysis, little background data exists on indoor bioaerosols, and no guidelines have been published. Thus, relatively intensive investigations are usually required, and difficult decisions must be made with respect to sampling strategy.

The total air environment in any situation cannot be sampled. Rather, a statistically adequate sample must be collected and overall patterns of exposure must be inferred. A necessary or suitable size sample has not been determined for bioaerosols. Such a determination would require that multiple duplicate samples be taken with several different kinds of equipment. The samples would also have to be taken at many sites in the

environment and over a time period that included all the potential variability of the predicted aerosols.

5.1.1 Studying Indoor Microbial Ecology with Respect to Air Pollution

At the most basic level, indoor bioaerosols may be considered as an ecosystem. In this case, human exposure is not the concern, but rather factors controlling population dynamics of sources, the dynamics of dissemination, and the dynamics and biology of airborne populations are the concern. These studies provide the basis for all bioaerosol investigations. A few of the factors that must be considered are

- the role of environmental factors (substrate, climate) on microbial survival, growth, reproduction, and metabolism;
- factors controlling dissemination from reservoirs; and
- the taxonomy of environmental microbes (Dimmick and Akers, 1969).

5.1.2 Documenting Exposure/Dose/Symptom Relationships

To set standards or develop guidelines for interpretation of bioaerosol data, exposure-response information is necessary. These data may be generated in the laboratory from animal or human exposure studies, or epidemiologically, by collecting baseline data and evaluating symptoms in a wide variety of environments. Documenting exposure requires air sampling and assessment of particle size as well as other exposure parameters. Questionnaires and/or physical exams may be used to evaluate symptoms (Su et al., 1990; Burge and Garrison, 1989).

5.1.3 Documenting Unusual Exposure Situations

Documenting unusual exposure situations is the simplest approach to evaluating bioaerosol exposures and is usually appropriate for problem-solving investigations done in response to epidemics of disease or building-related symptoms. Field investigations designed to approach specific building-related problems may be divided into two steps: a careful analysis of the biological status of the environment and an evaluation of the environmental data with respect to health risks.

Ideally, air sampling should be used to assess airborne exposure levels. Studies that include air sampling need to be designed with appropriate controls, and need to include enough samples so that reasonably valid interpretations may be made. Minimally, it is essential to have duplicate samples in the environment of symptomatic people, asymptomatic people in the same environment, and samples of the outdoor air near air intakes. Sampling before and after disturbing potential reservoirs/amplifiers is also extremely useful.

A second approach involves searching for potential reservoirs/amplifiers, assessing contamination (visually or through assay techniques), and either providing logical hypotheses for dissemination/exposure or conducting air sampling in association with postulated dissemination conditions and comparing air sample results to those obtained with samples from the potential source. Concluding that an unusual exposure situation exists is relatively straightforward if high levels of an organism, antigen, or toxin are found in indoor air and not outdoors or in other control situations. Unfortunately, proving the negative case is rarely, if ever, possible even when sampling protocols are multifaceted and extensive (American Conference of Governmental and Industrial Hygienists, 1989b).

5.2 AIR SAMPLE COLLECTION TECHNOLOGY

Air sampling for biological aerosols is usually done to estimate the potential for human exposure. Occasionally, bioaerosol sampling is done for legal reasons. In both cases, but especially in the latter, it is essential that the sampling procedures can withstand legal challenge. This means that either the best available sampling strategy, instrumentation and methods for analysis, must be used or one must be able to document the validity of using less efficient or less reliable methods.

Sampling can be either personal or ambient. Personal sampling (i.e., sampling near the breathing zone of affected individuals) would be ideal. However, personal samplers that reliably estimate most microbial aerosols have yet to be developed (American Conference of Governmental and Industrial Hygienists, 1989a). Ambient sampling is most often used for both microbial and antigen aerosols. Ambient sampling, if properly done, reflects average exposures for all occupants, but can underestimate individual doses. To be effective, ambient sampling must be done at all the levels and sites where people work or live and over a time

period that covers potential cyclic changes in the aerosols and activity patterns of the people (American Conference of Governmental and Industrial Hygienists, 1989a).

Air sampling may be performed either over short periods (grab samples) or over longer periods (average or integrated samples). Grab samples usually represent 5 minutes or less of sampling time, whereas integrated samples are collected over a longer exposure period. All of the methods commonly used for viable microorganisms collect grab samples. It is difficult to maintain viability and/or prevent growth of viable microorganisms over longer sampling periods.

Size-selective sampling has always been considered important for bioaerosol-related studies because lung deposition has been considered necessary for efficient transmission of infectious disease. However, upper airway deposition of allergens clearly causes disease (Wilson et al., 1973). Except in research situations, most microbial samples can be collected as a unit on a single-stage sampling device. At most, two stages are useful for determining the so-called "respirable" fraction of the aerosol.

Accuracy and precision are factors that apply to all aerosol sampling methods. Accuracy is the amount by which the measured value deviates from the true value. Precision is the standard deviation of repeated measurements of the same variable. Accuracy is difficult to assess for most bioaerosols. For chemicals, accuracy of a particular method is measured by using another method and comparing results. Chamber studies that provide a continuously stable aerosol are necessary for most bioaerosols. Precision can be assessed by increasing the number of measurements at a particular site using the same methodology.

5.2.1 Principles of Aerosol Sampling

The term aerosol technically refers to the complex of particles suspended in a gaseous medium, although the popularly used definition is synonymous with "particle". Bioaerosols, then, are airborne particles with a biological origin that follow the physical rules for any aerosol. Principles of aerosol sampling have been reviewed elsewhere (Edmonds, 1979). Aerosols can be collected using gravitation, centrifugal force, inertial impaction, filtration, and electrostatic impaction (American Conference of Governmental and Industrial Hygienists, 1989a; Burge and Solomon 1987). The most commonly used modalities for collection of bioaerosols are inertial impaction and filtration. Regardless of the sampling mode, when

selecting sampling devices for biological aerosols, the efficiency with which particles are removed or collected from the air, the efficiency with which viability is protected, and the overall efficiency must be considered (American Conference of Governmental and Industrial Hygienists, 1989a).

Inertial impactors collect samples by drawing air through an orifice using suction (e.g., the sieve-plate, slit, and centrifugal impactors) or by rotating collecting surfaces rapidly through the air (e.g., rotorods). Suction devices require that the ambient airflow be directed into the sampler orifice, and that the rate of suction be equal to the ambient air speed. This is called isokinetic sampling. When suction sampling is not isokinetic, patterns of particle collection change. For example, if suction speed is greater than air speed, small particles will tend to be vacuumed from the air and will be over-represented in the sample (May, 1967; May, 1980). Similar kinds of effects occur if the sampler orifice is not directed towards the airflow. In the extreme case where the orifice is at right angles to the ambient airflow, smaller particles are likely to be preferentially sampled at all suction speeds (Akers and Won, 1969). In general, in most indoor environments, an isokinetic sampling condition can be ignored if the aerosol of interest is less than 10 μm and sampling rates (airflow through the sampling orifice) are low. Errors may be introduced when larger aerosols are being sampled. Many common allergens, including all pollen and many fungus spores, are larger than 10 μm.

Once the particles are in the sampler, impaction efficiency depends on the inertia of the particle as it hits the impaction surface. Larger particles have more inertia at a given particle speed and are more likely to impact. Thus, although suction samplers readily draw small spores into the sampling orifice, larger particles are more likely to impact on collection surfaces and be counted. Only a few samplers have been well characterized with respect both to aerosol-collection efficiency and particle-retrieval efficiency. Those that have not should be used with care.

Filtration devices also rely on suction for collection of the aerosol. Once in the device, theoretically, filtration samplers are 100% efficient at trapping particles larger than the rated filter pore size. Therefore, if suction conditions are chosen appropriately, very high sampling efficiency may be obtained using filtration.

The width of the collection surface controls the efficiency of small particle collection for the rotating impactors. Unless the collection surfaces are well under 1 mm in diameter, small particles are poorly collected by these devices (Edmonds, 1972).

5.2.2 Sampling for Viable Microorganisms

Some organisms must remain viable during sampling and analysis procedures. Many infectious agents, including all bacteria and many fungi, are only identifiable in the living state. Factors that affect viability in the air of these kinds of aerosols are covered elsewhere in this document. However, the process of sample collection, handling, and analysis also affects viability or culturability.

Sample collection can damage living cells in many ways, including mechanical effects caused by impaction and desiccation, as demonstrated by a decrease in bacterial recovery when slit-to-agar distance is decreased in a slit sampler, or when the slit width is decreased. The speed at which the particle hits the agar surface is increased by both of these changes and the losses are presumed due to mechanical breakage of the cells (Goldberg and Shechmeister, 1951). The effects of changes in relative humidity on microorganisms are complex and poorly studied. It has been shown that bacterial cells collected on a dry filter rapidly desiccate with resulting loss in viability (Wolochow, 1958). In addition, rehydration of aerosols of bacteria and viruses may cause cell damage (Hatch and Dimmick, 1966; Akers et al., 1966). Neither of these effects have been well studied for fungi.

It should be apparent that the media into which cells are collected should not adversely impact viability. Wetting agents, growth inhibitors, antibacterial, and/or antifungal agents all have adverse effects on some organisms (Burge et al., 1977b). In addition, culture media must be chosen that either will support the growth of the organism directly, or will protect its viability until transfer to appropriate media can be undertaken. In general, rich media (e.g., nutrient or casein soy peptone agar for bacteria, malt extract agar for fungi) are used in air samplers. However, use of minimal media may allow stressed organisms to recover, and recoveries will vary depending on the culture medium used (Akers and Won, 1969). The cultural sampling data is in the form "colony-forming units" rather than spores or cells unless dilution methods (e.g., liquid impingers) are used. There is no way of knowing how many original cells contributed to a single colony on a culture plate impactor.

Culture plate impactors (preseeded with living cells for viruses), liquid impingers, membrane filter cassettes, and high-volume electrostatic devices have been used to collect viable microorganisms. Viruses, infectious bacteria, fungi, and protozoa are usually sampled by methods that allow their culture both in vitro and in vivo. Therefore, viability is of concern. Although viruses are submicronic, they usually travel through the air on larger particles and the same sampling devices may be used as for larger bacteria and fungi. Bacteria also tend to travel on rafts (masses of organic material). Indoor human-source bacteria generally travel on particles larger than 20 μm. The thermophilic actinomycetes and the fungi, on the other hand, appear to travel as single spores and may be as small as 1 to 2 μm.

When choosing a sampler for collection of a particular viable aerosol, the size of the particles, the relative fragility of the organisms, and the expected concentrations should be considered. For very small particles (small fungus spores, thermophilic actinomycetes), suction devices and isokinetic sampling should be used and impaction efficiency within the sampler should be well characterized. Larger particles also require isokinetic conditions if suction sampling is used. However, impaction efficiency becomes somewhat less of a concern.

A hypothetical "order of decreasing fragility" for common indoor microbial aerosols is likely protozoa > vegetative bacterial cells > viruses > fungal spores > bacterial endospores (including thermophilic actinomycete spores). Therefore, relatively gentle methods should be used for protozoa and vegetative bacteria, whereas more aggressive techniques may be used for fungal spores and bacterial endospores. In practice, the same sampling devices are generally used for all, and careful consideration of the relative potential for loss of viability must be taken when the more fragile organisms are sampled.

Intercellular interactions are well known for fungi and have also been reported for bacteria. It is clear that the more fungal spores that impact on an agar surface, the fewer will germinate and produce a recognizable colony. Therefore, it is essential to select sampling rates and volumes that will not overload the sampling surface when using a culture plate impactor. In addition, it should be noted that some fungi and actinomycetes can produce diffusible antibiotics that will kill many bacteria and some fungi. When sampling dense aerosols of any type, it is probably advisable to use a method where dilution culture can

easily be used to minimize damage from these compounds (e.g., liquid impingers or filter cassettes).

5.2.3 Sampling for Microscopically Identifiable Organisms

Both bacteria and fungi can be sampled by methods that allow for direct microscopic examination. These methods produce total counts of individual cells in contrast to viable colony-forming units. Commonly used methods include suction slit impactors, membrane filter cassette samplers, and rotating arm impactors. The suction sampler readily overestimates small aerosolized particles in still air, and underestimates them in air moving faster than the sampler flow rate (May, 1967). However, because viability is not a concern, samples can be collected over long periods of time for analysis by direct microscopy. Thus, one slit impactor collects a time-discriminated sample over a 7-day period (the Burkard version of the Hirst spore trap). Filter cassettes can be used at low flow rates over an entire work shift, allowing integrated 8-hour exposure assessments to be made.

The rotating arm impactors are the most commonly used samplers in the United States for outdoor bioaerosols (with emphasis on pollen). They are highly efficient at collecting particles in the size range of most pollen and are easy to use. However, if the collecting surface is >1.5-μm wide, they are less than 5% efficient for fungal spores smaller than 5 μm (Edmonds, 1972). In dense viral aerosols where infectivity is not of concern, it is also possible to collect the samples on a membrane filter for subsequent examination by electron microscopy. Some interest has centered on the use of filter cassettes with subsequent direct microscopic examination for total bacteria and/or fungi or with elution and culture. Bacteria must be stained for microscopic analysis. However, these methods appear to underestimate both viable and nonviable aerosol concentrations. They are useful for endotoxins, and possibly for other toxins and allergens, provided sample volumes are of sufficient size.

5.2.4 Sampling for Amorphous Particles

Many of the allergens, antigens, and toxins that may accumulate in the indoor environment are carried on particles that do not grow in culture and are not readily identifiable microscopically. Collection of these particles from air, although requiring adherence to the same aerosol collection rules that apply to other particles, also must enable

the particles themselves, the soluble adhering material, or other associated material to be eluted for assay or assayed directly from the sampling medium. For water soluble materials that are not hydrophobic, liquid impingement is a possible choice. Most investigators, however, use some kind of filter medium in a suction sampler. For endotoxins, smooth surface polycarbonate filters minimize the risk of permanent adherence of the toxins to filter surfaces (Milton et al., 1990). Adherence of other materials to filters has not been carefully tested.

Mite, cat, and small mammal antigens have all been collected from indoor air using high-volume filtration devices (Swanson et al., 1985; Platts-Mills et al., 1989). The suction rate used in these devices is high and small particles are preferentially collected. It is also probable that the high suction rate changes the environment by, in effect, cleaning the air. These two factors make high-volume filtration sampling in indoor air a qualitative rather than a quantitative procedure.

5.2.5 Sampling for Volatile Aerosols

Both fungi and bacteria produce volatile compounds that are odoriferous and may cause irritation and other health risks. Sampling for these compounds requires prior knowledge of their nature. Water soluble volatiles are collectible with a liquid impinger. Various adsorbents (e.g., charcoal, tenax) may be used for some volatiles. Cold traps (i.e., liquid nitrogen) are used to collect a total volatile component. These methods, however, have not yet been applied to microbial volatiles in other than research settings.

5.2.6 Source Sampling

Source sampling involves collecting materials from suspected reservoirs for analysis using any of the methods discussed below (e.g., culture, microscopic observation, immunoassay, biological assay, or chemical assays). Reservoirs that are commonly assessed include house dust, fluid reservoirs, soft materials, and surfaces.

House dust mite allergens, cockroach allergens, fungi, and bacteria are commonly assayed from samples of house dust collected with vacuum devices (Gravesen, 1978; Platts-Mills et al., 1986b). In general, principles of aerosol collection have been ignored in these studies. Results are presented as antigen, mites, or colony-forming units of fungi or

bacteria per gram of dust. Unfortunately, the size of particles collected as well as the location within the reservoir (i.e., carpeting) is controlled by the amount of suction at the vacuum orifice, and the size of particles retained depends on the pore size of the collection bag. As a result, although individual studies using a single vacuum device allow comparisons from site to site, studies using different collection devices are not comparable. Also, results using poorly characterized collectors do not accurately represent the potential for exposure.

Sampling from fluid reservoirs entails collecting the sample (water/slime) in a bottle or syringe for subsequent analysis. Where water samples are concerned, a reasonably valid sample can be obtained if the reservoir is well mixed prior to sample collection. Slime that collects on wet surfaces can be scraped into a collection container. These samples are likely to represent local conditions; however, multiple sites should be sampled to assess reliability.

Soft materials such as carpeting and other fabrics, fiberglass and wall paper, and other paper materials may be collected for microscopic examination or elution for other assay methods. Obtaining a representative sample is a considerable challenge. Often one collects parts of these materials supporting visible fungi growth only for determination of the kind of fungi and not the quantity present.

Where removable, hard materials can be taken to the laboratory for analysis. Usually, however, scrapings must be collected (from areas of obvious growth) or in situ surface samples must be taken. Transparent tape pressed against obvious contaminated surfaces allows straightforward microscopic examination. The tape can be dissolved with acetone without damaging collected spores. Surface cultures can be obtained either using sterile swabs or contact plates. Neither of these methods is quantitative, although they are often used by inexperienced investigators to evaluate levels of contamination. Obtaining a representative sample, especially of organisms growing on the surface (rather than having settled), is nearly impossible. Table 5-1 briefly summarizes sampling modalities commonly used to evaluate biological contamination of the indoor environment.

5.3 AIR- AND SOURCE-SAMPLE ANALYSIS METHODS

Although sample collection principles are common to both biological and nonbiological aerosols, analysis methods are quite different and require procedures specific to each different

TABLE 5-1. SUMMARY OF SAMPLING MODALITIES USEFUL FOR INDOOR BIOAEROSOLS

Method	Viruses	Bacteria	Fungi	Pollen	Antigens	Toxins
Cultural						
Impaction	X	X	X			
Impingement	X	X				
Direct Visualization						
Impaction			X	X		
Filtration	X	X	X	X		
Impingement	X	X				
Chemical Assay						
Filtration					X	X
Impingement					X	X

bioaerosol. Bioaerosol analysis methods fall into five general categories: direct microscopy, culture, immunoassay, bioassays, and chemical analysis.

5.3.1 Direct Microscopy

Fungi spores can be counted microscopically from any sample that either is collected onto an optically suitable surface (transparent tape, microscope slide, plastic rotorod), onto a surface that can be made transparent (some filter media), or into a medium that can subsequently be filtered (e.g., a liquid impinger). Many fungal spores are identifiable using microscopic examination without staining. Bacteria, including *Legionella*, are countable microscopically only if stained. The type of stain used depends on the chemical nature of the organism (e.g., acridine orange fluorescent staining for bacteria, basic fuschsin or phenosaffranin stains for pollen) (Palmgren et al., 1986; Muilenberg, 1989), or the antigenic composition (e.g., fluorescent antibody stains used for *Legionella*) (Winn, 1985). Most particle collection methods can be used with subsequent staining, providing the impaction medium (often silicone grease), when present, does not interfere with the staining. Although pollen can readily be stained after impaction in grease on a rotorod or Burkard trap slide, acridine orange and fluorescent antibody stains do not penetrate well through a layer of grease. For these, filter collection media or liquid collection are more appropriate.

Scanning electron microscopy (SEM) must be used to visualize viruses. Although useful for determining surface characteristics, SEM does not usually significantly add to the information necessary for bacterial or fungal spore identification.

Although attempts have been made to count spores and pollen using digitized microscopic images fed into computer programs, so far these methods are impractical because of the enormous morphological variability in most bioaerosols. Likewise, particle counters, including those using advanced laser techniques, do not allow differentiation between the bioaerosol fraction and the total particulate fraction in air samples.

5.3.2 Culture

Many viable organisms (fungi and bacteria) that are collected by impaction on a culture plate surface can reproduce and produce visible colonies. Each colony may represent one or more original cells, so that one colony is called a colony-forming unit. This is in contrast to liquid impingers, where cells are usually separated into single units before culture. Culture plate impaction methods will always underestimate actual airborne cell counts both because of this possible multiple cell bias and because not all airborne cells are ever viable and no single medium will support the growth of all organisms. The conditions of incubation must be appropriate and the culture medium must not be overloaded.

For air sampling, relatively rich culture media are generally used. These media usually contain necessary carbohydrates in a simple form (e.g., glucose) and amino acids in a natural form (e.g., peptone, blood, soy digests, etc.). On these media most healthy organisms will grow, given time. The organisms actually recovered, however, are those that grow the most rapidly. Organisms that have been damaged before sampling (i.e., stressed organisms) often begin growth very slowly and are overrun on rich media. For these, very restrictive and dilute culture media, which slow the growth of all organisms, should be used. If an organism that is able to utilize an unusual carbon source is suspected, a culture medium providing only that source may allow that organism to compete more successfully. For example, *Stachybotrys atra*, a relatively common toxigenic fungus, grows very slowly in culture and may not be efficiently recovered when other organisms are also present in a sample. However, *S. atra* can utilize pure cellulose and culture media containing cellulose as the sole carbohydrate source provide a competitive advantage to *Stachybotrys*.

Another means for reducing competition between organisms is to add biocides that prevent the growth of particular organisms. Streptomycin and rose bengal are often added to media designed to recover fungi from environments heavily contaminated with bacteria. It should be noted that rose bengal becomes a general biocide and will kill fungi, as well as bacteria, if it becomes light-activated (Burge et al., 1977b).

Culturing of viruses requires that living cells be present on the culture medium. For bacterial viruses, bacteria are spread on the plate before the viruses are introduced. For human viruses, human cell cultures are required. Viruses are usually incubated at 37 °C (Fields, 1986).

Bacteria can usually grow on nutrient media, although some are fastidious and require highly specific and complex conditions (e.g., *Legionella*). Most bacteria will grow at 30 °C, a few are thermophilic, and some can grow at room temperature and below. Incubation temperatures for bacteria depend on the source. Bacteria that apparently were growing in a cold water reservoir should be incubated at room temperature. Human source bacteria are often incubated at 35 °C. The thermophilic bacteria, including the actinomycetes, require a temperature of 56 to 60 °C. Identification of bacterial taxa requires Gram-staining, microscopic examination, and several physiological tests (e.g., production of oxidase and catalase). For some bacteria (including *Legionella*), fluorescent antibodies are available and can be used to microscopically identify species (American Conference of Governmental and Industrial Hygienists, 1989b).

Fungi will also grow on artificial nutrient media. Fungal culture medium is usually more acidic than that preferred by most bacteria. Media that will support the growth of many environmental bacteria are usually not good for fungi and vice versa. Most fungi grow best at room temperature. A few can compete well at colder temperatures (psychrophiles). Most of the fungi that are able to invade human tissue are thermotolerant (i.e., they can grow at a wide range of temperatures). A few fungi are thermophilic, requiring temperatures in excess of 45 °C for growth (Cooney and Emerson, 1964). Fungi are identified by their gross appearance in culture and by the morphology of spore production (Barnett and Hunter, 1987).

Amoebae can be grown in culture if they are provided with a living nutrient source (usually bacteria). It is thought that amoebae ingest *Legionella*, which remains alive and infectious inside the amoebal cell and is protected from the action of biocides. Amoebae are

identified by their morphology and ability to cause disease when injected into a suitable host. Fluorescent antibodies are also available for identifying some amoeba species.

5.3.3 Immunoassay

Proteins derived from such sources as cats, cockroaches, and dust mites can be measured by immunoassay. Most immunoassays are based on extraction of the water-soluble fraction of a specific antigen source (i.e., each immunoassay is specific for the antigens used to develop the assay). For example, mite antigen assays are specific only for mite antigens that are soluble in the systems used, and that will attach to the solid phase used. Recent developments allow assays of antigen materials electrically transferred to a solid substrate without initial water extraction (immunoblotting) (Hoyer et al., 1990). Three types of immunoassays are currently available:

(1) Radioallergosorbent test (RAST) or ELISA inhibition assays,
(2) direct RAST or ELISA assays, and
(3) immunoblot assays.

The RAST and ELISA inhibition assays involve coating a solid phase, usually paper discs, cellulose beads, or microtiter plates, with a water extract of a known antigen source and mixing the same antigen (in a series of dilutions) or an unknown sample with human serum that contains antibodies against the specific antigen. The serum/antigen mixture is incubated with the solid phase, and the solid phase is examined for human antibodies using an anti-human antibody labelled with a radioisotope (RAST) or an enzyme (ELISA) (Agarwal et al., 1981; Jensen et al., 1989; Swanson et al., 1985). These techniques only require an allergen extract and serum from one or a group of individuals who are highly allergic to the relevant allergen. However, these assays are very difficult to standardize and, because the units are arbitrary, the results for one allergen cannot be compared with those for another allergen.

Direct RAST and ELISA assays depend on the biochemical purification of specific antigens from a given source and the development of monoclonal antibodies against each specific antigen. The monoclonal antibody is then attached to the solid phase, incubated with

both known quantities of the relevant antigen and with unknown samples, and then incubated with a second monoclonal to the same antigen labelled as for the inhibition assays. The assumption is that the quantity of one protein will act as a guide to the presence of that source. It is implied that the protein being measured is in a reasonably constant relationship to other proteins from the same source. In addition, it is necessary that the protein being assayed be reasonably stable under the conditions of collection and storage. There are two main advantages of specific allergen assays. First, because the assays can be adapted to give results in absolute units (i.e., micrograms), the results for one protein can be compared with proteins from another allergen source. Second, the reagents are sufficiently simple to allow complete standardization of the assay. Thus, measurements of specific major allergens can be made in micrograms and standardized relative to a national or international standard. There are now at least three allergens where measurements have been made over a period of 10 years or more using a consistent standardization system: the cat allergen Fel d I (= Cat I), the ragweed pollen allergen Amb a I (= Antigen E), and the dust mite allergen Der p I (Ohman et al., 1987; King and Norman, 1962; Chapman and Platts-Mills, 1980). Originally, these assays were dependent on polyclonal antisera. Recently, they have been made easier to standardize and simpler to perform by the introduction of monoclonal antibodies (Chapman et al., 1987; Luczynska et al., 1989; Pollart et al., 1989b). There are definite problems with using a single allergen as a marker for the multiple proteins derived from a complex source such as dust mites or cockroaches. However, assays of single allergens represent enormous advantages in terms of standardization, simplicity, and cost. At present, these are the only assays that offer the possibility of establishing threshold or risk levels for exposure associated with disease.

Immunoblotting techniques, which are currently in the experimental stage, utilize the same monoclonal antibodies (or polyclonal where necessary) as the direct RAST and ELISA techniques, but do not require extraction of antigen sources before assay. Extraction procedures can denature antigens and change their relative concentrations. Immunoblotting involves electrically transferring proteins directly from air sample filter material or source onto nitrocellulose membranes. The membranes are then incubated with the monoclonals and secondary antibodies labelled with enzymes. The enzymes react with substrate to form a

colored precipitate which is visible to the eye and can be optically scanned for quantitation (Hoyer et al., 1990).

5.3.4 Bioassays and Chemical Analysis

Most commonly used assays for endotoxin depend on the fact that endotoxin causes a dose-dependent gel reaction in horseshoe crab (*Limulus*) amoebocytes. Three general types of *Limulus* (LAL) assays are available (Jacobs, 1989): the gel-clot method, the turbidimetric-kinetic assay, and the chromogenic assay. The gel-clot method is simple, more or less qualitative, and depends on the formation of gel in a tube. The turbidimetric-kinetic assay evaluates the rate of the gel reaction, as measured by carefully controlled densitometer readings. The chromogenic assay depends on the production of a pigment released from a substrate by the clotting enzyme of LAL. Recent developments have included mathematical approaches to assay analysis that make the LAL assay more sensitive and reliable (Milton et al., 1990). The assays also measure cell-bound endotoxins less well than free endotoxins. Unfortunately, in many cases, most exposures to endotoxins may be related to intact bacterial cells. For example, although cotton dust is known to contain high levels of gram-negative bacteria as well as endotoxin, human symptoms correlate more closely with bacterial levels than with measured endotoxin levels. For this reason, assessment of gram-negative bacterial levels may be an appropriate surrogate until more accurate methods for measuring endotoxins have been developed.

Bioassays are necessary to determine pathogenicity of specific strains of infectious viruses, bacteria, and fungi. Sentinel animals have been used to document the presence of infectious *Legionella*. Environmental isolates of *Histoplasma* do not do well in culture until they have been passed through a living organism.

Thin layer or high pressure liquid chromatography are used for the detection of many mycotoxins, including the aflatoxins and the macrocyclic trichothecenes (e.g., satratoxins) (Shank, 1981). Gas/liquid chromatography and mass spectroscopy (GC/MS), used for the *Fusarium* toxins, is being investigated as a tool for endotoxin analysis and is the method of choice for volatile organic compounds derived from microbial growth. All of these methods are readily applicable to air samples, but have not been extensively used.

Attempts have been made to biochemically "fingerprint" specific bacterial taxa and to use pyrolysis and GC/MS to identify the fingerprinted compounds. The number of individual taxa that have been studied with respect to these methods is extremely small. In addition, significant variability in fingerprint compounds exists between strains of a single taxon (Sonesson et al., 1988).

6. CONTROL OF BIOAEROSOL-INDUCED DISEASE

Very little actual research has gone into addressing the control of bioaerosol-induced disease, except in the area of immunization. A large amount of literature is available on immunization to prevent infectious disease (Ruben, 1987) and immunotherapy for the control of atopic disease (Ohman, 1989). These topics will not be discussed here. Other approaches that are used, often in the absence of direct evidence of their efficacy, may be grouped as follows (American Conference of Governmental and Industrial Hygienists, 1989b):

- building design that provides for bioaerosol control,
- maintenance of indoor spaces so that contamination does not occur, and
- remedial actions to control existing contamination.

6.1 BUILDING DESIGN

Buildings, including those for both public and private use, can be designed to prevent penetration of outside aerosols to the indoor environment and to minimize the establishment of indoor sources of biocontaminants. The purpose of the ASHRAE Standard 62-1989 (American Society of Heating, Refrigerating and Air-Conditioning Engineers, 1989) is "to specify minimum ventilation rates and indoor air quality that will be acceptable to human occupants and are intended to avoid adverse health effects". The standard classifies procedures for obtaining acceptable indoor air quality into two categories: the ventilation rate procedure that specifies specific ventilation rates for given environments (Section 4.1) and the indoor air quality procedure (Section 4.2) that requires control of known and specifiable contaminants. Also specified are design criteria that are aimed at preventing penetration of the outdoor aerosol and contamination of the ventilation system itself and controlling relative humidity (see Chapter 5). Although supported by very little experimental or epidemiological data, the standards, when applied to buildings without significant indoor sources, other than human occupants, will go far in providing adequate air quality with respect to human source aerosols (Brundage et al., 1988) and problems related to relative humidity (Arundel et al.,

1986; Carpenter et al., 1985). Unfortunately, the standards are rarely applied to domestic buildings, and the use of the "indoor air quality" procedures do not work for bioaerosols because there are no standards to use as guidelines. However, some control of residential air quality can be obtained by the use of central or room air-conditioning (Spiegelman and Friedman, 1968; Solomon et al., 1980; Carpenter et al., 1985). Air-conditioning allows homes to be closed, preventing penetration of outdoor aerosols, and acts to reduce relative humidity.

6.2 BUILDING MAINTENANCE

Even if a building is properly designed, lack of adequate maintenance will inevitably result in bioaerosol problems. Buildings must be maintained free of leaks and excessive moisture. Filters cannot be allowed to build up a layer of organic material that will support the growth of fungi or bacteria (Burge and Garrison, 1989; Baxter, 1982). Air conditioners, as well as controlling bioaerosols, may act as sources if not properly maintained (Banaszak et al., 1970). Unfortunately, maintenance of home air-conditioners is rarely simple. Humidifiers, whether centrally installed or console, are always contaminated with microorganisms and often provide a built-in dissemination mechanism. A relatively large amount of literature has been developed on the role of humidification in outbreaks of infectious disease, HP, and sick building syndrome (Burge et al., 1987; Finnegan et al., 1984; Kreiss and Hodgson, 1984; Kreiss, 1989). Less clear are methods for preventing contamination and dissemination of organisms and antigens from these reservoirs.

Dust control measures may also help to prevent biological contamination of the indoor environment. High-efficiency vacuuming of carpeting may help to prevent explosion of mite populations (Walshaw and Evans, 1986; Arlian et al., 1982). Limiting indoor food and water sources for cockroaches can prevent severe infestations in some parts of the country.

6.3 REMEDIAL ACTIONS

Once contamination has occurred, several approaches may be taken to rid the environment of the pollutant: air cleaning, air disinfection, and source control. Air cleaning

or particle removal is a function of the efficiency of the removal apparatus (e.g., filter, electrostatic precipitator), the rate of flow through the device, and the strengths and rates of emission of sources in the environment. To date, insufficient research has been conducted to allow a prediction of the effectiveness of air cleaning in the presence of active sources (Nelson et al., 1988). However, it probably more efficiently removes small particles that remain airborne for long periods of time. Also of concern is the downflow from the cleaner, which can disturb settled dust and entrained antigens and microorganisms.

The use of ultraviolet light has been studied as a means of removing infectious agents from the indoor environment (Perkins et al., 1947; Rentschler and Nagy, 1942; Riley and Kaufman, 1971; Wells et al., 1942). Ultraviolet radiation is dangerous and human contact should be avoided. Also, ultraviolet lights only emit the appropriate wavelengths when completely clean. Placing units in ductwork to kill recirculated organisms works only so long as dust has not accumulated on the bulb surfaces.

Germicidal sprays have also been proposed for killing infectious organisms in air (Kethley et al., 1956). These methods are not practical for occupied environments and all residual disinfectant must be removed before occupancy is resumed. Biocides should never be sprayed into ventilation systems in occupied buildings.

Source control involves removing the contaminated substrate from the environment, removing the contamination, or killing organisms without removal. The first is usually the most effective, although often not always practical. Immediate removal of soft materials that have come in contact with contaminated water is usually recommended because soft material that is impregnated with fungal or bacterial growth is not retrievable. Removing living antigen sources (household pets) is by far the best method for control of the contamination they produce. Contamination can often be removed from hard surfaces. The recommendation that smooth-surfaced flooring be used in bedrooms to prevent house dust accumulation is a case in point. Dust can be removed from smooth floors by vacuuming or wet mopping, whereas it inevitably accumulates in carpeting. Finally, biocides can be used to kill organisms, although few biocides safe for use in occupied environments will prevent recurrence of contamination (American Conference of Governmental and Industrial Hygienists, 1989b).

7. REFERENCES

Aas, K.; Backman, A.; Belin, L.; Weeke, B. (1978) Standardization of allergen extracts with appropriate methods: the combined use of skin prick testing and radio-allergosorbent tests. Allergy (Copenhagen) 33: 130-137.

Agarwal, M. K.; Yunginger, J. W.; Swanson, M. C.; Reed, C. E. (1981) An immunochemical method to measure atmospheric allergens. J. Allergy Clin. Immunol. 68: 194-200.

Agarwal, M. K.; Jones, R. T.; Yunginger, J. W. (1982) Shared allergenic and antigenic determinants in *Alternaria* and *Stemphylium* extracts. J. Allergy Clin. Immunol. 70: 437-444.

Ainsworth, G. C.; Sussman, A. S., eds. (1965) The fungi: an advanced treatise, v. 1, the fungal cell. New York, NY: Academic Press.

Aisner, J.; Schimpff, S. C.; Bennett, J. E.; Young, V. M.; Wiernik, P. H. (1976) *Aspergillus* infections in cancer patients: association with fireproofing materials in a new hospital. JAMA J. Am. Med. Assoc. 235: 411-412.

Ajello, L. (1971) The medical mycological iceberg. HSMHA Health Rep. 86: 437-448.

Akers, A. B.; Won, W. D. (1969) Assay of living, airborne microorganisms. In: Dimmick, R. L.; Akers, A. B., eds. An introduction to experimental aerobiology. New York, NY: Wiley-Interscience; pp. 59-99.

Akers, T. G.; Bond, S.; Goldberg, L. J. (1966) Effect of temperature and relative humidity on survival of airborne Columbia SK group viruses. Appl. Microbiol. 14: 361-364.

Alexopoulos, C. J. (1962) Introductory mycology. 2nd ed. New York, NY: John Wiley & Sons, Inc.

American Conference of Governmental Industrial Hygienists. (1984) TLVs: Threshold limit values for chemical substances and physical agents in the work environment and biological exposure indices with intended changes for 1984-85. Cincinnati, OH: American Conference of Governmental and Industrial Hygienists.

American Conference of Governmental Industrial Hygienists. (1989a) Air sampling instruments for evaluation of atmospheric contaminants. 7th ed. Cincinnati, OH: American Conference of Governmental and Industrial Hygienists.

American Conference of Governmental Industrial Hygienists. (1989b) Guidelines for the assessment of bioaerosols in the indoor environment. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc. (1989) Ventilation for acceptable indoor air quality. Atlanta, GA: American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc.; ASHRAE standard no. 62-1989.

Arlian, L. G. (1977) Humidity as a factor regulating feeding and water balance of the house dust mites *Dermatophagoides farinae* and *D. pteronyssinus* (Acari: Pyroglyphidae). J. Med. Entomol. 14: 484-488.

Arlian, L. G.; Bernstein, I. L.; Gallagher, J. S. (1982) The prevalence of house dust mites, *Dermatophagoides* spp, and associated environmental conditions in homes in Ohio. J. Allergy Clin. Immunol. 69: 527-532.

Arnow, P. M.; Andersen, R. L.; Mainous, P. D.; Smith, E. J. (1978) Pulmonary aspergillosis during hospital renovation. Am. Rev. Respir. Dis. 118: 49-53.

Artenstein, M. S.; Miller, W. S. (1966) Air sampling for respiratory disease agents in Army recruits. Bacteriol. Rev. 30: 571-575.

Arundel, A. V.; Sterling, E. M.; Biggin, J. H.; Sterling, T. D. (1986) Indirect health effects of relative humidity in indoor environments. Environ. Health Perspect. 65: 351-361.

Aukrust, L.; Borch, S. M. (1979) Partial purification and characterization of two *Cladosporium herbarum* allergens. Int. Arch. Allergy Appl. Immunol. 60: 68-79.

Banaszak, E. F.; Thiede, W. H.; Fink, J. N. (1970) Hypersensitivity pneumonitis due to contamination of an air conditioner. N. Engl. J. Med. 283: 271-276.

Barbee, R. A.; Brown, W. G.; Kaltenborn, W.; Halonen, M. (1981) Allergen skin-test reactivity in a community population sample: correlation with age, histamine skin reactions, and total serum immunoglobulin E. J. Allergy Clin. Immunol. 68: 15-19.

Barnett, H. L.; Hunter, B. B., eds. (1987) Illustrated genera of imperfect fungi. 4th ed. New York, NY: MacMillan Publishing Co.

Baskerville, A.; Fitzgeorge, R. B.; Broster, M.; Hambleton, P.; Dennis, P. J. (1981) Experimental transmission of Legionnaires' disease by exposure to aerosols of Legionella pneumophila. Lancet (8260/61): 1389-1390.

Baur, X.; Dewair, M.; Fruhmann, G.; Aschauer, H.; Pfletschinger, J.; Braunitzer, G. (1982) Hypersensitivity to chironomids (non-biting midges): localization of the antigenic determinants within certain polypeptide sequences of hemoglobins (erythrocruorins) of *Chironomus thummi thummi* (Diptera). J. Allergy Clin. Immunol. 69: 66-76.

Baxter, P. J. (1982) An outbreak of allergic-type symptoms among staff of an elementary school: a hazard of moldy air filters? Atlanta, GA: U. S. Centers for Disease Control, Center for Environmental Health.

Baxter, C. S.; Wey, H. E.; Burg, W. R. (1981) A prospective analysis of the potential risk associated with inhalation of aflatoxin-contaminated grain dusts. Food Cosmet. Toxicol. 19: 765-769.

Bayer, R. A. (1982) Q fever as an occupational illness at the National Institutes of Health. Public Health Rep. 97: 58-60.

Bernstein, R. S.; Sorenson, W. G.; Garabrant, D.; Reaux, C.; Treitman, R. D. (1983) Exposures to respirable, airborne *Penicillium* from a contaminated ventilation system: clinical, environmental and epidemiological aspects. Am. Ind. Hyg. Assoc. J. 44: 161-169.

Bernton, H. S.; Brown, H. (1967) Cockroach allergy II: the relation of infestation to sensitization. South. Med. J. 60: 852-855.

Berrens, L.; Guikers, C. L. H. (1972) An immunochemical study of pigeon-breeder's disease: III. the interaction of pigeon intestinal enzyme with human serum and a study of serum inhibitors. Int. Arch. Allergy Appl. Immunol. 43: 337-346.

Berrens, L.; Maesen, F. P. V. (1972a) An immunochemical study of pigeon-breeder's disease: I. the aselective antigens. Int. Arch. Allergy Appl. Immunol. 43: 289-304.

Berrens, L.; Maesen, F. P. V. (1972b) An immunochemical study of pigeon-breeder's disease: II. the specific antigens. Int. Arch. Allergy Appl. Immunol. 43: 327-336.

Bice, D. E.; McCarron, K.; Hoffman, E. O.; Salvaggio, J. (1977) Adjuvant properties of *Micropolyspora faeni*. Int. Arch. Allergy Appl. Immunol. 55: 267-274.

Blackley, C. H. (1873) Experimental researches on the causes and nature of catarrhus aestivus (hay-fever or hay-asthma). London, United Kingdom: Bailliere, Tindall and Cox.

Bloch, A. B.; Orenstein, W. A.; Ewing, W. M.; Spain, W. H.; Mallison, G. F.; Herrmann, K. L.; Hinman, A. R. (1985) Measles outbreak in a pediatric practice: airborne transmission in an office setting. Pediatrics 75: 676-683.

Blyth, W. (1978) The occurrence and nature of alveolitis-inducing substances in *Aspergillus clavatus*. Clin. Exp. Immunol. 32: 272-282.

Booij-Noord, H.; de Vries, K.; Sluiter, H. J.; Orie, N. G. M. (1972) Late bronchial obstructive reaction to experimental inhalation of house dust extract. Clin. Allergy 2: 43-61.

Boyd, D. H. A. (1971) The incidence of farmer's lung in Caithness. Scot. Med. J. 16: 261-262.

Boyer, R. S.; Klock, L. E.; Schmidt, C. D.; Hyland, L.; Maxwell, K.; Gardner, R. M.; Renzetti, A. D., Jr. (1974) Hypersensitivity lung disease in the turkey raising industry. Am. Rev. Respir. Dis. 109: 630-635.

Bringhurst, L. S.; Byrne, R. N.; Gershon-Cohen, J. (1959) Respiratory disease of mushroom workers: farmer's lung. JAMA J. Am. Med. Assoc. 171: 15-18.

Brown, F.; Wilson, G., eds. (1984) Volume 4: virology. In: Wilson, G.; Miles, A.; Parker, M. T., eds. Topley and Wilson's principles of bacteriology, virology and immunity. 7th ed. Baltimore, MD: Williams & Wilkins.

Brundage, J. F.; Scott, R. M.; Lednar, W. M.; Smith, D. W.; Miller, R. N. (1988) Building-associated risk of febrile acute respiratory diseases in Army trainees. JAMA J. Am. Med. Assoc. 259: 2108-2112.

Buchanan, T. M.; Hendricks, S. L.; Patton, C. M.; Feldman, R. A. (1974) Brucellosis in the United States, 1960-1972: an abattoir-associated disease. Part III. Epidemiology and evidence for acquired immunity. Medicine (Baltimore) 53: 427-439.

Buckland, F. E.; Tyrrell, D. A. J. (1962) Loss of infectivity on drying various viruses. Nature (London) 195: 1063-1064.

Buechner, H. A.; Prevatt, A. L.; Thompson, J.; Blitz, O. (1958) Bagassosis: a review, with further historical data, studies of pulmonary function, and results of adrenal steroid therapy. Am. J. Med. 25: 234-247.

Burg, W. R.; Shotwell, O. L. (1984) Aflatoxin levels in airborne dust generated from contaminated corn during harvest and at an elevator in 1980. J. Assoc. Off. Anal. Chem. 67: 309-312.

Burg, W. R.; Shotwell, O. L.; Saltzman, B. E. (1981) Measurements of airborne aflatoxins during the handling of contaminated corn. Am. Ind. Hyg. Assoc. J. 42: 1-11.

Burg, W. R.; Shotwell, O. L.; Saltzman, B. E. (1982) Measurements of airborne aflatoxins during the handling of 1979 contaminated corn. Am. Ind. Hyg. Assoc. J. 43: 580-586.

Burge, H. A. (1985) Fungus allergens. Clin. Rev. Allergy 3: 319-329.

Burge, H. A. (1987) Toxigenic potential of indoor microbial aerosols. In: Sandhu, S. S.; DeMarini, D. M.; Mass, M. J.; Moore, M. M.; Mumford, J. L., eds. Short-term bioassays in the analysis of complex environmental mixtures V: proceedings of a symposium; October 1986; Durham, NC. New York, NY: Plenum Press; pp. 391-397.

Burge, H. A. (1989a) Airborne allergenic fungi: classification, nomenclature, and distribution. Immunol. Allergy Clin. North Am. 9: 307-319.

Burge, H. A. (1989b) Indoor air and infectious disease. In: Cone, J. E.; Hodgson, M. J., eds. Problem buildings: building-associated illness and the sick building syndrome. Occup. Med.: State of the Art Rev. 4: 713-721.

Burge, H. A. (1990) Risks associated with indoor infectious aerosols. Toxicol. Ind. Health 6: 263-274.

Burge, H. A.; Garrison, R. P. (1989) Bioaerosol investigations: Columbia Plaza. Morgantown, WV: U. S. Department of Health and Human Services, National Institute of Occupational Safety and Health, Division of Respiratory Disease Studies; contract no. 200-86-2921. Available from: NTIS, Springfield, VA; PB91-108191.

Burge, H. A.; Solomon, W. R. (1987) Sampling and analysis of biological aerosols. Atmos. Environ. 21: 451-456.

Burge, H. A.; Solomon, W. R. (1990) Outdoor allergens. Clin. Rev. Allergy: in press.

Burge, H. P.; Boise, J. R.; Rutherford, J. A.; Solomon, W. R. (1977a) Comparative recoveries of airborne fungus spores by viable and non-viable modes of volumetric collection. Mycopathologia 61: 27-33.

Burge, H. P.; Solomon, W. R.; Boise, J. R. (1977b) Comparative merits of eight popular media in aerometric studies of fungi. J. Allergy Clin. Immunol. 60: 199-203.

Burge, S.; Hedge, A.; Wilson, S.; Bass, J. H.; Robertson, A. (1987) Sick building syndrome: a study of 4373 office workers. Ann. Occup. Hyg. 31: 493-504.

Burge, H. A.; Hoyer, M. E.; Solomon, W. R.; Simmons, E. G.; Gallup, J. (1989) Quality control factors for *Alternaria* allergens. Mycotaxon 34: 55-63.

Burke, G. W.; Carrington, C. B.; Strauss, R.; Fink, J. N.; Gaensler, E. A. (1977) Allergic alveolitis caused by home humidifiers: unusual clinical features and electron microscopic findings. JAMA J. Am. Med. Assoc. 238: 2705-2708.

Burr, M. L.; Dean, B. V.; Merrett, T. G.; Neale, E.; St. Leger, A. S.; Verrier-Jones, E. R. (1980) Effects of anti-mite measures on children with mite-sensitive asthma: a controlled trial. Thorax 35: 506-512.

Caldwell, J. R.; Pearce, D. E.; Spencer, C.; Leder, R.; Waldman, R. H. (1973) Immunologic mechanisms in hypersensitivity pneumonitis: I. evidence for cell-mediated immunity and complement fixation in pigeon breeders' disease. J. Allergy Clin. Immunol. 52: 225-230.

Campbell, J. A.; Kryda, M. J.; Treuhaft, M. W.; Marx, J. J., Jr.; Roberts, R. C. (1983) Cheese worker's hypersensitivity pneumonitis. Am. Rev. Respir. Dis. 127: 495-496.

Carpenter, G. B.; Win, G. H.; Furumizo, R. T.; Massey, D. G.; Ortiz, A. A. (1985) Air conditioning and house dust mite. J. Allergy Clin. Immunol. 75: 121.

Cartier, A.; Thomson, N. C.; Frith, P. A.; Roberts, R.; Hargreave, F. E. (1982) Allergen-induced increase in bronchial responsiveness to histamine: relationship to the late asthmatic response and change in airway caliber. J. Allergy Clin. Immunol. 70: 170-177.

Castellan, R. M.; Olenchock, S. A.; Kinsley, K. B.; Hankinson, J. L. (1987) Inhaled endotoxin and decreased spirometric values: an exposure-response relation for cotton dust. N. Engl. J. Med. 317: 605-610.

Chapman, M. D.; Platts-Mills, T. A. E. (1980) Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus*-antigen P_1. J. Immunol. 125: 587-592.

Chapman, M. D.; Heymann, P. W.; Wilkins, S. R.; Brown, M. J.; Platts-Mills, T. A. E. (1987) Monoclonal immunoassays for major dust mite (*Dermatophagoides*) allergens, *Der p* I and *Der f* I, and quantitative analysis of the allergen content of mite and house dust extracts. J. Allergy Clin. Immunol. 80: 184-194.

Chapman, M. D.; Aalberse, R. C.; Brown, M. J.; Platts-Mills, T. A. E. (1988) Monoclonal antibodies to the major feline allergen *Fel d* I: II. single step affinity purification of *Fel d* I, N-terminal sequence analysis, and development of a sensitive two-site immunoassay to assess *Fel d* I exposure. J. Immunol. 140: 812-818.

Charpin, D.; Kleisbauer, J.-P.; Lanteaume, A.; Razzouk, H.; Vervloet, D.; Toumi, M.; Faraj, F.; Charpin, J. (1988) Asthma and allergy to house-dust mites in populations living in high altitudes. Chest 93: 758-761.

Chatigny, M. A.; Dimmick, R. L. (1979) Transport of aerosols in the intramural environment. In: Edmonds, R. L., ed. Aerobiology: the ecological systems approach. Stroudsburg, PA: Dowden, Hutchinson & Ross, Inc.; pp. 95-109. (US/IBP synthesis series 10).

Christensen, C. M. (1975) Airborne fungus spores, plant disease, and respiratory allergy. In: Molds, mushrooms, and mycotoxins. Minneapolis, MN: University of Minnesota Press; pp. 114-142, 252.

Christensen, L. T.; Schmidt, C. D.; Robbins, L. (1975) Pigeon breeders' disease—a prevalence study and review. Clin. Allergy 5: 417-430.

Chua, K. Y.; Stewart, G. A.; Thomas, W. R.; Simpson, R. J.; Dilworth, R. J.; Plozza, T. M.; Turner, K. J. (1988) Sequence analysis of cDNA coding for a major house dust mite allergen, *Der p* I: homology with cysteine proteases. J. Exp. Med. 167: 175-182.

Cinkotai, F. F.; Lockwood, M. G.; Rylander, R. (1977) Airborne micro-organisms and prevalence of byssinotic symptoms in cotton mills. Am. Ind. Hyg. Assoc. J. 38: 554-559.

Clark, C. S.; Rylander, R.; Larsson, L. (1983a) Levels of gram-negative bacteria, *Aspergillus fumigatus*, dust, and endotoxin at compost plants. Appl. Environ. Microbiol. 45: 1501-1505.

Clark, S.; Rylander, R.; Larsson, L. (1983b) Airborne bacteria, endotoxin and fungi in dust in poultry and swine confinement buildings. Am. Ind. Hyg. Assoc. J. 44: 537-541.

Cockcroft, A.; Edwards, J.; Bevan, C.; Campbell, I.; Collins, G.; Houston, K.; Jenkins, D.; Latham, S.; Saunders, M.; Trotman, D. (1981a) An investigation of operating theatre staff exposed to humidifier fever antigens. Br. J. Ind. Med. 38: 144-151.

Cockcroft, A.; McCarthy, P.; Edwards, J.; Andersson, N. (1981b) Allergy in laboratory animal workers. Lancet (8224): 827-830.

Cooney, D. G.; Emerson, R. (1964) Thermophilic fungi: an account of their biology, activities, and classification. San Francisco, CA: W. H. Freeman and Company.

Couch, R. B. (1981) Viruses and indoor air pollution. Bull. N. Y. Acad. Med. 57: 907-921.

Croft, W. A.; Jarvis, B. B.; Yatawara, C. S. (1986) Airborne outbreak of trichothecene toxicosis. Atmos. Environ. 20: 549-552.

Cunningham, A. S.; Fink, J. N.; Schlueter, D. P. (1976) Childhood hypersensitivity pneumonitis due to dove antigens. Pediatrics 58: 436-442.

Dahlgren, C. M.; Buchanan, L. M.; Decker, H. M.; Freed, S. W.; Phillips, C. R.; Brachman, P. S. (1960) *Bacillus anthracis* aerosols in goat hair processing mills. Am. J. Hyg. 72: 24-31.

Dashek, W. V.; Eadie, T.; Llewellyn, G. C.; Olenchock, S. A.; Wirtz, G. H. (1983) Thin layer chromatographic analysis of possible aflatoxins within grain dusts. JAOCS J. Am. Oil Chem. Soc. 60: 563-566.

Davis, G. S.; Winn, W. C., Jr. (1987) Legionnaires' disease: respiratory infections caused by Legionella bacteria. Clin. Chest Med. 8: 419-439.

Davis, B. D.; Dulbecco, R.; Eisen, H. N.; Ginsburg, H. S. (1980) Virology. In: Microbiology. 3rd ed. New York, NY: Harper and Row; sec. 5.

Davis, G. S.; Winn, W. C., Jr.; Gump, D. W.; Craighead, J. E.; Beaty, H. N. (1982) Legionnaires' pneumonia after aerosol exposure in guinea pigs and rats. Am. Rev. Respir. Dis. 126: 1050-1057.

Davis, R. M.; Orenstein, W. A.; Frank, J. A., Jr.; Sacks, J. J.; Dales, L. G.; Preblud, S. R.; Bart, K. J.; Williams, N. M.; Hinman, A. R. (1986) Transmission of measles in medical settings: 1980 through 1984. JAMA J. Am. Med. Assoc. 255: 1295-1298.

Davis, W. E.; Horner, W. E.; Salvaggio, J. E.; Lehrer, S. B. (1988) Basidiospore allergens: analysis of *Coprinus quadrifidus* spore, cap, and stalk extracts. Clin. Allergy 18: 261-267.

de Groot, H.; Goei, K. G. H.; Aalberse, R. C. (1990) Monoclonal antibodies directed to three dog allergens. J. Allergy Clin. Immunol. 85: 279.

Deger, G. E. (1976) Aflatoxin—human colon carcinogenesis? Ann. Intern. Med. 85: 204.

DeLucca, A. J., II; Godshall, M. A.; Palmgren, M. S. (1984) Gram-negative bacterial endotoxins in grain elevator dusts. Am. Ind. Hyg. Assoc. J. 45: 336-339.

Dewair, M.; Baur, X.; Ziegler, K. (1985) Use of immunoblot technique for detection of human IgE and IgG antibodies to individual silk proteins. J. Allergy Clin. Immunol. 76: 537-542.

Dick, E. C.; Jennings, L. C.; Mink, K. A.; Wartgow, C. D.; Inhorn, S. L. (1987) Aerosol transmission of rhinovirus colds. J. Infect. Dis. 156: 442-448.

Dimmick, R. L.; Akers, A. B., eds. (1969) An introduction to experimental aerobiology. New York, NY: Wiley-Interscience.

Dixon, R. E. (1985) Economic costs of respiratory tract infections in the United States. Am. J. Med. 78(suppl. 6B): 45-51.

Doebbeling, B. N.; Wenzel, R. P. (1987) The epidemiology of *Legionella pneumophila* infections. Semin. Respir. Infect. 2: 206-221.

doPico, G. A.; Reddan, W.; Flaherty, D.; Tsiatis, A.; Peters, M. E.; Rao, P.; Rankin, J. (1977) Respiratory abnormalities among grain handlers: a clinical, physiologic, and immunologic study. Am. Rev. Respir. Dis. 115: 915-927.

doPico, G. A.; Flaherty, D.; Bhansali, P.; Chavaje, N. (1982) Grain fever syndrome induced by inhalation of airborne grain dust. J. Allergy Clin. Immunol. 69: 435-443.

doPico, G. A.; Reddan, W.; Anderson, S.; Flaherty, D.; Smalley, E. (1983) Acute effects of grain dust exposure during a work shift. Am. Rev. Respir. Dis. 128: 399-404.

Dreborg, S. K. G.; Einarsson, R. (1990) Does biological units (BU/ML) correlate to major allergen content? J. Allergy Clin. Immunol. 85: 151.

Dvorackova, I.; Pichova, V. (1986) Pulmonary interstitial fibrosis with evidence of aflatoxin B_1 in lung tissue. J. Toxicol. Environ. Health 18: 153-157.

Edmonds, R. L. (1972) Collection efficiency of rotorod samplers for sampling fungus spores in the atmosphere. Plant Dis. Rep. 56: 704-708.

Edmonds, R. L., ed. (1979) Aerobiology: the ecological systems approach. Stroudsburg, PA: Dowden, Hutchinson & Ross, Inc. (US/IBP synthesis series 10).

Edwards, J. H. (1972) The isolation of antigens associated with farmer's lung. Clin. Exp. Immunol. 11: 341-355.

Edwards, J. H. (1980) Microbial and immunological investigations and remedial action after an outbreak of humidifier fever. Br. J. Ind. Med. 37: 55-62.

Edwards, C.; Luntz, G. (1974) Budgerigar-fancier's lung: a report of a fatal case. Br. J. Dis. Chest 68: 57-64.

Edwards, J. H.; Fink, J. N.; Barboriak, J. J. (1969) Excretion of pigeon serum proteins in pigeon droppings. Proc. Soc. Exp. Biol. Med. 132: 907-911.

Edwards, J. H.; Barboriak, J. J.; Fink, J. N. (1970) Antigens in pigeon breeders' disease. Immunology 19: 729-734.

Edwards, J. H.; Griffiths, A. J.; Mullins, J. (1976) Protozoa as sources of antigen in "humidifier fever". Nature (London) 264: 438-439.

Eichner, R. D.; Mullbacher, A. (1984) Hypothesis: fungal toxins are involved in aspergillosis and AIDS. Aust. J. Exp. Biol. Med. Sci. 62: 479-484.

Ellakkani, M. A.; Alarie, Y. C.; Weyel, D. A.; Mazumdar, S.; Karol, M. H. (1984) Pulmonary reactions to inhaled cotton dust: an animal model for byssinosis. Toxicol. Appl. Pharmacol. 74: 267-284.

Emanuel, D. A.; Wenzel, F. J.; Bowerman, C. I.; Lawton, B. R. (1964) Farmer's lung: clinical, pathologic and immunologic study of twenty-four patients. Am. J. Med. 37: 392-401.

Emanuel, D. A.; Wenzel, F. J.; Lawton, B. R. (1966) Pneumonitis due to *Cryptostroma corticale* (maple-bark disease). N. Engl. J. Med. 274: 1413-1418.

Emmons, C. W. (1955) Saprophytic sources of *Cryptococcus neoformans* associated with the pigeon (*Columba livia*). Am. J. Hyg. 62: 227-232.

Emmons, C. W. (1962) Natural occurrence of opportunistic fungi. Lab. Invest. 11: 1026-1034.

Enarson, D. A.; Vedal, S.; Chan-Yeung, M. (1985) Rapid decline in FEV_1 in grain handlers: relation to level of dust exposure. Am. Rev. Respir. Dis. 132: 814-817.

England, A. C., III; Fraser, D. W.; Plikaytis, B. D.; Tsai, T. F.; Storch, G.; Broome, C. V. (1981) Sporadic legionellosis in the United States: the first thousand cases. Ann. Intern. Med. 94: 164-170.

Enterline, P. E.; Keleti, G.; Sykora, J. L.; Lange, J. H. (1985) Endotoxins, cotton dust, and cancer. Lancet (8461): 934-935.

Feeley, J. C. (1985) Impact of indoor air pathogens on human health. In: Gammage, R. B.; Kaye, S. V., eds. Indoor air and human health. Chelsea, MI: Lewis Publishers, Inc.; pp. 183-187.

Feldman, H. A.; Silverman, A. C.; Adair, C. V. (1950) An epidemic of Q fever among employees of a rendering plant in Syracuse, New York. J. Clin. Invest. 29: 812.

Fergusson, R. J.; Milne, L. J. R.; Crompton, G. K. (1984) Penicillium allergic alveolitis: faulty installation of central heating. Thorax 39: 294-298.

Fields, B. N., ed. (1986) Fundamental virology. New York, NY: Raven Press.

Fink, J. N. (1983) Hypersensitivity pneumonitis. In: Middleton, E., Jr.; Reed, C. E.; Ellis, E. F., eds. Allergy: principles and practice, v. 2. 2nd ed. St. Louis, MO: The C. V. Mosby Company; pp. 1085-1099.

Fink, J. N. (1988) Hypersensitivity pneumonitis. In: Middleton, E., Jr.; Reed, C. E.; Ellis, E. F.; Adkinson, N. F., Jr.; Yunginger, J. W., eds. Allergy: principles and practice, v. II. 3rd ed. St. Louis, MO: The C. V. Mosby Company; pp. 1237-1252.

Fink, J. N.; Resnick, A. J.; Salvaggio, J. (1971) Presence of thermophilic actinomycetes in residential heating systems. Appl. Microbiol. 22: 730-731.

Fink, J. N.; Schlueter, D. P.; Sosman, A. J.; Unger, G. F.; Barboriak, J. J.; Rimm, A. A.; Arkins, J. A.; Dhaliwal, K. S. (1972) Clinical survey of pigeon breeders. Chest 62: 277-281.

Finnegan, M. J.; Pickering, C. A. C.; Burge, P. S. (1984) The sick building syndrome: prevalence studies. Br. Med. J. 289: 1573-1575.

Fletcher, S. M.; Rondle, C. J. M.; Murray, I. G. (1970) The extracellular antigens of *Micropolyspora faeni*: their significance in farmer's lung disease. J. Hyg. 68: 401-409.

Fontana, V. J.; Wittig, H.; Holt, L. E., Jr. (1963) Observations on the specificity of the skin test: the incidence of positive skin tests in allergic and nonallergic children. J. Allergy 34: 348-353.

Foster, J. W. (1949) Chemical activities of fungi. New York, NY: Academic Press, Inc.

Fox, J. P.; Elveback, L.; Scott, W.; Gatewood, L.; Ackerman, E. (1971) Herd immunity: basic concept and relevance to public health immunization practices. Am. J. Epidemiol. 94: 179-189.

Fraser, D. W. (1984) Sources of legionellosis. In: Thornsberry, C.; Balows, A.; Feeley, J. C.; Jakubowski, W., eds. Legionella: proceedings of the 2nd international symposium. Washington, DC: American Society for Microbiology; pp. 277-280.

Fredricks, W. W.; Tebo, T. H. (1975) An antigen of pigeon breeders' disease. Fed. Proc. Fed. Am. Soc. Exp. Biol. 34: 984.

Furcolow, M. L. (1958) Recent studies on the epidemiology of histoplasmosis. Ann. N. Y. Acad. Sci. 72: 127-163.

Gardner, D. E. (1982) Use of experimental airborne infections for monitoring altered host defenses. Environ. Health Perspect. 43: 99-107.

Garibaldi, R. A. (1985) Epidemiology of community-acquired respiratory tract infections in adults: incidence, etiology, and impact. Am. J. Med. 78(suppl. 6B): 32-37.

Gelber, L.; Pollart, S.; Chapman, M.; Seltzer, L.; Platts-Mills, T. (1990) Serum IgE antibodies and allergen exposure as risk factors for acute asthma. In: World conference on lung health; May; Boston, MA. Am. Rev. Respir. Dis. 141 (4, pt. 2): A146.

Gerone, P. J.; Couch, R. B.; Keefer, G. V.; Douglas, R. G.; Derrenbacher, E. B.; Knight, V. (1966) Assessment of experimental and natural viral aerosols. Bacteriol. Rev. 30: 576-588.

Glick, T. H.; Gregg, M. B.; Berman, B.; Mallison, G.; Rhodes, W. W., Jr.; Kassanoff, I. (1978) Pontiac fever, an epidemic of unknown etiology in a health department: I. clinical and epidemiologic aspects. Am. J. Epidemiol. 107: 149-160.

Goldberg, L. J.; Shechmeister, I. L. (1951) Studies on the experimental epidemiology of respiratory infections: V. evaluation of factors related to slit sampling of air-borne bacteria. J. Infect. Dis. 88: 243-247.

Goodlow, R. J.; Leonard, F. A. (1961) Viability and infectivity of microorganisms in experimental airborne infection. Bacteriol. Rev. 25: 182-187.

Grant, I. W. B.; Blyth, W.; Wardrop, V. E.; Gordon, R. M.; Pearson, J. C. G.; Mair, A. (1972) Prevalence of farmer's lung in Scotland: a pilot survey. Br. Med. J. (5799): 530-534.

Gravesen, S. (1978) Identification and prevalence of culturable mesophilic microfungi in house dust from 100 Danish homes: comparison between airborne and dust-bound fungi. Allergy (Copenhagen) 33: 268-272.

Green, W. F. (1972) Precipitins against a fungus, *Phoma violacea*, isolated from a mouldy shower curtain in sera from patients with suspected allergic interstitial pneumonitis. Med. J. Aust. 1: 696-698.

Greene, W. H.; Andriole, V. T. (1980) An outbreak of nosocomial invasive aspergillosis. In: 20th interscience conference on antimicrobial agents and chemotherapy; September; New Orleans, LA. Washington, DC: American Society for Microbiology; abstract no. 546.

Gregory, P. H. (1957) Electrostatic charges on spores of fungi in air. Nature (London) 180: 330.

Gregory, P. H. (1961) The microbiology of the atmosphere. London, United Kingdom: Leonard Hill [Books] Limited.

Gregory, P. H. (1973) The microbiology of the atmosphere. 2nd ed. New York, NY: John Wiley & Sons.

Gregory, P. H.; Lacey, M. E. (1963) Mycological examination of dust from mouldy hay associated with farmer's lung disease. J. Gen. Microbiol. 30: 75-88.

Gregory, P. H.; Lacey, M. E.; Festenstein, G. N.; Skinner, F. A. (1963) Microbial and biochemical changes during the moulding of hay. J. Gen. Microbiol. 33: 147-174.

Grieble, H. G.; Colton, F. R.; Bird, T. J.; Toigo, A.; Griffith, L. G. (1970) Fine-particle humidifiers: source of *Pseudomonas aeruginosa* infections in a respiratory-disease unit. N. Engl. J. Med. 282: 531-535.

Gross, N. J. (1980) Allergy to laboratory animals: epidemiologic, clinical, and physiologic aspects, and a trial of cromolyn in its management. J. Allergy Clin. Immunol. 66: 158-165.

Guiguet, M.; Pierre, J.; Brun, P.; Berthelot, G.; Gottot, S.; Gibert, C.; Valleron, A. J. (1987) Epidemiological survey of a major outbreak of nosocomial legionellosis. Int. J. Epidemiol. 16: 466-471.

Gustafson, T. L.; Lavely, G. B.; Brawner, E. R., Jr.; Hutcheson, R. H., Jr.; Wright, P. F.; Schaffner, W. (1982) An outbreak of airborne nosocomial varicella. Pediatrics 70: 550-556.

Habel, K. (1945) Mumps and chickenpox as air-borne diseases. Am. J. Med. Sci. 209: 75-78.

Hales, C. A.; Rubin, R. H. (1979) Case records of the Massachusetts General Hospital: case 47-1979 [a 34-year-old man with recurrent pulmonary infiltrates]. N. Engl. J. Med. 301: 1168-1174.

Halim, A. F.; Narciso, J. A.; Collins, R. P. (1975) Odorous constituents of *Penicillium decumbens*. Mycologia 67: 1158-1165.

Hatch, M. T; Dimmick, R. L. (1966) Physiological responses of airborne bacteria to shifts in relative humidity. Bacteriol. Rev. 30: 597-603.

Hendricks, S. L.; Borts, I. H.; Hereen, R. H.; Hausler, W. J.; Held, J. R. (1962) Brucellosis outbreak in an Iowa packing house. Am. J. Public Health 52: 1166-1178.

Hesse, U. J.; Fryd, D. S.; Chatterjee, S. N.; Simmons, R. L.; Sutherland, D. E. R.; Najarian, J. S. (1986) Pulmonary infections. The Minnesota randomized prospective trial of cyclosporine vs azathioprine-antilymphocyte globulin for immunosuppression in renal allograft recipients. Arch. Surg. 121: 1056-1060.

Hook, W. A.; Powers, K.; Siraganian, R. P. (1984) Skin tests and blood leukocyte histamine release of patients with allergies to laboratory animals. J. Allergy Clin. Immunol. 73: 457-465.

Horner, W. E.; Ibanez, M. D.; Lehrer, S. B. (1989) Immunoprint analysis of *Calvatia cyathiformis* allergens: I. reactivity with individual sera. J. Allergy Clin. Immunol. 83: 784-792.

Houk, V. N. (1980) Spread of tuberculosis via recirculated air in a naval vessel: the *Byrd* study. In: Kundsin, R. B., ed. Airborne contagion. Ann. N. Y. Acad. Sci. 353: 10-24.

Hoyer, M.; Arscott, P.; Burge, H.; Klapper, D.; Solomon, W.; Baker, J. R., Jr. (1990) Detection and quantitation of airborne ragweed allergens using a new immunoblotting technique. J. Allergy Clin. Immunol. 85: 227.

Huddleson, I. F.; Munger, M. (1940) A study of an epidemic of brucellosis due to Brucella melitensis. Am. J. Public Health 30: 944-954.

Huebner, R. J. (1947) Report of an outbreak of Q fever at the National Institute of Health: II. epidemiological features. Am. J. Public Health 37: 431-440.

Hulett, A. C.; Dockhorn, R. J. (1979) House dust, mite (D. farinae) and cockroach allergy in a midwestern population. Ann. Allergy 42: 160-165.

Ibanez, M. D.; Horner, W. E.; Liengswangswong, V.; Sastre, J.; Lehrer, S. B. (1988) Identification and analysis of basidiospore allergens from puffballs. J. Allergy Clin. Immunol. 82: 787-795.

Ingold, C. T. (1971) Fungal spores: their liberation and dispersal. Oxford, United Kingdom: Clarendon Press.

Ishizaka, K.; Ishizaka, T. (1967) Identification of γE-antibodies as a carrier of reaginic activity. J. Immunol. 99: 1187-1198.

Jacobs, R. R. (1989) Airborne endotoxins: an association with occupational lung disease. Appl. Ind. Hyg. 4 (2): 50-56.

Jensen, J.; Poulsen, L. K.; Mygind, K.; Weeke, E. R.; Weeke, B. (1989) Immunochemical estimations for allergenic activities from outdoor aero-allergens, collected by a high-volume air sampler. Allergy (Copenhagen) 44: 52-59.

Johnson, C. L.; Bernstein, I. L.; Gallagher, J. S.; Bonventre, P. F.; Brooks, S. M. (1980) Familial hypersensitivity pneumonitis induced by *Bacillus subtilis*. Am. Rev. Respir. Dis. 122: 339-348.

Joklik, W. K.; Willett, H. P.; Amos, D. B., eds. (1984) Basic virology and clinical virology. In: Zinsser microbiology. 18th ed. Norwalk, CT: Appleton-Century-Crofts; sections IV-V.

Kalakoutskii, L. V.; Agre, N. S. (1973) Endospores of actinomycetes: dormancy and germination. In: Sykes, G.; Skinner, F. A., eds. Actinomycetales: characteristics and practical importance. New York, NY: Academic Press; pp. 179-195. (The Society for Applied Bacteriology symposium series no. 2).

Kaminski, E.; Libbey, L. M.; Stawicki, S.; Wasowicz, E. (1972) Identification of the predominant volatile compounds produced by *Aspergillus flavus*. Appl. Microbiol. 24: 721-726.

Kaminski, E.; Stawicki, S.; Wasowicz, E. (1974) Volatile flavor compounds produced by molds of *Aspergillus*, *Penicillium*, and *Fungi imperfecti*. Appl. Microbiol. 27: 1001-1004.

Kang, B.; Vellody, D.; Homburger, H.; Yunginger, J. W. (1979) Cockroach cause of allergic asthma: its specificity and immunologic profile. J. Allergy Clin. Immunol. 63: 80-86.

Karahadian, C.; Josephson, D. B.; Lindsay, R. C. (1985) Volatile compounds from *Pencillium sp.* contributing musty-earthy notes to Brie and Camembert cheese flavors. J. Agric. Food Chem. 33: 339-343.

Karim, Y. G.; Ijaz, M. K.; Sattar, S. A.; Johnson-Lussenburg, C. M. (1985) Effect of relative humidity on the airborne survival of rhinovirus-14. Can. J. Microbiol. 31: 1058-1061.

Kark, J. D.; Lebiush, M.; Rannon, L. (1982) Cigarette smoking as a risk factor for epidemic $A(H_1N_1)$ influenza in young men. N. Engl. J. Med. 307: 1042-1046.

Kaufman, L.; Blumer, S. (1978) Cryptococcosis: the awakening giant. In: Proceedings of the fourth international conference on the mycoses: the black and white yeasts; June 1977; Brasilia, Brazil. Washington, DC: Pan American Health Organization; scientific publication no. 356; pp. 176-182.

Kaufmann, A. F.; Fox, M. D.; Boyce, J. M.; Anderson, D. C.; Potter, M. E.; Martone, W. J.; Patton, C. M. (1980) Airborne spread of brucellosis. In: Kundsin, R. B., ed. Airborne contagion. Ann. N. Y. Acad. Sci. 353: 105-114.

Keller, R. H.; Swartz, S.; Schlueter, D. P.; Bar-Sela, S.; Fink, J. N. (1984) Immunoregulation in hypersensitivity pneumonitis: phenotypic and functional studies of bronchoalveolar lavage lymphocytes. Am. Rev. Respir. Dis. 130: 766-771.

Kelsen, S. G.; McGuckin, M. (1980) The role of airborne bacteria in the contamination of fine particle nebulizers and the development of nosocomial pneumonia. Ann. N. Y. Acad. Sci. 353: 218-229.

Kendrick, B. (1985) The fifth kingdom. Waterloo, ON, Canada: Mycologue Publications.

Kennedy, S. M.; Christiani, D. C.; Eisen, E. A.; Wegman, D. H.; Greaves, I. A.; Olenchock, S. A.; Ye, T.-T.; Lu, P.-L. (1987) Cotton dust and endotoxin exposure-response relationships in cotton textile workers. Am. Rev. Respir. Dis. 135: 194-200.

Kent, D. C. (1967) Tuberculosis as a military epidemic disease and its control by the Navy Tuberculosis Control Program. Dis. Chest 52: 588-594.

Kent, D. C.; Reid, D.; Sokolowski, J. W.; Houk, V. N. (1967) Tuberculin conversion: the iceberg of tuberculous pathogenesis. Arch. Environ. Health 14: 580-584.

Kern, R. A. (1921) Dust sensitization in bronchial asthma. Med. Clin. North Am. 5: 751-758.

Kerrebijn, K. F. (1970) Endogenous factors in childhood CNSLD: methodological aspects in population studies. In: Orie, N. G. M.; van der Lende, R., eds. Bronchitis III: proceedings of the third international symposium on bronchitis; September 1969; Groningen, The Netherlands. Assen, The Netherlands: Royal Vangorcum; pp. 38-48.

Kethley, T. W.; Fincher, E. L.; Cown, W. B. (1956) A system for the evaluation of aerial disinfectants. Appl. Microbiol. 4: 237-243.

Kethley, T. W.; Fincher, E. L.; Cown, W. B. (1957) The effect of sampling method upon the apparent response of airborne bacteria to temperature and relative humidity. J. Infect. Dis. 100: 97-102.

Kim, S. J.; Chaparas, S. D. (1978) Characterization of antigens from Aspergillus fumigatus: I. preparation of antigens from organisms grown in completely synthetic medium. Am. Rev. Respir. Dis. 118: 547-551.

Kingston, D.; Warhurst, D. C. (1969) Isolation of amoebae from the air. J. Med. Microbiol. 2: 27-36.

Kirkland, T. N.; Fierer, J. (1985) Genetic control of resistance to *Coccidioides immitis*: a single gene that is expressed in spleen cells determines resistance. J. Immunol. 135: 548-552.

Kitchen, M. S.; Reiber, C. D.; Eastin, G. B. (1977) An urban epidemic of North American blastomycosis. Am. Rev. Respir. Dis. 115: 1063-1066.

Kjellman, B.; Pettersson, R. (1983) The problem of furred pets in childhood atopic disease: failure of an information program. Allergy (Copenhagen) 38: 65-73.

Klein, B. S.; Vergeront, J. M.; Weeks, R. J.; Kumar, U. N.; Mathai, G.; Varkey, B.; Kaufman, L.; Bradsher, R. W.; Stoebig, J. F.; Davis, J. P. (1986) Isolation of *Blastomyces dermatitidis* in soil associated with a large outbreak of blastomycosis in Wisconsin. N. Engl. J. Med. 314: 529-534.

Knight, V. (1980) Viruses as agents of airborne contagion. In: Kundsin, R. B., ed. Airborne contagion. Ann. N. Y. Acad. Sci. 353: 147-156.

Knysak, D. (1989) Animal aeroallergens. Immunol. Allergy Clin. North Am. 9: 357-364.

Kobayashi, M.; Stahmann, M. A.; Rankin, J.; Dickie, H. A. (1963) Antigens in moldy hay as the cause of farmer's lung. Proc. Soc. Exp. Biol. Med. 113: 472-476.

Kohler, P. F.; Gross, G.; Salvaggio, J.; Hawkins, J. (1976) Humidifier lung: hypersensitivity pneumonitis related to thermotolerant bacterial aerosols. Chest 69: 294-296.

Korn, D. S.; Florman, A. L.; Gribetz, I. (1968) Recurrent pneumonitis with hypersensitivity to hen litter. JAMA J. Am. Med. Assoc. 205(1): 44-45.

Korsgaard, J. (1982) Preventive measures in house-dust allergy. Am. Rev. Respir. Dis. 125: 80-84.

Korsgaard, J. (1983) Mite asthma and residency: a case-control study on the impact of exposure to house-dust mites in dwellings. Am. Rev. Respir. Dis. 128: 231-235.

Krasinski, K.; Holzman, R. S.; Hanna, B.; Greco, M. A.; Graff, M.; Bhogal, M. (1985) Nosocomial fungal infection during hospital renovation. Infect. Control 6: 278-282.

Kreiss, K. (1989) The epidemiology of building-related complaints and illness. Occup. Med. 4: 575-592.

Kreiss, K.; Hodgson, M. J. (1984) Building-associated epidemics. In: Walsh, P. J.; Dudney, C. S.; Copenhaver, E. D., eds. Indoor air quality. Boca Raton, FL: CRC Press, Inc.; pp. 87-106.

Krieg, N. R.; Holt, J. G., eds. (1984) Bergey's manual of systematic bacteriology, v. 1. Baltimore, MD: Williams & Wilkins.

Kumar, P.; Marier, R.; Leech, S. H. (1981) Hypersensitivity pneumonitis due to contamination of a car air conditioner. N. Engl. J. Med. 305: 1531-1532.

LaBerge, D. E.; Stahmann, M. A. (1966) Antigens from moldy hay involved in farmer's lung. Proc. Soc. Exp. Biol. Med. 121: 458-462.

Lacey, J.; Lacey, M. E. (1964) Spore concentrations in the air of farm buildings. Trans. Br. Mycol. Soc. 47: 547-552.

Land, C. J.; Hult, K.; Fuchs, R.; Hagelberg, S.; Lundstrom, H. (1987) Tremorgenic mycotoxins from *Aspergillus fumigatus* as a possible occupational health problem in sawmills. Appl. Environ. Microbiol. 53: 787-790.

Langmuir, A. D. (1980) Changing concepts of airborne infection of acute contagious diseases: a reconsideration of classic epidemiologic theories. Ann. N. Y. Acad. Sci. 353: 35-44.

Lantz, R. C.; Birch, K.; Hinton, D. E.; Burrell, R. (1985) Morphometric changes of the lung induced by inhaled bacterial endotoxin. Exp. Mol. Pathol. 43: 305-320.

Larsen, R. A.; Jacobson, J. A.; Morris, A. H.; Benowitz, B. A. (1985) Acute respiratory failure caused by primary pulmonary coccidioidomycosis: two case reports and a review of the literature. Am. Rev. Respir. Dis. 131: 797-799.

Leclair, J. M.; Zaia, J. A.; Levin, M. J.; Congdon, R. G.; Goldmann, D. A. (1980) Airborne transmission of chickenpox in a hospital. N. Engl. J. Med. 302: 450-453.

Lee, T. H.; Wraith, D. G.; Bennett, C. O.; Bentley, A. P. (1983) Budgerigar fancier's lung: the persistence of budgerigar precipitins and the recovery of lung function after cessation of avian exposure. Clin. Allergy 13: 197-202.

Leitermann, K.; Ohman, J. L., Jr. (1984) Cat allergen 1: biochemical, antigenic, and allergenic properties. J. Allergy Clin. Immunol. 74: 147-153.

Lenhart, S. W.; Olenchock, S. A.; Cole, E. C. (1982) Viable sampling for airborne bacteria in a poultry processing plant. J. Toxicol. Environ. Health 10: 613-619.

Lentino, J. R.; Rosenkranz, M. A.; Michaels, J. A.; Kurup, V. P.; Rose, H. D.; Rytel, M. W. (1982) Nosocomial aspergillosis: a retrospective review of airborne disease secondary to road construction and contaminated air conditioners. Am. J. Epidemiol. 116: 430-437.

Lockey, R. F.; Benedict, L. M.; Turkeltaub, P. C.; Bukantz, S. C. (1987) Fatalities from immunotherapy (IT) and skin testing (ST). J. Allergy Clin. Immunol. 79: 660-677.

Longbottom, J. L. (1980) Purification and characterization of allergens from the urines of mice and rats. In: Oehling, A.; Mathov, E.; Glazer, I.; Arbesman, C., eds. Advances in allergology and clinical immunology. Oxford, United Kingdom: Pergamon Press; pp. 483-490.

Longini, I. M., Jr.; Koopman, J. S.; Monto, A. S.; Fox, J. P. (1982) Estimating household and community transmission parameters for influenza. Am. J. Epidemiol. 115: 736-751.

Longini, I. M., Jr.; Koopman, J. S.; Haber, M.; Cotsonis, G. A. (1988) Statistical inference for infectious diseases: risk-specific household and community transmission parameters. Am. J. Epidemiol. 128: 845-859.

Lorusso, J. R.; Moffat, S.; Ohman, J. L., Jr. (1986) Immunologic and biochemical properties of the major mouse urinary allergen (Mus m 1). J. Allergy Clin. Immunol. 78: 928-937.

Luczynska, C. M.; Arruda, L. K.; Platts-Mills, T. A. E.; Miller, J. D.; Lopez, M.; Chapman, M. D. (1989) A two-site monoclonal antibody ELISA for the quantification of the major *Dermatophagoides* spp. allergens, *Der p* I and *Der f* I. J. Immunol. Methods 118: 227-235.

Lundholm, I. M. (1982) Comparison of methods for quantitative determinations of airborne bacteria and evaluation of total viable counts. Appl. Environ. Microbiol. 44: 179-183.

Lutsky, I. I.; Neuman, I. (1975) Laboratory animal dander allergy: I. an occupational disease. Ann. Allergy 35: 201-205.

Marinkovich, V. A.; Hill, A. (1975) Hypersensitivity alveolitis. JAMA J. Am. Med. Assoc. 231: 944-947.

Mathews, K. P. (1989) Inhalant insect-derived allergens. Immunol. Allergy Clin. North Am. 9: 321-338.

May, K. R. (1967) Physical aspects of sampling airborne microbes. In: Gregory, P. H.; Monteith, J. L., eds. Airborne microbes: seventeenth symposium of the Society for General Microbiology; April; London, United Kingdom. London, United Kingdom: Cambridge University Press; pp. 60-80.

May, K. R. (1980) Assessment of viable airborne particles. In: Mercer, T. T.; Morrow, P. E.; Stober, W., eds. Assessment of airborne particles. Springfield, IL: Charles C. Thomas; pp. 480-494.

May, K. R.; Pomeroy, N. P. (1973) Bacterial dispersion from the body surface. In: Hers, J. F. Ph.; Winkler, K. C., eds. Airborne transmission and airborne infection: concepts and methods presented at the IVth international symposium on aerobiology; Enschede, The Netherlands. New York, NY: John Wiley and Sons; pp. 426-432.

Meiklejohn, G.; Reimer, L. G.; Graves, P. S.; Helmick, C. (1981) Cryptic epidemic of Q fever in a medical school. J. Infect. Dis. 144: 107-113.

Mendoza, J.; Snyder, R. D. (1970) Cockroach sensitivity in children with bronchial asthma. Ann. Allergy 28: 159-163.

Meyer, R. D. (1983) Legionella infections: a review of five years of research. Rev. Infect. Dis. 5: 258-278.

Michel, O.; Le Bon, B.; Duchateau, J.; Vertongen, F.; Sergysels, R. (1989) Domestic endotoxin exposure in asthma. In: Scientific meeting of the contact group "Respiratory Physiopathology" of the "National Fund for Scientific Research, " invited papers on: inhalation pathology; June; Leuven, Belgium. Eur. Respir. J. 2: 1019-1020.

Milton, D. K.; Gere, R. J.; Feldman, H. A.; Greaves, I. A. (1990) Endotoxin measurement: aerosol sampling and application of a new Limulus method. Am. Ind. Hyg. Assoc. J. 51: 331-337.

Mirocha, C. J.; Pawlosky, R. A.; Chatterjee, K.; Watson, S.; Hayes, W. (1983) Analysis for *Fusarium* toxins in various samples implicated in biological warfare in Southeast Asia. J. Assoc. Off. Anal. Chem. 66: 1485-1499.

Mitchell, E. B.; Wilkins, S.; McCallum Deighton, J.; Platts-Mills, T. A. E. (1985) Reduction of house dust mite allergen levels in the home: use of the acaricide, pirimiphos methyl. Clin. Allergy 15: 235-240.

Monto, A. S. (1987) Influenza: quantifying morbidity and mortality. Am. J. Med. 82(suppl. 6A): 20-25.

Moore, V. L.; Pedersen, G. M.; Hauser, W. C.; Fink, J. N. (1980) A study of lung lavage materials in patients with hypersensitivity pneumonitis: in vitro response to mitogen and antigen in pigeon breeders' disease. J. Allergy Clin. Immunol. 65: 365-370.

Moser, M. R.; Bender, T. R.; Margolis, H. S.; Noble, G. R.; Kendal, A. P.; Ritter, D. G. (1979) An outbreak of influenza aboard a commercial airliner. Am. J. Epidemiol. 110: 1-6.

Muder, R. R.; Yu, V. L.; Woo, A. H. (1986) Mode of transmission of *Legionella pneumophila*: a critical review. Arch. Intern. Med. 146: 1607-1612.

Muilenberg, M. L. (1989) Aeroallergen assessment by microscopy and culture. Immunol. Allergy Clin. North Am. 9: 245-268.

Murray, A. B.; Ferguson, A. C. (1983) Dust-free bedrooms in the treatment of asthmatic children with house dust or house dust mite allergy: a controlled trial. Pediatrics 71: 418-422.

Nelson, H. S.; Hirsch, S. R.; Ohman, J. L., Jr.; Platts-Mills, T. A. E.; Reed, C. E.; Solomon, W. R. (1988) Recommendations for the use of residential air-cleaning devices in the treatment of allergic respiratory diseases. J. Allergy Clin. Immunol. 82: 661-669.

Nevalainen, A. (1989) Bacterial aerosols in indoor air. Helsinki, Finland: National Public Health Institute.

O'Brien, I. M.; Bull, J.; Creamer, B.; Sepulveda, R.; Harries, M.; Burge, P. S.; Pepys, J. (1978) Asthma and extrinsic allergic alveolitis due to *Merulius lacrymans*. Clin. Allergy 8: 535-542.

O'Neil, C. E.; Hughes, J. M.; Butcher, B. T.; Salvaggio, J. E.; Lehrer, S. B. (1988) Basidiospore extracts: evidence for common antigenic/allergenic determinants. Int. Arch. Allergy Appl. Immunol. 85: 161-166.

Ohman, J. L., Jr. (1978) Allergy in man caused by exposure to mammals. J. Am. Vet. Med. Assoc. 172: 1403-1406.

Ohman, J. L., Jr. (1989) Allergen immunotherapy in asthma: evidence for efficacy. J. Allergy Clin. Immunol. 84: 133-140.

Ohman, J. L., Jr.; Kendall, S.; Lowell, F. C. (1977) IgE antibody to cat allergens in an allergic population. J. Allergy Clin. Immunol. 60: 317-323.

Ohman, J. L., Jr.; Lorusso, J. R.; Lewis, S. (1987) Cat allergen content of commercial house dust extracts: comparison with dust extracts from cat-containing environment. J. Allergy Clin. Immunol. 79: 955-959.

Page, F. C. (1976) An illustrated key to freshwater and soil amoebae. Sci. Publ. Freshwater Biol. Assoc. (34): 1-155.

Palchak, R. B.; Cohen, R.; Ainslie, M.; Hoerner, C. L. (1988) Airborne endotoxin associated with industrial-scale production of protein products in gram-negative bacteria. Am. Ind. Hyg. Assoc. J. 49: 420-421.

Palmgren, M. S.; Lee, L. S.; Delucca, A. J., II; Ciegler, A. (1983) Preliminary study of mycoflora and mycotoxins in grain dust from New Orleans area grain elevators. Am. Ind. Hyg. Assoc. J. 44: 485-488.

Palmgren, U.; Strom, G.; Blomquist, G.; Malmberg, P. (1986) Collection of airborne micro-organisms on Nuclepore filters, estimation and analysis—CAMNEA method. J. Appl. Bacteriol. 61: 401-406.

Parrott, W. F.; Blyth, W. (1980) Another causal factor in the production of humidifier fever. J. Soc. Occup. Med. 30: 63-68.

Pathak, V. K.; Pady, S. M. (1965) Numbers and viability of certain airborne fungus spores. Mycologia 57: 301-310.

Patriarca, P. A.; Weber, J. A.; Parker, R. A.; Orenstein, W. A.; Hall, W. N.; Kendal, A. P.; Schonberger, L. B. (1986) Risk factors for outbreaks of influenza in nursing homes: a case-control study. Am. J. Epidemiol. 124: 114-119.

Patterson, R.; Sommers, H.; Fink, J. N. (1974) Farmer's lung following inhalation of *Aspergillus flavus* growing in mouldy corn. Clin. Allergy 4: 79-86.

Patterson, R.; Fink, J. N.; Miles, W. B.; Basich, J. E.; Schleuter, D. B.; Tinkelman, D. G.; Roberts, M. (1981) Hypersensitivity lung disease presumptively due to *Cephalosporium* in homes contaminated by sewage flooding or by himidifier water. J. Allergy Clin. Immunol. 68: 128-132.

Peat, J. K.; Salome, C. M.; Sedgwick, C. S.; Kerrebijn, J.; Woolcock, A. J. (1989) A prospective study of bronchial hyperresponsiveness and respiratory symptoms in a population of Australian schoolchildren. Clin. Exp. Allergy 19: 299-306.

Pepys, J. (1969) Hypersensitivity diseases of the lungs due to fungi and organic dusts. In: Kallos, P.; Hasek, M.; Inderbitzin, T. M.; Miescher, P. A.; Waksman, B. H., eds. Monographs in allergy, vol. 4. Basel, Switzerland: S. Karger.

Pepys, J.; Jenkins, P. A. (1965) Precipitin (F.L.H.) test in farmer's lung. Thorax 20: 21-35.

Perkins, J. E.; Bahlke, A. M.; Silverman, H. F. (1947) Effect of ultra-violet irradiation of classrooms on spread of measles in large rural central schools: preliminary report. Am. J. Public Health 37: 529-537.

Petitti, D. B.; Friedman, G. D. (1985) Respiratory morbidity in smokers of low- and high-yield cigarettes. Prev. Med. 14: 217-225.

Platts-Mills, T. A. E.; Chapman, M. D. (1987) Dust mites: immunology, allergic disease, and environmental control. J. Allergy Clin. Immunol. 80: 755-775.

Platts-Mills, T. A. E.; de Weck, A. L. (1989) Dust mite allergens and asthma—a worldwide problem. J. Allergy Clin. Immunol. 83: 416-427.

Platts-Mills, T. A. E.; Tovey, E. R.; Mitchell, E. B.; Moszoro, H.; Nock, P.; Wilkins, S. R. (1982) Reduction of bronchial hyperreactivity during prolonged allergen avoidance. Lancet (8300): 675-677.

Platts-Mills, T. A. E.; Heymann, P. W.; Chapman, M. D.; Hayden, M. L.; Wilkins, S. R. (1986a) Cross-reacting and species-specific determinants on a major allergen from *Dermatophagoides pteronyssinus* and *D. farinae*: development of a radioimmunoassay for antigen P_1 equivalent in house dust and dust mite extracts. J. Allergy Clin. Immunol. 78: 398-407.

Platts-Mills, T. A. E.; Heymann, P. W.; Longbottom, J. L.; Wilkins, S. R. (1986b) Airborne allergens associated with asthma: particle sizes carrying dust mite and rat allergens measured with a cascade impactor. J. Allergy Clin. Immunol. 77: 850-857.

Platts-Mills, T. A. E.; Hayden, M. L.; Chapman, M. D.; Wilkins, S. R. (1987) Seasonal variation in dust mite and grass-pollen allergens in dust from the houses of patients with asthma. J. Allergy Clin. Immunol. 79: 781-791.

Platts-Mills, T. A. E.; Chapman, M. D.; Heymann, P. W.; Luczynska, C. M. (1989) Measurements of airborne allergen using immunoassays. Immunol. Allergy Clin. North Am. 9: 269-283.

Plouffe, J. F.; Para, M. F.; Maher, W. E.; Hackman, B.; Webster, L. (1983) Subtypes of Legionella pneumophila serogroup 1 associated with different attack rates. Lancet (8351): 649-650.

Pollart, S. M.; Chapman, M. D.; Fiocco, G. P.; Rose, G.; Platts-Mills, T. A. E. (1989a) Epidemiology of acute asthma: IgE antibodies to common inhalant allergens as a risk factor for emergency room visits. J. Allergy Clin. Immunol. 83: 875-882.

Pollart, S. M.; Platts-Mills, T. A. E.; Chapman, M. D. (1989b) Identification, quantification and purification of cockroach (CR) allergens using monoclonal antibodies (mAb). J. Allergy Clin. Immunol. 83: 293.

Price, J. A.; Longbottom, J. L. (1987) Allergy to mice. I. Identification of two major mouse allergens (Ag 1 and Ag 3) and investigation of their possible origin. Clin. Allergy 17: 43-53.

Reed, C. E.; Sosman, A.; Barbee, R. A. (1965) Pigeon-breeders' lung: a newly observed interstitial pulmonary disease. JAMA J. Am. Med. Assoc. 193(4): 261-265.

Rentschler, H. C.; Nagy, R. (1942) Bactericidal action of ultraviolet radiation on air-borne organisms. J. Bacteriol. 44: 85-94.

Richman, P. G.; Khan, H. A.; Turkeltaub, P. C.; Malveaux, F. J.; Baer, H. (1984) The important sources of German cockroach allergens as determined by RAST analyses. J. Allergy Clin. Immunol. 73: 590-595.

Riley, E. C. (1980) The role of ventilation in the spread of measles in an elementary school. Ann. N. Y. Acad. Sci. 353: 25-34.

Riley, R. L. (1982) Indoor airborne infection. Environ. Int. 8: 317-320.

Riley, R. L.; Kaufman, J. E. (1971) Air disinfection in corridors by upper air irradiation with ultraviolet. Arch. Environ. Health 22: 551-553.

Riley, R. T.; Kemppainen, B. W.; Norred, W. P. (1985) Penetration of aflatoxins through isolated human epidermis. J. Toxicol. Environ. Health 15: 769-777.

Rinaldi, M. G. (1983) Invasive aspergillosis. Rev. Infect. Dis. 5: 1061-1077.

Rippon, J. W. (1982) Medical mycology: the pathogenic fungi and the pathogenic actinomycetes. 2nd ed. Philadelphia, PA: W. B. Saunders Company.

Rippon, J. W. (1988) Medical mycology: the pathogenic fungi and the pathogenic actinomycetes. 3rd ed. Philadelphia, PA: W. B. Saunders Company.

Rippon, J. W.; Conway, T. P.; Domes, A. L. (1965) Pathogenic potential of *Aspergillus* and *Penicillium* species. J. Infect. Dis. 115: 27-32.

Rodricks, J. V.; Hesseltine, C. W.; Mehlman, M. A. (1977) Mycotoxins in human and animal health. Park Forest South, IL: Pathotox Publishers, Inc.

Rolfsen, W.; Schroder, H.; Tibell, C.; Tibell, M. (1987) Detection of specific IgE antibodies towards cat flea (*Ctenocephalides felis felis*) in patients with suspected cat allergy. Allergy (Copenhagen) 42: 177-181.

Ruben, F. L. (1987) Prevention and control of influenza: role of vaccine. Am. J. Med. 82(suppl. 6A): 31-34.

Rylander, R.; Haglind, P. (1984) Airborne endotoxins and humidifier disease. Clin. Allergy 14: 109-112.

Rylander, R.; Haglind, P.; Lundholm, M.; Mattsby, I.; Stenqvist, K. (1978) Humidifier fever and endotoxin exposure. Clin. Allergy 8: 511-516.

Rylander, R.; Haglind, P.; Lundholm, M. (1985) Endotoxin in cotton dust and respiratory function decrement among cotton workers in an experimental cardroom. Am. Rev. Respir. Dis. 131: 209-213.

Salvaggio, J. E. (1987) Robert A. Cooke memorial lecture: hypersensitivity pneumonitis. J. Allergy Clin. Immunol. 79: 558-571.

Salvaggio, J. E.; Buechner, H. A.; Seabury, J. H.; Arquembourg, P. (1966) Bagassosis: I. precipitins against extracts of crude bagasse in the serum of patients. Ann. Intern. Med. 64: 748-758.

Schachter, J.; Sung, M.; Meyer, K. F. (1971) Potential danger of Q fever in a university hospital environment. J. Infect. Dis. 123: 301-304.

Schlichting, H. E., Jr. (1969) The importance of airborne algae and protozoa. J. Air Pollut. Control Assoc. 19: 946-951.

Schlueter, D. P.; Fink, J. N.; Hensley, G. T. (1972) Wood-pulp workers' disease: a hypersensitivity pneumonitis caused by *Alternaria*. Ann. Intern. Med. 77: 907-914.

Schmidt, C. D.; Jensen, R. L.; Christensen, L. T.; Crapo, R. O.; Davis, J. J. (1988) Longitudinal pulmonary function changes in pigeon breeders. Chest 93: 359-363.

Schoenbaum, S. C. (1987) Economic impact of influenza: the individual's perspective. Am. J. Med. 82(suppl. 6A): 26-30.

Schou, C.; Lowenstein, H. (1990) Purification and characterization of the important dog allergen Can f I (Ag 13). J. Allergy Clin. Immunol. 85: 170.

Schulaner, F. A. (1970) Sensitivity to the cockroach in three groups of allergic children. Pediatrics 45: 465-466.

Schulman, J. L. (1968) The use of an animal model to study transmission of influenza virus infection. Am. J. Public Health 58: 2092-2096.

Schumacher, M. J.; Jeffery, S. E. (1976) Variability of *Alternaria alternata*: biochemical and immunological characteristics of culture filtrates from seven isolates. J. Allergy Clin. Immunol. 58: 263-277.

Scully, R. E., ed. (1979) Case records of the Massachusetts General Hospital: weekly clinicopathological exercises; case 47-1979. N. Engl. J. Med. 301: 1168-1174.

Selby, P., ed. (1976) Influenza: virus, vaccines, and strategy. New York, NY: Academic Press.

Shank, R. C., ed. (1981) Mycotoxins and N-nitroso compounds: environmental risks. Boca Raton, FL: CRC Press, Inc. 2v.

Shimazu, K.; Ando, M.; Sakata, T.; Yoshida, K.; Araki, S. (1984) Hypersensitivity pneumonitis induced by *Trichosporon cutaneum*. Am. Rev. Respir. Dis. 130: 407-411.

Sigel, M. M.; Scott, T. F. M.; Henle, W.; Janton, O. H. (1950) Q fever in a wool and hair processing plant. Am. J. Public Health 40: 524-532.

Slavin, R. G. (1983) Allergic bronchopulmonary aspergillosis. In: Middleton, E., Jr.; Reed, C. E.; Ellis, E. F., eds. Allergy: principles and practice, v. 2. 2nd ed. St. Louis, MO: The C. V. Mosby Company; pp. 1067-1083.

Smith, P. W.; Massamari, R. M. (1977) Room humidifiers as the source of *Acinetobacter* infections. JAMA J. Am. Med. Assoc. 237: 795-797.

Sneath, P. H. A.; Mair, N. S.; Sharpe, M. E.; Holt, J. G., eds. (1986) Bergey's manual of systematic bacteriology, v. 2. Baltimore, MD: Williams & Wilkins.

Solley, G. O.; Hyatt, R. E. (1980) Hypersensitivity pneumonitis induced by *Penicillium* species. J. Allergy Clin. Immunol. 65: 65-70.

Solomon, W. R.; Burge, H. P.; Boise, J. R. (1978) Airborne Aspergillus fumigatus levels outside and within a large clinical center. J. Allergy Clin. Immunol. 62: 56-60.

Solomon, W. R.; Burge, H. A.; Boise, J. R. (1980) Exclusion of particulate allergens by window air conditioners. J. Allergy Clin. Immunol. 65: 305-308.

Sonesson, A.; Larsson, L.; Fox, A.; Westerdahl, G.; Odham, G. (1988) Determination of environmental levels of peptidoglycan and lipopolysaccharide using gas chromatography with negative-ion chemical-ionization mass spectrometry utilizing bacterial amino acids and hydroxy fatty acids as biomarkers. J. Chromatogr. 431: 1-15.

Sorenson, W. G.; Simpson, J. P.; Peach, M. J., III; Thedell, T. D.; Olenchock, S. A. (1981) Aflatoxin in respirable corn dust particles. J. Toxicol. Environ. Health 7: 669-672.

Spendlove, J. C.; Fannin, K. F. (1983) Source, significance, and control of indoor microbial aerosols: human health aspects. Public Health Rep. 98: 229-244.

Spengler, J. D.; Sexton, K. (1983) Indoor air pollution: a public health perspective. Science (Washington, DC) 221: 9-17.

Spiegelman, J.; Friedman, H. (1968) The effect of central air filtration and air conditioning on pollen and microbial contamination. J. Allergy 42: 193-202.

Sporik, R.; Holgate, S. T.; Platts-Mills, T. A. E.; Cogswell, J. J. (1990) Exposure to house-dust mite allergen (*Der p* I) and the development of asthma in childhood: a prospective study. N. Engl. J. Med. 323: 502-507.

Stankus, R. P.; O'Neil, C. E. (1988) Antigenic/allergenic characterization of American and German cockroach extracts. J. Allergy Clin. Immunol. 81: 563-570.

Stanwick, R. S. (1986) Balancing the risks: *Legionella pneumophila* pneumonia and tap water scalds in the home. Can. Med. Assoc. J. 135: 1251-1252.

Stiehm, E. R.; Reed, C. E.; Barbee, R. A.; Tooley, W. H. (1966) Pigeon breeders' disease in children. J. Pediatr. (St. Louis) 69: 980.

Stiehm, E. R.; Reed, C. E.; Tooley, W. H. (1967) Pigeon breeder's lung in children. Pediatrics 39: 904-915.

Storch, G.; Baine, W. B.; Fraser, D. W.; Broome, C. V.; Clegg, H. W., II.; Cohen, M. L.; Goings, S. A. J.; Politi, B. D.; Terranova, W. A.; Tsai, T. F.; Plikaytis, B. D.; Shepard, C. C.; Bennett, J. V. (1979) Sporadic community-acquired Legionnaires' disease in the United States: a case-control study. Ann. Intern. Med. 90: 596-600.

Su, H. J.; Burge, H. A.; Spengler, J. D. (1990) Indoor saprophytic aerosols and respiratory health. J. Allergy Clin. Immunol. 85: 248.

Sussman, A. S.; Halvorson, H. O. (1966) Spores: their dormancy and germination. New York, NY: Harper & Row.

Svartengren, M.; Falk, R.; Linnman, L.; Philipson, K.; Camner, P. (1987) Deposition of large particles in human lung. Exp. Lung Res. 12: 75-88.

Swanson, M. C.; Agarwal, M. K.; Reed, C. E. (1985) An immunochemical approach to indoor aeroallergen quantitation with a new volumetric air sampler: studies with mite, roach, cat, mouse, and guinea pig antigens. J. Allergy Clin. Immunol. 76: 724-729.

Sweet, L. C.; Anderson, J. A.; Callies, Q. C.; Coates, E. O., Jr. (1971) Hypersensitivity pneumonitis related to a home furnace humidifier. J. Allergy Clin. Immunol. 48: 171-178.

Task Group on Lung Dynamics. (1966) Deposition and retention models for internal dosimetry of the human respiratory tract. Health Phys. 12: 173-207.

Tebo, T. H.; Moore, V. L.; Fink, J. N. (1977) Antigens in pigeon breeder's disease: the use of pigeon dropping antigens in detecting antibody activity. Clin. Allergy 7: 103-108.

Tourville, D. R.; Weiss, W. I.; Wertlake, P. T.; Leudemann, G. M. (1972) Hypersensitivity pneumonitis due to contamination of home humidifier. J. Allergy Clin. Immunol. 49: 245-251.

Truitt, G. W. (1951) The mushroom fly as a cause of bronchial asthma. Ann. Allergy 9: 513-516.

Tsujino, G.; Sako, M.; Takahashi, M. (1984) Varicella infection in a children's hospital: prevention by vaccine and an episode of airborne transmission. Biken J. 27: 129-132.

Twarog, F. J.; Picone, F. J.; Strunk, R. S.; So, J.; Colten, H. R. (1977) Immediate hypersensitivity to cockroach: isolation and purification of the major antigens. J. Allergy Clin. Immunol. 59: 154-160.

U.S. Department of Health and Human Services. (1991) Guidelines for the diagnosis and management of asthma. Bethesda, MD: Public Health Service, National Institutes of Health, National Asthma Education Program; publication no. 91-3042.

van Assendelft, A. H. W.; Raitio, M.; Turkia, V. (1985) Fuel chip-induced hypersensitivity pneumonitis caused by Penicillium species. Chest 87: 394-396.

Viander, M.; Valovirta, E.; Vanto, T.; Koivikko, A. (1983) Cross-reactivity of cat and dog allergen extracts: RAST inhibition studies with special reference to the allergenic activity in saliva and urine. Int. Arch. Allergy Appl. Immunol. 71: 252-260.

Vincken, W.; Roels, P. (1984) Hypersensitivity pneumonitis due to *Aspergillus fumigatus* in compost. Thorax 39: 74-75.

Voorhorst, R.; Spieksma, F. Th. M.; Varekamp, H.; Leupen, M. J.; Lyklema, A. W. (1967) The house-dust mite (*Dermatophagoides pteronyssinus*) and the allergens it produces. Identity with the house-dust allergen. J. Allergy 39: 325-339.

Voorhorst, R.; Spieksma, F. Th. M.; Varekamp, N. (1969) House-dust atopy and the house-dust mite *Dermatophagoides pteronyssinus* (Trouessart 1897). Leiden, The Netherlands: Stafleu's Scientific Publishing Company.

Walls, A. F.; Longbottom, J. L. (1983) Quantitative immunoelectrophoretic analysis of rat allergen extracts: fur, urine and saliva studied by crossed redio-immunoelectrophoresis. Allergy (Copenhagen) 38: 501-512.

Walls, A. F.; Newman Taylor, A. J.; Longbottom, J. L. (1985) Allergy to guinea pigs: II. identification of specific allergens in guinea pig dust by crossed radio-immunoelectrophoresis and investigation of the possible origin. Clin. Allergy 15: 535-546.

Walsh, T. J.; Pizzo, P. A. (1988) Nosocomial fungal infections: a classification for hospital-acquired fungal infections and mycoses arising from endogenous flora or reactivation. Annu. Rev. Microbiol. 42: 517-545.

Walshaw, M. J.; Evans, C. C. (1986) Allergen avoidance in house dust mite sensitive adult asthma. Q. J. Med. 58: 199-215.

Warner, J. O.; Price, J. F.; Soothill, J. F.; Hey, E. N. (1978) Controlled trial of hyposensitisation to Dermatophagoides pteronyssinus in children with asthma. Lancet (8096): 912-915.

Weiss, N. S.; Soleymani, Y. (1971) Hypersensitivity lung disease caused by contamination of an air-conditioning system. Ann. Allergy 29: 154-156.

Wells, M. W.; Holla, W. A. (1950) Ventilation in the flow of measles and chickenpox through a community: progress report, January 1, 1946 to June 15, 1949, airborne infection study, Westchester County Department of Health. JAMA J. Am. Med. Assoc. 142: 1337-1344.

Wells, W. F.; Wells, M. W.; Wilder, T. S. (1942) The environmental control of epidemic contagion: I. an epidemiologic study of radiant disinfection of air in day schools. Am. J. Hyg. 35: 97-121.

Wenzel, F. J.; Emanuel, D. A. (1965) Experimental studies of farmer's lung. N. Y. State J. Med. 65: 3032-3037.

Westphal, O.; Westphal, U.; Sommer, T. (1977) The history of pyrogen research. In: Schlessinger, D., ed. Microbiology—977. Washington, DC: American Society for Microbiology; pp. 221-238.

Westphal, O.; Jann, K.; Himmelspach, K. (1983) Chemistry and immunochemistry of bacterial lipopolysaccharides as cell wall antigens and endotoxins. Prog. Allergy 33: 9-39.

Wicklow, D. T.; Shotwell, O. L. (1983) Intrafungal distribution of aflatoxins among conidia and sclerotia of *Aspergillus flavus* and *Aspergillus parasiticus*. Can. J. Microbiol. 29: 1-5.

Williams, D. M.; Krick, J. A.; Remington, J. S. (1976) Pulmonary infection in the compromised host: part 1. Am. Rev. Respir. Dis. 114: 359-394.

Wilson, A. F.; Novey, H. S.; Berke, R. A.; Surprenant, E. L. (1973) Deposition of inhaled pollen and pollen extract in human airways. N. Engl. J. Med. 288: 1056-1058.

Winn, W. C., Jr. (1985) Legionella and legionnaires' disease: a review with emphasis on environmental studies and laboratory diagnosis. Crit. Rev. Clin. Lab. Sci. 21: 323-381.

Wolochow, H. (1958) The membrane filter technique for estimating numbers of viable bacteria: some observed limitations with certain species. Appl. Microbiol. 6: 201-206.

Wood, R. A.; Chapman, M. D.; Adkinson, N. F., Jr.; Eggleston, P. A. (1989) The effect of cat removal on allergen content in household-dust samples. J. Allergy Clin. Immunol. 83: 730-734.

Wray, B. B.; O'Steen, K. G. (1975) Mycotoxin-producing fungi from house associated with leukemia. Arch. Environ. Health 30: 571-573.

Yasueda, H.; Mita, H.; Yui, Y.; Shida, T. (1989) Comparative analysis of physicochemical and immunochemical properties of the two major allergens from *Dermatophagoides pteronyssinus* and the corresponding allergens from *Dermatophagoides farinae*. Int. Arch. Allergy Appl. Immunol. 88: 402-407.

Yocom, J. E.; Clink, W. L.; Cote, W. A. (1971) Indoor/outdoor air quality relationships. J. Air Pollut. Control Assoc. 21: 251-259.

Yocum, M. W.; Saltzman, A. R.; Strong, D. M.; Donaldson, J. C.; Ward, G. W., Jr.; Walsh, F. M.; Cobb, O. M., Jr.; Elliott, R. C. (1976) Extrinsic allergic alveolitis after Aspergillus fumigatus inhalation: evidence of a type IV immunologic pathogenesis. Am. J. Med. 61: 939-945.

Young, L. S.; Feeley, J. C.; Brachman, P. S. (1970) Vaporized formaldehyde treatment of a textile mill contaminated with *Bacillus anthracis*. Arch. Environ. Health 20: 400-403.

Yunginger, J. W.; Jones, R. T.; Nesheim, M. E.; Geller, M. (1980) Studies on *Alternaria* allergens: III. isolation of a major allergenic fraction (ALT-1). J. Allergy Clin. Immunol. 66: 138-147.