ACKNOWLEDGEMENTS

CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

J. LINDSEY CHALK.   Laboratory animal allergy:
industrial hygiene groundwork for a prospective
epidemiologic study.   (Under the direction of
DR. JOHN M. DEMENT)

This paper discusses some basic principles of allergy and asthma, and provides an overview of laboratory animal allergy and aeroallergen quantitation methods. Laboratory animal worker exposures to total and respirable particulate concentrations were determined from personal samples (both time-weighted average and task specific) and area samples. All results were below threshold limit values for nuisance dust (10 mg/M^3-total, 5 mg/M^3-respirable) and hardwood dust (1 mg/M^3). The highest particulate exposures existed in the area on the clean side of the automatic cage washers (0.97 mg/M^3-total, 0.05 mg/M^3-respirable, TWAs) where hardwood bedding chips were dispensed into clean cages. The respirable percent of the samples varied widely (5 to 100%). The air samples collected will be analyzed by RAST inhibition for rat urinary protein and rat dander, and will be used for dose determination in a prospective study on the development of laboratory animal allergy and asthma. A system of dose calculation and data presentation is suggested for the follow-up epidemiology.

INTRODUCTION

The Status of Aeroallergen Sampling in LAA

This study was intended to address some of the questions raised by current LAA (laboratory animal allergy) related research. Personal air sampling has not been used to quantitate an individual's breathing zone exposure to allergenic material, nor have specific tasks involved with animal work been evaluated for their exposure potential. Concentrations of specific airborne allergens in laboratory animal holding rooms have been measured, but actual personal inhalation exposure is still a matter of conjecture. In order for critera for a recommended standard to be developed, exposures need to be linked to effects (or dose to response). Only within the past few years has it been possible to measure aeroallergen dose meaningfully. Now that dose can be correlated to a response, criteria for development of a standard will be a matter of examining the evidence to discover the cogent picture.

The NIEHS Project

The dose-response correlation will be accomplished by a NIEHS (National Institute of Environmental Health Sciences) clinical/epidemiologic survey which incorporates the quantitative results begun by this study and relates exposure to response data elucidated by skin prick tests, RAST inhibition tests, and questionnaires.

Exposures are most meaningful if expressed as 8-hour time-weighted averages, and in addition to identifying average task-specific peak

exposures (e.g., cage washing, necropsy, lavage, etc.), these para-
meters identify the higher risk jobs and tasks, thereby enhancing
accurate, quick recognition, evaluation and control of hazards. Also
the relationship of gravimetric results with RAST inhibition results
for specific antigens should be evaluated. A positive correlation may
be significant, since gravimetric sampling and measuring techniques are
much simpler than the latter, but may be useful as an index of allergen
activity. Particulate concentration may also profoundly effect
the allergen activity in some hitherto unrecognized way.

Objectives

The objectives of the present study were to: 1) determine per-
sonal exposures to total and respirable dusts in laboratory animal
handling areas, 2) establish a hierarchy of tasks/jobs by hazard, or
dose intensity, for use in future industrial hygiene and epidemiologic
studies, 3) investigate the total particulate / respirable particulate
relationship of LAA-inducing aerosol, 4) identify interim control stra-
tegies in view of the current data, and 5) provide preliminary exposure
data to be used in a concomitant clinical/epidemiologic study.

LITERATURE REVIEW

Theory and Mechanisms of Allergy and Asthma

Allergies and asthma are among the most common of all health problems experienced in this country. It has been estimated that about one out of every six Americans suffers from at least one allergic problem. (48) The direct and indirect costs to these afflicted persons exceeds $1.8 billion. The cost to Americans and to American industry and business for approximately 35 million annual sick days and more than 90.5 million days of restricted activity each year is incalculable. (48) Despite explosive advances in recent years of our understanding, diagnosis, and treatment of asthma and allergic diseases, additional research is needed before the debilitating effects of these disorders are brought under control.

Allergy may be defined as unfavorable physiologic events mediated by a variety of different immunologic reactions. Allergic reactions can involve any organ system in the body. Allergy is a harmful hypersensitivity to a specific substance; it is the antithesis of immunity. The concept of immunity centers on the body's ability to recognize foreign, "nonself" material, such as microorganisms, pollen, and dust, and react in such a way as to control or destroy the invading material. However, when the same ordinarily protective system produces a harmful reaction we refer to this as an allergic response.

An "antigen" is any substance which elicits a reaction from the immune system. The immune system recognizes structures on the antigen

as being nonself and reacts against them. The reaction may be mediated by the action of cells (cell-mediated immunity) or by proteins in the blood called antibodies (humoral immunity).

Discoveries in the early 1960's led to a hypothesis that is currently one of the central dogmas of immunology today. This hypothesis states that there are two distinct types of immunocompetent white blood cells: one which requires the thymus, a lymph gland in the chest, for development and is responsible for cell-mediated immunity (T-lymphocytes); and the other, developing independent from the thymus, is responsible for the mediation of the antibody responses (B-lymphocytes). (18) Phagocytes, a third type of white blood cell, participate in the immune response. They are scavengers and include macrophages, neutrophils, and eosinophils. T cells, B cells, and pha- gocytes all originate in the bone marrow.

T cells can directly attack an antigen, and produce chemical "mediators" that attract or activate other parts of the immmune system. Cell-mediated immunity is a major factor in the rejection of transplanted organs, tissue grafts, and may be important in cancer pro- tection. B cells interact with T cells; they divide and change into cells that can produce large numbers of specific "antibodies" when they react with an antigen and may be assisted in this by T cells. The antibodies produced are specific to the B cell that produced them. This illustrates why vaccination works by injecting dead or weakened antigens which prime the immmune system for a rapid response to a real attack.

Antibodies are the mediators of humoral immunity. They are defined as proteins that are formed by the host in response to an anti-

gen and react specifically with that antigen. The special group of five classes of proteins comprising the antibodies are called the "immunoglobulins": IgG, IgA, IgM, IgD, and IgE. IgG is the most abundant immunoglobulin and includes the primary serum antibodies against bacteria, viruses, and exogenous toxins. IgG antibodies are at times involved with certain phagocytes to facilitate phagocytosis. IgG is the only class of antibodies that crosses the human placenta to become the principle antibody of the newborn. IgA antibodies, found mainly in body secretions such as saliva, tears, and nasal secretions, serve as a first line of defense against inhaled or ingested microorganisms. IgD's function is not well understood. IgM is large and binds most efficiently to antigens and to "Cl". Cl is the first component of the "complement system", a group of blood enzymes that play a vital role in immunity and will be discussed shortly. IgG can also activate the complement system. IgE (reagenic) antibodies were discovered in 1921 by Prausnitz and Kustner when they determined that serum could transfer cutaneous sensitivity, but were not isolated until 1966. Most IgE is on the surface of basophils and mast cells, not free in blood serum (see Figure 1). Antigens that bind to IgE on these cells are called "allergens". Examples include airborne pollens, fungi, and animal dander. Thus, most of the allergic reactions that plague many people are due to IgE.

The lysosomes of tissue mast cells and circulating basophils contain most of the histamine in the human body. Histamine, which regulates the permeability of capillaries, is released with other mediators when antigens bind to the IgE on basophils and mast cells. This is the beginning of the allergic reaction. The released mediators then begin their harmful work: fluid leaks into extravascular spaces, smooth

Figure 1. NORMAL IMMUNOGLOBULIN LEVELS

Class	Serum concentration (mg/ml)
IgG	12.5
IgA	2.2
IgM	1.1
IgD	0.02
IgE	0.00004

Reference: Merrett, 1981

muscle has spasms, phagocytic cells are attracted, and platelets are activated.

The complement system may enhance the effects of antibodies or may directly interact with microbes. It is the primary humoral response to the binding of antigen and antibody, and one of the principal causes of inflammation induced by the immune system (20). The main functions of the complement system in killing bacteria are 1) the attraction of phagocytes, 2) the increased adherence of phagocytes to antigens, and 3) cell lysis. Late in the nineteenth century Bordet first described the complement system. This series of proteins has come to be known as the "classic" pathway, since in 1954 an alternate, but related pathway was described by Pillemer and his colleagues. The alternate pathway is triggered in the absence of antibodies but the results are the same: an amplification loop, inflammation and host defense effects, and ultimate destruction of the invasive material.

Earlier it was pointed out that the immune pathways that function as the protective mechanisms can also cause injury. The most widely used classification of the hypersensitivity, or allergic, reaction has four groups. Type I is applicable to LAA, and will be discussed last. Type II, cytotoxic hypersensitivity, occurs when circulating antibodies develop against a cell membrane or an antigen associated with a cell membrane. Autoimmune hemolytic anemia and drug-induced hemolytic anemia are examples, where antibodies to red cells or antibodies to a drug bound to a red cell may be found. A transfusion with an incompatible blood type will also incite a Type II reaction. The complement system is often involved with the cytotoxic reaction, usually activated by IgG or IgM.

Type III, immune complex hypersensitivity, occurs from hours to several days after exposure to antigen. In this case, antibodies combine with soluble antigens in blood, forming immune complexes. The immune complexes pass into extravascular spaces, and deposit in the walls of blood vessels or kidneys. The complement system is also involved in producing the disease state. An example of this type of reaction is serum sickness-- a reaction to an injection of an animal serum.

Type IV, delayed hypersensitivity, is cell-mediated, whereas Types I, II, and III are humoral responses. This type of reaction reaches its peak about two days after exposure. Here T cells react directly with the antigen, initiating a complex process of cell production and release of lymphokines which bring more cells into the reaction to attack the invader. Examples of the Type IV reaction can be found in tuberculin immunity, graft rejection, and contact dermatitis, such as poison ivy.

Type I, immediate hypersensitivity, goes by many names (anaphylactic or reagin-dependent hypersensitivity, etc.) and is the immmune response that is synonymous with allergy. Within seconds or minutes of exposure to antigen, this mechanism produces its effect; examples of Type I reactions include hay fever, some asthma and hives, some food and drug reactions, insect-sting reactions, and anaphylactic shock (a severe, potentially fatal reaction). The bridging of two adjacent IgE antibodies on the surface of a mast cell or basophil triggers a series of intracellular events resulting in the release of numerous preformed inflammatory mediators, such as histamine, and the synthesis of new mediators, such as slow-reacting substances (the

leukotrienes), platelet activating factor, and prostaglandins. These substances cause edema, spasm of smooth muscle, granulocyte attraction, activation of platelets, and increased production of mucous.

The first necessary step in developing an allergy is exposure to the antigen, which may be a pollen, dust, a mold, animal dander, animal urinary protein, or some other substance. IgE antibodies specific to the antigen are formed, usually having a molecular weight of about 190,000; IgE is a monomer of two short and two long polypeptide chains-- the basic building block of all immunoglobulins. The principle site of IgE synthesis is the lymphatic tissue of the respiratory and gastrointestinal systems, but especially the tonsils and adenoids. IgE is found in a lower concentration in the blood than any other immunoglobulin class. Evidently IgE synthesis by B cells is under careful and constant regulation by both helper and suppressor T cells with suppression usually predominating. It is thought by some that allergic diseases represent a "breakthrough" phenomenon in which the suppressive mechanisms that normally keep IgE levels low are abrogated (4).

Up to 500,000 IgE antibodies can attach to a mast cell or basophil. In humans mast cells far outnumber basophils; mast cells are found primarily in tissues such as the skin, the respiratory tract, and the gastrointestinal tract, while basophils are found in the blood. When the allergic subject encounters the specific antigen again, it binds only to the specific antibodies now sitting on the surfaces of cells, bridging them. This signals the cell to release its granules of chemical mediators, which leads to symptoms such as wheezing, sneezing, runny eyes, itching, abdominal pain, wretching, or diarrhea. The mediators lead to tissue injury either by direct effect on blood

vessels, nerve fibers, smooth muscles in the lungs, or by attracting other damage-producing cells to the site. Mediators released from human mast cells and basophils include: 1) histamine--causes itching, constriction of bronchial tube smooth muscle, and increase of blood vessel permeability, 2) slow-reacting substance of anaphylaxis (SRS-A)-- the most powerful constrictor of human bronchial tube smooth muscle known, 3) eosinophil chemotactic factor of anaphylaxis (ECF-A)-- attracts eosinophils, 4) platelet activating factor (PAF)-- stimulates the release of secondary mediators from platelets and causes platelet aggregation (33), 5) neutrophil chemotactic factor (NCF)--attracts neutrophils, 6) superoxide (O_2^-)-- possibly deteriorates the mucous membrane, as in severe asthma, 7) bradykinin-- a potent blood-vessel dilator, 8) prostaglandins-- tissue reactions such as bronchodilation and bronchoconstriction. Some mediators are preformed while others are newly synthesized following basophil or mast cell membrane activation. Of the preformed mediators, some are eluted rapidly from granules, others slowly; of the newly formed mediators, some are formed within the mast cell or basophil, others are generated secondarily by other cells or from extracellular fluid. Mediators are still being investi-gated and defined. It is important to recognize that these harmful cells and chemicals are intended for protecting the body, and do, in a normal immune response.

The allergic response may be complicated by ear inflammation or sinusitis, but the most common allergic condition is allergic rhinitis, inflammation of the nasal mucous membranes. Although never fatal, rhi-nitis significantly impairs normal function of the sufferer, often causing absences from work and school. Type I allergic response can

range from mild effects to life-threatening; other Type I disorders include bronchial asthma, anaphylaxis, and urticaria.

The skin prick test is the most common diagnostic procedure used to demonstrate immediate hypersensitivity. A drop of antigen concentrate is placed on the skin, then the skin is pricked with a needle. The degree of sensitivity is correlated by the wheal-and-flare response after 15 minutes. If the skin prick test is negative, intradermal injection of antigen may be used to demonstrate sensitivity. Although not a precise indicator of allergic conditions, the radioimmunosorbent test (RIST) may be used to measure total IgE. The radioallergosorbent test (RAST), which measures antigen-specific IgE, is more helpful. Both methods can measure IgE levels to 1 ng/ml. The RAST test will be discussed in more depth later.

Allergies are controlled by a combination of genetic and nongenetic factors. One may inherit a tendency to be allergic, and usually someone suffering from an allergic disease has a close relative who also has some allergic disease.

Advances in drug therapy have resulted in much relief for allergy and asthma sufferers. However, side effects and erratic responses to the drugs do occur, since, as many drugs, they are mostly developed through experiment and observation of their effect on symptoms, rather than with a detailed knowledge of the molecular activity taking place in vivo. Immunosuppression-- the suppression of the immune system by drugs or irradiation-- is also under investigation. Immunotherapy, or desensitization, often referred to as "allergy shots", is also an effective treatment. This technique is one of gradually increasing the amount of injected allergen which is known to trigger the allergic

response of the patient. Originally this was thought to reduce or eliminate IgE, but it has been determined that IgG is proliferated and blocks the antigen from reacting with IgE, thus avoiding an allergic reaction.

Although difficult to define, asthma is a special aspect of allergic reaction that warrants some discussion. Asthma is a condition characterized by widespread narrowing of the bronchial airways, which changes in severity over short periods of time either spontaneously or under treatment, and is not due to cardiovascular disease. During an attack, the asthmatic suffers shortness of breath and wheezing. Bronchodilator drugs offer relief in most cases. There are many and varied causes of asthma. Allergy can be a sufficient but not an exclusive cause of asthma. Some causes involve an allergic reaction (extrinsic asthma) and for others no outside factor such as an allergen can be determined (intrinsic asthma). Millions of Americans suffer from asthma. Asthma and allergic diseases are the leading chronic disorders in children under 17. If asthma attacks are seasonal and appear to be related to allergy, skin tests are often employed to help determine the causal allergen and to help in treatment planning.

Airway obstruction can be present even when the asthmatic has no symptoms. During an asthmatic attack, the smooth muscle of the bronchioles constrict and narrow the airway. The bronchiole linings themselves become swollen and filled with eosinophils. Excess mucous is produced, further plugging the airways. Breathing becomes forced, and the rush of air through narrowed airways and the vibration of mucous there produces wheezing sounds. Though the anatomical changes are well described, the underlying mechanism is still not well

understood. It appears that all the layers of the airway wall have physical alterations. The size of the bronchial smooth muscles of asthmatics is increased, possibly due to prolonged constriction or simply to increased susceptibility to contraction. Allergen-induced asthma usually causes an immediate decrease in lung function, which may be followed by a secondary phase of bronchoconstriction developing 4 to 6 hours later. Rarely does the late reaction develop without the immediate reaction.

In an acute asthma attack, the trapped air due to narrowed airway passages causes uneven ventilation and perfusion which leads to decreased arterial blood oxygen (hypoxemia). Hyperventilation first decreases carbon dioxide in the blood then the CO_2 level increases as obstruction worsens. Severe asthma attacks can be life-threatening. Also, the overinflation of the lungs and the increased pressures required for inhalation in an asthma attack put considerable stress on the cardiovascular system.

Histamine plays a role in asthma, but antihistamines, which are so helpful in controlling hay fever, are usually ineffective in treating asthma. However, immunotherapy and drug therapy are used widely with considerable success. Subjects sensitive to a specific allergen will find their symptoms greatly reduced with removal of the antigen, but controlling the environment is often difficult. So it is with allergic and asthmatic subjects whose symptoms are triggered by laboratory animals.

Laboratory Animal Allergy

With a working knowledge of the theory and mechanisms of allergy and asthma attention may now be focused on the problem of laboratory

animal allergy (LAA). LAA may be described as an allergic disorder characterized by conjunctivitis, rhinitis, urticaria, or asthma, or a combination of these symptoms, and is causally related to exposure to allergens derived from laboratory animals. The syndrome has the typical characteristics of an IgE mediated condition, but can trigger a type III (arthus-type) asthma attack hours after exposure. Although the list of animals used by scientific and medical workers is endless, five species comprise 99% of laboratory animals in use today: rats, mice, guinea pigs, rabbits, and hamsters. Personnel in contact with laboratory animals are a diverse occupational group with a wide range of socioeconomic and educational background. Included are veterinarians, physicians, dentists, senior scientists, animal research technicians, laboratory technicians, geneticists, breeders, and animal caretakers. A typical employee having acquired LAA has been described as a person of either sex, a scientific or technical worker with laboratory animals, 23 to 32 years of age, with a family history of atopy, one who has developed LAA within three years of the start of close contact with laboratory animals, exhibits an immediate hypersensitivity (Type I) reaction in less than 10 minutes, has at least 3 clinical symptoms, such as rhinitis, asthma, and cough, and may be allergic to more than one species (25).

Although allergy acquired from laboratory animals has been documented for over a century, it has only recently come to be considered a significant occupational disease. In Great Britain animal asthma is now a prescribed industrial disease under national industrial injuries laws, entitling the affected worker to compensation from central government funds (8,10,26). It has been estimated that 35,000 workers

and scientists in the United States are regularly exposed to laboratory
animals, and that there are 4,700-8,600 cases of LAA and 2,400-3,700
laboratory animal asthmatics in the UK alone (13). Many of these
employees changed jobs or specific animal contact. Many others resigned
employment. There is apparently no uniform policy regarding the
problem of allergy to laboratory animals in US animal facilities.
While 103 of 155 surveyed animal research facilities required a
preemployment medical examination, only six of these included hypersen-
sitivity screening (26). The attention to and knowledge of LAA has,
nevertheless, been growing steadily. Examples in the literature
include an LAA case discussed by Sorrell and Gottesman in 1957 (41), 10
cases in Sweden discussed by Rajka in 1961 (35), and a review of the
LAA problem by Patterson in 1964 (34). This attention to LAA and new
knowledge pertaining to it has virtually exploded in the last ten
years, due in part to the advancement of the radioallergosorbent test
(RAST), which will be discussed. Despite the mounting numbers of ani-
mal allergy case histories reported in the literature (17) and the
knowledge explosion, allergy texts have barely begun to lend more than
a perfunctory comment on the topic (32,28).

Numerous studies of the prevalence of LAA have emerged (6,38,21,
24,8,25,31,12), and with them many of the patterns of LAA are becoming
defined. It appears that 15-30% of exposed workers suffer from LAA.
However it is likely that the problem is greater than it appears, since
job migration and active avoidance of animal exposure jobs doubtless
occur. The study performed by Davies and McArdle found that nearly
half of those who worked with laboratory animals and developed symptoms
had to stop work permanently or temporarily. The frequencies of

hayfever, eczema, and bronchitis were not elevated among exposed workers, but asthma, nasal symptoms and eye symptoms were elevated (13). Urine has been found to be an important source of allergens (30,46), and does not appear to be strain specific (27). Atopy, or predisposition (by familial or personal history of asthma or allergy), skin testing, and RAST scores all seem quite dissociated from the development of post-employment allergy, but closely associated with the development of asthma due to LAA (13,31,12). The practical value of pre-employment hypersensitivity screening, therefore, appears to be somewhat limited.

Slovak and Hill proposed that LAA occurs in two distinct forms: regional LAA syndrome and progressive LAA syndrome (38). In this model, regional LAA is characterized by rhinitis with negative skin prick tests, and progressive LAA consists of rhinitis leading progressively to asthma with positive prick tests. Atopes appear not to be at special risk of developing LAA but if they do it is more likely to progress to asthma. Slovak and Hill suggest the need for a prospective study of LAA to establish the incidence and prevalence of LAA. They state, "Such a study could be definitive, especially if it were designed to run alongside a properly constituted occupational hygiene survey, so that some quantitative correlation could be made with incidence and prevalence. Only then would it be possible to offer an opinion on the establishment of hygiene standards...." Such prospective studies will allow specific recommendations to be made about the protective efficacy of specific designs for cages, animal rooms, experimental rooms, ventilation systems, and other protective equipment.

Quantitation of Antigen Exposures

Since the problem of LAA cannot be contained in a purely clinical or medical arena, contributions are required from the field of industrial hygiene. A brief overview of the history of aerobiologic measurement techniques follows.

Early investigations of pollen in the nineteenth century emphasized an approach utilizing the principle of gravitational settling of particles upon adhesive-coated slides. Such methods were severely limited for deriving quantitative results since the tendency of a particle to cross airflow streamlines is almost exclusively a function of its size (40). Nevertheless, traditional reliance on gravitational collection was reinforced in 1946 when a covered slide support proposed by Durham (14) was adopted as a standard pollen sampler. Such samplers owe their usefulness to low cost, durability, and power source independence. Though attempts have been made to derive air volume by this method, particles enumerated on gravity slides or open culture plates represent an unknown and incalculable volume of air.

Impaction type samplers have also been used, in which the collection surface acts upon the air mass, rather than vice versa. This group of devices includes the Rotorod sampler, which has two upright arms rotating at about 2,500 rpm on a small motor. The arms are coated with adhesive and microscopically examined after collection. They are most useful for collection of particles larger than 5-10 um. These samplers are less susceptible to anisokinetic sampling error than suction type impactors.

Suction type impactors force impaction of small particles by drawing air through specially designed flow channels which cause the air to

undergo rapid directional changes. Examples of such instruments include the Andersen sieve impingers, cascade impactors, and the Hirst automatic spore trap.

Filter samplers, especially Hi-Vol fiber filter sampling, is often used as well. Though high volume and a large filter are advantageous, microscopic inspection is difficult. Membrane filters lend themselves better to morphological examination.

As industrial hygienists have participated more in the occupational health and clinical work support, a broader range of devices has been put to use, including electrostatic collectors, thermal precipitators, and elutriators (7). Personal samplers using porous membrane filters and/or size selective cyclones have gained wider usage, and are the choice of this study. These samplers provide a truer picture of actual human inhalation exposure.

Laboratory animal derived aeroallergens present a broad particle size distribution and diverse morphology. This picture is supplemented by a nondescript, complex mixture of wood chip bedding, animal feed, dust, and microorganisms. Gravimetric and RAST (described below) are the most effective means of quantifying animal-derived allergens, since the allergens are not viable and bioamplification is therefore not possible, and since microscopic inspection is tedious, difficult, and only vaguely quantitative.

In the 1960's a remarkable serological assay was introduced for in vitro measurement of IgE antibodies: the RAST (radioallergosorbent test). RAST greatly facilitated fundamental research in allergy, but launched a growing controversy as a replacement for the time-honored

Prausnitz-Kustner (P-K) skin test (29,2,1,5). Work continues as investigators refine and perfect the RAST and its applications to LAA. A vast amount of new immunologic information on animal allergens has been generated in the last ten years. RAST tests using urinary proteins from mice, rats, guinea pigs, and rabbits have been developed and used in the diagnosis of LAA (15). A variation on the RAST, called the "mini-RAST", was developed which in some cases allows the use of much less allergen without compromising sensitivity (19).

But what is the RAST, and how does it work? The test for antibody content of a serum must first be understood. The serum antibody content can be assessed by its ability to bind to antigen which has been insolubilized either by coupling to an immunoadsorbent or by physical adsorption to a plastic tube; the bound immunoglobulin may then be estimated by addition of a radiolabelled anti-Ig raised in another species. That is, a patient's serum is added to a plastic tube coated with antigen; the antibodies will bind to the antigen and remaining serum proteins can be readily washed away. Bound antibody can now be estimated by addition of radiolabelled purified anti-human Ig; after rinsing out excess unbound reagent, the radioactivity of the tube will be a measure of the antibody content of the patient's serum. The distribution of antibody in different Ig classes can be determined by using specific antisera. In the RAST test for IgE antibodies in allergic patients, the allergen (e.g., rat urinary protein extract) is covalently coupled to a paper disc which is then treated with patient's serum. The amount of specific IgE bound to the paper is then estimated by addition of labelled anti-IgE (Figure 2).

But how is antigen measured? This is done by a slightly different test called "RAST inhibition". The binding of radioactively labelled
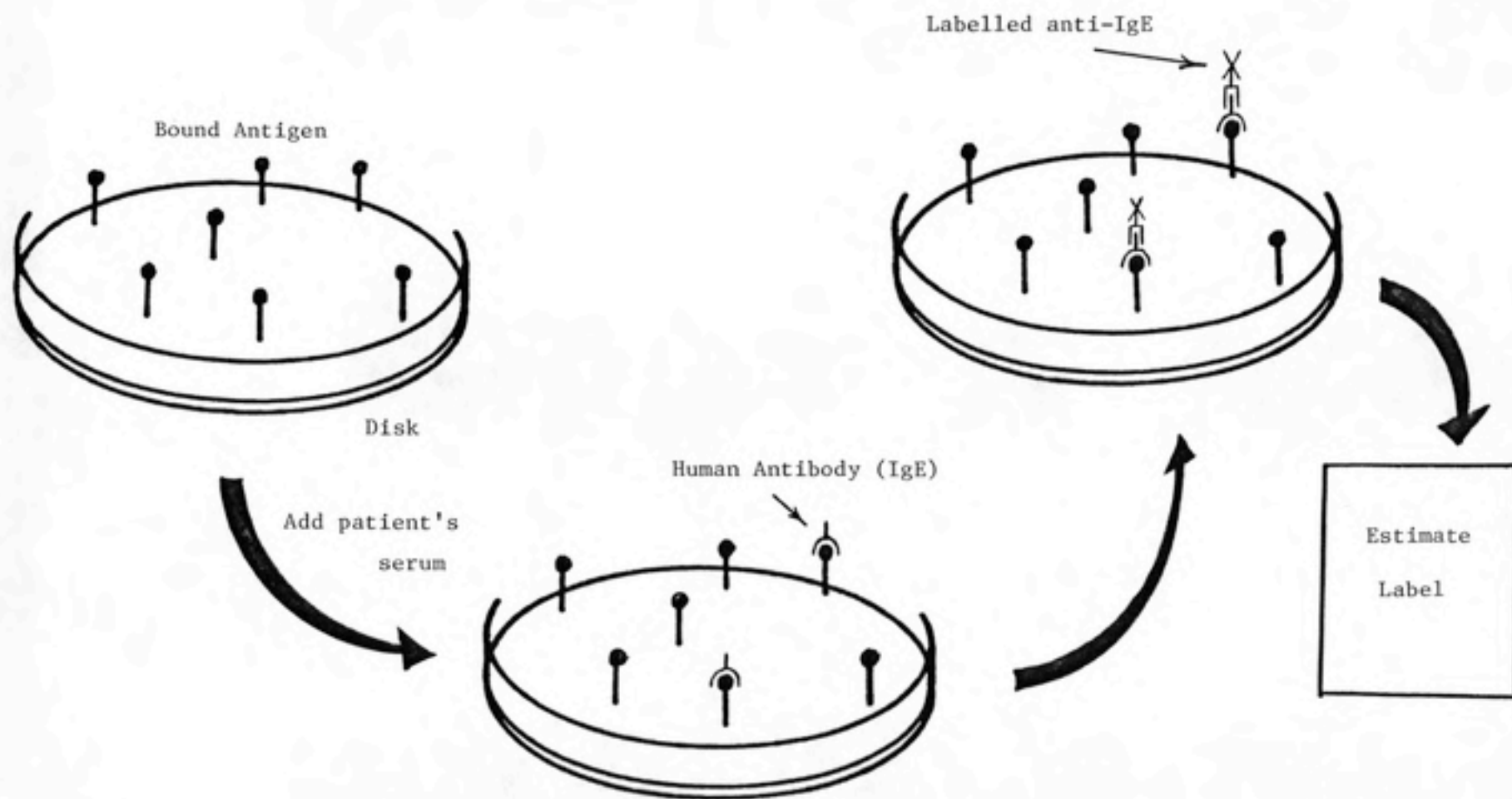
FIGURE 2. The RAST Test for Quantitative Determination of Antibody IgE. (Adapted from Roitt, 1980.)

Figure 3. Principle of radioimmunoassay (simplified by assuming a very highly avid antibody and one combining site per antibody molecule).

		Free Ag	Bound Ag	Ratio Free:Bound radioactivity
A)	15 *Ag + 10 Ab ⟶	5*Ag	+ 10*AgAb	1:2
B)	15 *Ag 15 Ag + 10 Ab ⟶	10*Ag } 10 Ag }	+ 5*AgAb } 5 AgAb }	2:1

% Radioactivity Bound — 100 — 0

Concentration of test Ag

---
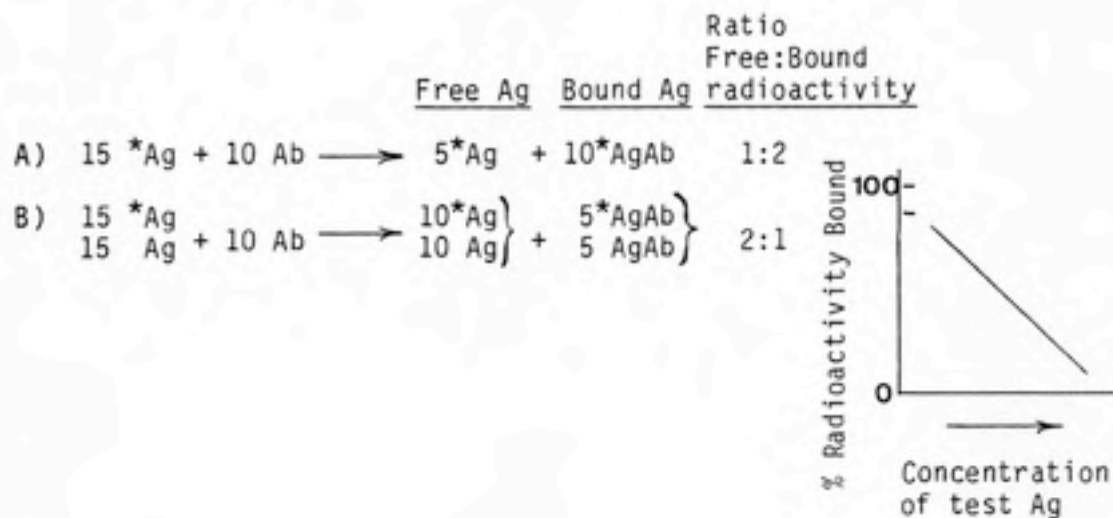
*Ag = radioactive antigen
Ag = unlabelled antigen
Ab = antibody

A) If we add 15 mol of radiolabelled Ag to 10 mol of Ab, 5 mol of Ag will be free and 10 bound to Ab. The ratio of the counts of free to bound will be 1:2.

B) If we now add 15 mol of unlabelled Ag plus 15 mol radio Ag to the Ab, again only 10 mol of total Ag will be bound, but since the Ab cannot distinguish labelled from unlabelled Ag, half will be radioactive. The remaining antigen will be free and the ratio free:bound radioactivity changes to 2:1. This ratio will vary with the amount of unlabelled Ag added and this enables a calibration curve to be constructed.

Reference: Roitt, 1980

antigen to a limited fixed amount of antibody can be partially inhi-
bited by addition of unlabelled antigen and the extent of this inhibi-
tion can be used as a measure of the unlabelled material added (Figure
3) (36). Methods vary in the means used to separate free antigen from
that bound to antibody.

With the development of methods for labelling antigens to a high
specific activity, very low concentrations down to the 10^{-12} g/ml level
can be detected. A disadvantage for some applications is that these
methods cannot distinguish active protein molecules from biologically
inactive fragments which still retain antigenic determinants.
Nevertheless, RAST has been a great aid to immunology, and more speci-
fically, to the investigation of LAA.

The first attempts to quantify animal allergens in the workplace
were made at Oak Ridge National Laboratory in Tennessee in 1974 (24).
Three techniques were used for mouse-derived allergens: morphologic
identification in air samples collected on Millipore filters, quan-
tification of dust and extractable protein nitrogen determined by the
micro-Kjeldahl technique in dust collected in the same way, and double
diffusion in agar gel using rabbit antisera to mouse serum, dander, and
urine. The results of the first two methods showed a positive correla-
tion with the size of the animal population in the area, but the tech-
niques were not specific with regard to allergen. The last technique
was mainly qualitative, but did seem to indicate strong allergenicity
of urine as compared to serum and the low allergenicity of food and
bedding. The study also illuminated the allergenic importance of
soluble material on dander.

Then in 1979 a RAST inhibition technique was used to demonstrate mouse allergen in the dust from a mouse-inhabited room (37), though, again, airborne allergen concentrations were not quantified.

Finally, in 1981 researchers at the Allergic Diseases Research Laboratory of the Mayo Clinic in Rochester, Minnesota, adapted the RAST inhibition technique to measure airborne allergens (3). This involved collection of ragweed pollen or Alternaria fungal spores on the filter sheet of a Hi-Vol air sampler. Particles greater than 0.3 um diameter were captured and eluted, then dialyzed and lyophilized. The eluates were reconstituted and assayed for specific allergen activity by a RAST inhibition assay. Their results showed a positive correlation with pollen and spore counts by traditional morphologic identification. About a year later the same laboratory published the results of applying the technique to the laboratory animal allergy problem (45). Airborne mouse pelt and urine allergen concentrations were investigated in a mouse-care room and immunology laboratory and were found to range from 1.8 to 825 ng/M^3, varying with the number of animals in the room and the degree of work activity. This was accomplished using a high-volume air sampler to collect the air samples on fiber filter sheets.

In a recent study on three guinea-pig-derived allergens (42), urine, pelt, and albumin, the first two were found to be cross-reactive while the albumin allergen was not. Hi-Vol air samples were collected in the animal housing room, a research laboratory, a library, and out-side. Guinea pig urine allergen activity was detected in all indoor samples by RAST inhibition, pelt allergen activity was detected in the former two indoor samples, and no guinea pig albumin was detected in the air samples. Sizing of airborne particles with an Andersen cascade

impactor demonstrated that most of the allergen activity was associated with particles of a diameter greater than 5 um and less than 0.8 um. The study implicated urine as being the major source of guinea pig allergens, and showed that it is present in airborne particles small enough to penetrate the lower respiratory tract when inhaled.

Another important recent study extending the knowledge of laboratory animal-derived aeroallergens in the work environment was published in 1983 by a group of researchers in the UK (16). That study employed air samplers drawing air at 2 Lpm for 5 hours in four different rooms, each room devoted primarily to a different species of animal, then RAST inhibition assay of the samples. Their methods appeared reliable, and so were extended to the evaluation of the effects of air change rates and humidity on airborne allergen levels in the rat room. Their findings, in general, demonstrated that reducing the air changes increased allergen levels, while increasing the humidity from 54% to 77% caused a significant reduction in allergen levels.

In an unpublished paper presented at the Inhaled Particles Conference in September 1985, M. Corn, et al, reported the results of an air sampling study for rat derived allergens (11). This study evaluated particle size distributions in an animal facility, as well as total particulate. Urinary and salivary antigen was quantified by indirect ELISA (enzyme-linked immunosorbent assay), rather than RAST inhibition. Personal samples yielded airborne antigen concentrations about one order of magnitude higher than corresponding area samples, but since the extraction efficiencies of the antigenic proteins were unknown, the results were lower limit estimates of concentrations. Other interesting observations from the study were that 1) antigen to par-

ticulate weight-to-weight ratio appeared to be approximately 1/1,000 (possibly the lowest known mass ratio of active chemical/contaminant airborne particulate to carrier), 2) changes in antigen concentration levels and total particulate levels did not coincide, and 3) approximately 70% of the antigen by weight was associated with respirable particles. Their study did not isolate samples by specific tasks.

Recent literature has shown more industrial hygiene involvement in the problem of LAA: the potential value of a powered, air-purifying helmet type respirator to protect laboratory animal workers has been examined (39), and the first attempt has recently been made at a viable volumetric air sampler designed specifically for immunochemical aeroallergen quantitation (43).

METHODS

Description of Facilities Studied

The south campus facility of NIEHS at Research Triangle Park was the site of all air sampling except the lavage samples, which were collected at the north campus. The building consists of five modules, most of which consist of five stories including the basement, with interstitial space between all floors. Several completely separate ventilation systems provide all laboratories, surgical suites, and animal areas with about 15 air changes per hour of single-pass filtered air. The animal areas and the surgical suite rooms are in the basement of C and D Modules (see Figure 4) and are on ventilation systems separate from any laboratory or office areas. Animal breeding and holding room air is also preceded by a HEPA filter for the protection of the animals. Air diffusers are centrally located in the ceiling of animal rooms and most laboratories, while exhaust vents are located low on the walls. Animal area air is strictly regulated at 72 plus or minus 2 °C, and 50% plus or minus 5% RH.

Holding and breeding rooms each have two doors. One opens from a regulated clean corridor under positive pressure with respect to the room, where clean cages, equipment, and food for animal husbandry is obtained; the other door opens to a "dirty", or "return" corridor, under negative pressure with respect to the room, where soiled cages and trash are left for subsequent removal. The contents of soiled cages are dumped in hoppers in the cage wash area and loaded in the cage
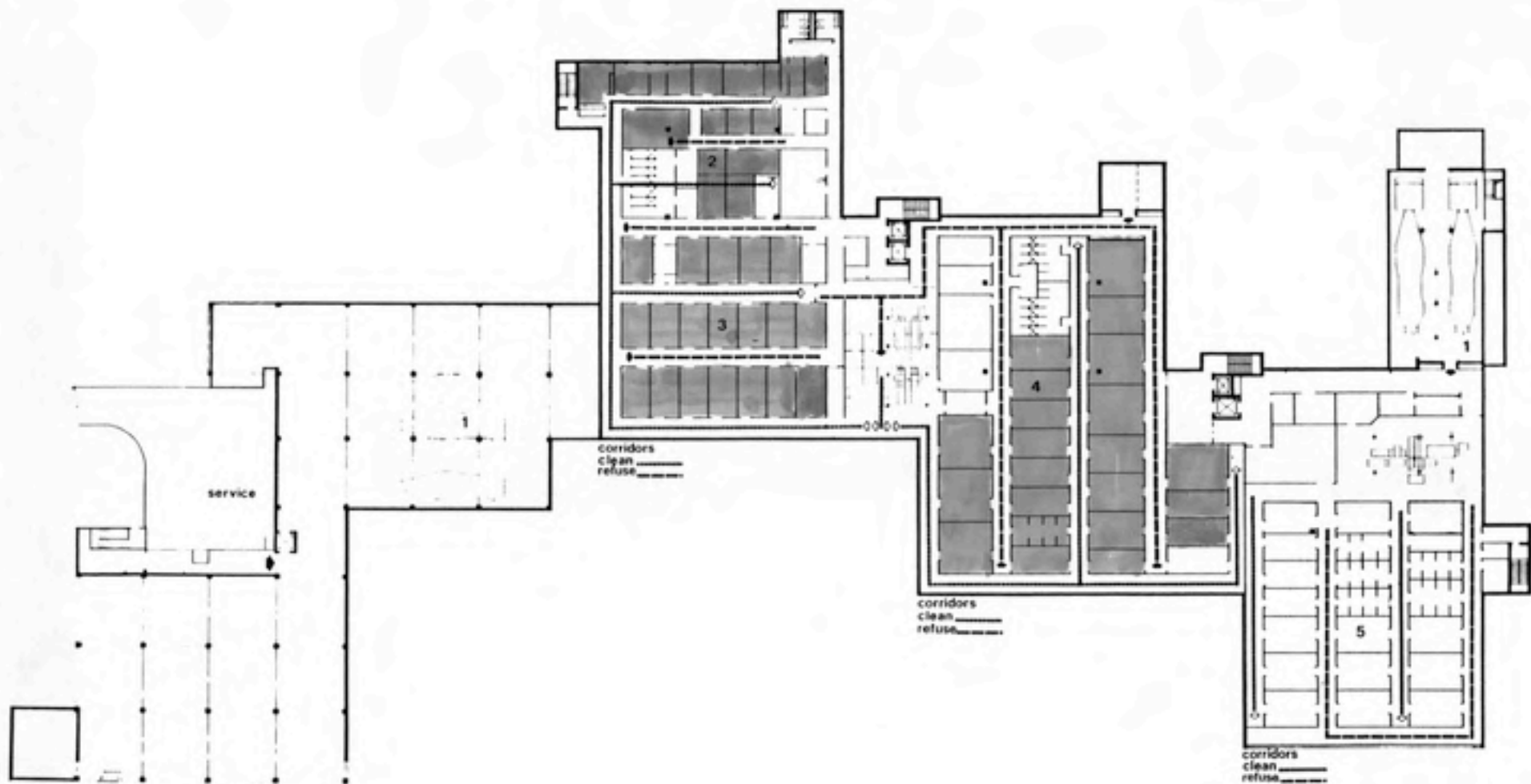
Basement Floor

Scale

FIGURE 4.

1 Mechanical
2 Surgery Suite
3 High Access Animal Holding Rooms
4 Limited Access Animal Holding Rooms
5 Quarantine Animal Holding Rooms

corridors
clean
refuse

service

washing machines. The clean cages emerge in the higher pressure clean side, are filled with bedding, and the cycle is repeated. Water bottles, racks, filter bonnets, and other equipment are circulated in the same fashion.

Large narrow racks on wheels line the walls and form rows in animal breeding and holding rooms. The racks have six shelves, but the top shelf is rarely used. Approximately 25 clear plastic cages are stored per rack, each with a HEPA filter bonnet, or cap. Food and water is available to the animals from a holder which fits between the cage and the filter. The rat population in a given holding room is usually about 200-250 animals, having 3 to 5 rats per cage. Breeding rooms generally have a much larger population. Bedding is changed every 3 or 4 days, along with food and water. Other species are bred or stored in the same facility, usually in rooms dedicated to one species.

Most experimentation with animals occurs in the surgical suite rooms, which are in the basement of the same modules. When animals are taken to other parts of the facility (i.e., user laboratories) they are not returned.

The bedding used is a carefully regulated mixture of beech, maple, and birch wood chips. From 85 to 90% by weight of the bedding particles are required to remain between the sieve sizes of 0.03 and 0.09 inches after a 5-minute shake. Both animal feed and bedding are autoclaved and pneumatically delivered from a warehouse to the clean side of the washroom for dispensing. The applicable TLV for airborne particulate in the clean side of the washroom is 1 mg/M^3 for hardwood

dust, while the TLV for nuisance dust (10 mg/M^3 total, 5 mg/M^3 respirable) would apply to all other areas.

Air Sampling/Analysis Methods

Due to the extremely low levels of particulate found in many areas where air samples were to be collected, a filter medium was needed which would provide gravimetric stability. Preliminary tests showed that accurate measurements were needed for as low as a 10 ug weight change or better. Glass fiber filters and mixed cellulose ester filters appeared to provide an accuracy of only plus or minus 100 ug at best, while polyvinyl chloride (PVC) filters provided an accuracy of up to plus or minus 1 ug. An extremely small pore size was desired, since antigenic material may be carried by particles of any size. The MSA (Mine Safety Appliances Company) type FWS-D polyvinyl chloride membrane filter (P/N 459733, 37 mm diameter) was used for this study owing to its small pore size (0.5 micron) and weight stability with changing ambient humidity conditions. These filters ranged in weight from about 12 to 17 mg.

A CAHN 26 electrobalance was used to weigh all samples. While set at the 50 mg range for all samples, the approximate lower limit of measurement for the balance was 1 ug. All filters were desiccated at least 24 hours prior to preweighing and postweighing. Triplicate gravimetric controls were weighed for all samples. In two cases, when the sample weight was less than the average of the three corresponding controls, the resulting weight and concentration is reported as zero. If one of the three controls appeared to be an out-lying datum, it was thrown out at the discretion of the investigator.

All air samples were collected with DuPont constant flow personal sample pumps (model # P2500) at 2 Lpm for total particulate samples and 1.7 Lpm for respirable samples. All filters were assembled with cellulose support pads in three-piece polystyrene filter holders (cassettes). Each respirable sample was collected through a MSA 10 cm nylon cyclone separator with a particle cut size of 10 um aerodynamic diameter. Pump calibrations were performed before and after each sampling period (unless they occurred on the same day) by the bubble buret method with an in-line filter, and an in-line cyclone for respirable particulate sampling. The pre- and post-sampling flowrates were averaged, and the volumes were corrected for temperature and pressure as follows:

$$V_C = [spl \ time(min) \ X \ ave \ flowrate(Lpm)] \ X$$

$$[P/P_S] \ X \ [T_S/T]$$

where
V_C = corrected volume
P = barometric pressure, day of sampling, mmHg (the Princo U.S. Signal Corps type barometer readings were corrected for temperature and latitude)
P_S = Standard pressure, 760 mmHg
T_S = Standard temperature, 298 °K
T = ambient temperature (usually 295 °K)

After postweighing, each filter (excluding most controls) were put in 50 ml centrifuge tubes, sealed with teflon tape, labeled, and stored at less than 0 °C. These samples would be analyzed by RAST inhibition assay at a later date. In order to limit the scope of this study all samples were to be RAST tested for two specific rat-derived antigens only: urinary protein and dander. Therefore, all air samples were

collected during work with rats. Rats used in this facility were almost exclusively "CD" or "F-344" rats.

Two "blanks", unweighed filters, were put directly into tubes and sealed. Three to five controls from various areas, which exhibited the typical slight weight change, were also sealed and frozen to examine the effects of contamination during weighing or diffusion contamination (the cassette plugs were removed during sampling time for most controls). Other controls were as follows: an outside air sample taken from the roof of the facility; an industrial hygiene laboratory air sample, where animals are never used, two floors above the animal areas and on a separate ventilation supply; and a mouse room air sample, to test for holding room cross contamination, as different species are usually housed in different rooms.

Sampling Strategy

Three basic types of samples were collected. Area samples were collected to gather information on ambient concentrations of contaminants in various areas, which has implications for casual, or indirect animal exposure, especially to persons in areas where concentration peaks in animal-derived air contamination are expected. Secondly, 8-hour time-weighted average concentrations were obtained for the most common and intense exposures, based upon observations and analysis of a wide diversity of animal-related work performed by employees at the NIEHS facility. Time-weighted averages were calculated assuming zero exposure for the remainder of the time for an 8-hour shift. Thirdly, specific work "tasks" were sampled. A task is a well-defined undertaking of usually short duration (less than a full 8-hour shift)

in which a peak exposure to airborne animal-derived allergen con-
centrations may be expected for a given employee. A task may be
something done routinely several times a day, very infrequently or
completely sporadically and irregularly; it may last only a few minutes
(e.g., guillotine 8 rats) or much longer than a full shift (e.g., a 19
hour neuroendocrinology experiment on live, active canulated rats).
Tasks, like TWA samples, were identified through observations and ana-
lysis of a wide range of animal-related work performed by the personnel
employed at this facility. Tasks for which particulate exposures were
determined (shown in Table 1) were chosen by considering a multiplicity
of factors, such as intensity of exposure, universality of exposure,
availability/practicality (very rare, brief, or sporadic tasks proved
too difficult to monitor), and nature or type of exposure.

All personal samples, whether "task" or "TWA", were collected in
duplicate, simultaneously collecting total and respirable particulate
on each monitored employee with both filters on the same lapel, the
side of the subject's favored dexterity. This system yielded
"matching" total/respirable samples, eliminating all confounding fac-
tors of exposure except particle size separation. Therefore,
"respirable percent" of all such samples was reportable. Most task
samples, due to the irregular or infrequent nature of the tasks, were
collected for multiple periods on different people; the filters were
the same and the exposure was cumulative. In such cases, the samples
were returned to freezer storage, along with their corresponding
controls, between periods of collection. An attempt was made to
achieve at least 5 hours of sampling time for all task samples.

Three controls were designated for each personal sample pair and
area sample. Where multiple samples were collected in one area on the

Table 1. Tasks for which particulate exposures were
determined, and a summary of their activities.

TASK	AREA(S) PERFORMED	ACTIVITIES INVOLVED
Cage unloading	washroom- clean side clean corridors	unload clean cages, bottles, racks, etc. stack cages, racks distribute cages, food and water clean up, hose down floor
Cage loading	washroom- return dirty corridors	dump and load soiled cages, etc. retrieve same from return corridors clean up, hose down floor
Animal care	animal holding rooms	change bedding, water, feed clean up, mop
Care/breeding	breeding rooms	change bedding, water, feed weaning, check plugs clean up, mop
Surgical implants	surgery suite	inject animals surgically insert/remove implant capsules clean up, mop
Kill, necropsy, tissue work	user laboratory	sacrifice, necropsy, tissue isolation and weighing
Live rat experimentation	user laboratory	bleeding canulated live rats
Weighing	holding room	weighing rats on electronic balance
Lavage	surgery suite	kill rats by injection canulate, hook up for lavage isolate lungs clean up
Metabolic cage experiments	holding room	gavage rats collect excrement from metabolic cage receptacles weigh animals, food

same day, only one set of three controls was used. For personal samples, controls were placed in the room most frequented by the employee.

Smoking was not allowed in any areas sampled other than the breakroom, however it was not enforced in the locker rooms or receiving until the time of the second sample in those areas.

Multiple samples were collected for most area samples and for a few task and TWA samples.

RESULTS/DISCUSSION

Air Sampling Results

Three logical groups relative to laboratory animal-derived antigen exposure emerged from the overall picture of air sampling: 1) animal husbandry-related exposures in animal areas, 2) animal surgery/manipulation- related exposures usually in adjacent rooms to the animal holding and breeding rooms, in the same modules and on the same floor as the animal areas, and 3) user activity centered in the investigator's own laboratory. (For data summaries, refer to Tables 2, 3, and 4.) Although work with metabolic cages was performed in husbandry rooms, it was usually performed by highly educated investigators and involved extensive manipulation of animals and their excrement; therefore it was grouped in the second category.

The highest particulate exposure by far existed in the clean side of the washroom. This was due to the aerosolization of autoclaved, fresh bedding as it was automatically dropped in a continuous curtain into washed cages passing underneath, and fresh feed which was dispensed into stainless steel containers from a height of several feet. The total dust time-weighted average for this area, 0.97 mg/M^3, was very near the threshold limit value of 1 mg/M^3 for hardwoods. The variability of some sample results in certain areas reflect the range of day to day activity, such as in the return side of the wash room. Other factors affecting the results included the number of animals, their activity level, the presence or absence of filter bonnets

Table 2. Particulate Concentrations of Area Samples
Among Laboratory Animal Workers

AREA	CONCENTRATION (ug/M^3) Mean	StDev	No. of Samples	INDIVIDUAL CONCENTRATIONS (ug/M^3)
Husbandry				
1 washroom- clean side	2354	378	2	2621, 2087
2 washroom- return side	78	56	2	117, 38
3 breakroom	70	41	2	99, 41
4 animal holding room	35	29	3	59, 42, 3
5 return corridor	18	5	2	21, 14
6 men's locker room	14	13	2	23, 4
7 clean side corridor	11	16	2	22, 0
8 receiving	10	1	2	11, 9
9 breeding room	8	7	3	12, 11, 0
Average of 4 & 9	21	24	6	
Surgery Suites				
1 lavage	55		1	55
2 metabolic cages	31	16	2	42, 19
3 surgical implanting	12	16	2	23, 0
All	28	21	5	
User Laboratories				
All	26	20	4	53, 29, 16, 6

Table 3. Particulate Exposure Among Laboratory
Animal Workers: TASK Samples

TASK	CONCENTRATION (ug/M^3) Total (Respirable)	% Respirable	No. of Samples	No. of Splg Periods	INDIVIDUAL CONCENTRATIONS (ug/M^3)
Cage unloading	3038 (174)	6	1(1)	5(5)	
Cage loading	276 (27)	10	1(1)	6(6)	
Animal care	272±83*(62)	23	2(1)	3,4(3)	331,213(62)
Care/breeding	181 (21)	12	1(1)	4(4)	
Kill, necropsy, tissue work	149 (74)	50	1(1)	1(1)	
Surgical implants	105 (44)	42	1(1)	2(2)	
Weighing rats	78 (4)	5	1(1)	2(2)	
Live rat experimentation	29 (29)	100	1(1)	1(1)	
Lavage	29 (23)	79	1(1)	3(3)	
Metabolic cage work	27 (2)	7	1(1)	4(4)	

*Mean ± StDev

over the cages, smoking in the area, and employee work practices. All
five TWAs measured were lower than their corresponding task samples, as
expected. The respirable percent of the samples ranged from 5 to 100%;
there was no clear pattern in these values.

Control of Antigen Exposure

Effective controls of human exposure to animal allergens in the
husbandry areas are commonly employed already; they exist for the pur-
pose of animal and human health protection. These controls include
filter caps for individual cages, the wearing of surgical masks by
employees, a reasonable room air exchange rate, frequent bedding
change, and personal protective equipment such as gloves, coveralls,
hair covers, and shoe covers. Tighter filter caps for aerosol reduc-
tion would amplify two of their inherent disadvantages, namely, reduced
gaseous exchange and extra labor (22). Increasing the ventilation
exchange rate would be uneconomical and upset an already delicate air
pressure balance system used in many facilities. Administrative
controls, such as worker rotation or transfer, is to a large degree the
problem precipitating this study, and consequently can in no way be
considered a proper control.

One important control method which is often overlooked may be
employee work practices. Rats belong to a group of animal species
which cause a high emission of particles and microorganisms (47). It
is evident that the concentration of aerosol depends partly on animal
density and activity patterns (44,47). When filter top cages are not
used the floor in the rooms with rats will be covered with a dense
layer of particles in the morning, due to their high level of nocturnal

activity. This is the time of lowest airborne concentrations. Where filter tops are not in use, the floor should be washed first before working in the room rather than washing the floor after working in the room, to avoid stirring up the dust. Cleaning and other work in the rodent rooms should be done in the early morning when the reduced aerosol concentrations constitute less threat to employee health. Any disturbance of animals will result in renewed aerosol emissions released to the room. If the worker is aware of this fact he will to some extent be able to control the emission by his quiet work and behavior towards the animals. This can apply to laboratory investigators as well.

The surgical masks which are routinely used in animal facilities are meant for animal protection rather than human protection, and cannot be considered a protective device as they are not approved for exposure even to dust and mist. Despite this fact, numerous allergy sufferers were observed using these masks for protection. Only twice were respirators approved for dust observed to be used, but even then they were worn in such a way as to void the approval. Allergy sufferers should as a last resort have respirators approved for dust, perhaps with high-efficiency filters, made available to them and should be trained in their limitations and proper use. Immunotherapy has also been an effective means of relief in some cases where source control is not feasible.

No conventional laminar flow or funnel systems attached to cages, filter racks, or ventilated cage racks successfully protect husbandry, housekeeping, or other animal workers from animal-derived aeroallergens as many animal manipulations must be done outside of the containment.

Only devices or systems which provide some form of physical or air barrier separating animals from personnel can constitute a viable engineering control. Unfortunately, this does not presently appear to be economically feasible when large numbers of animals are concerned. Also, ventilation is required for manipulation operations such as cage cleaning, sacrifice, and necropsy.

RECOMMENDATIONS FOR FUTURE STUDIES

RAST test results (in ng/M^3 of specific antigen) for samples collected during this study may vary widely and may not correspond closely with particulate results. The following system for assigning exposure levels for rat-exposed workers for this and other similar cohort studies is suggested.

Every task, TWA, and area sample will have an average concentration for each specific antigen tested (urine and pelt, in this case). The highest assay result of each specific antigen can be assigned a "severity index" of 1, a "full dose", and the others assigned proportionally. Each sample could then be described by a severity index for each specific allergen assay. For example, if four samples were taken, the results and severity indices may appear as follows:

SAMPLE	RAT DANDER/ SEVERITY	RAT URINE/ SEVERITY
Metabolic cage- task	900 ng/M^3 / 0.75	2700 ng/M^3 / 1.00
Metabolic cage- area	70 ng/M^3 / 0.06	270 ng/M^3 / 0.10
User- TWA	300 ng/M^3 / 0.25	490 ng/M^3 / 0.18
Surgical implanting- task	1200 ng/M^3 / 1.00	540 ng/M^3 / 0.20

As the prospective cohort study proceeds, the epidemiologist will want to periodically calculate the cumulative doses of the exposed group. The data from the questionnaire must elucidate certain information from the participants: what particular tasks with animals are performed, how many hours per day/week each task is performed, and the

symptomatology at the given point in time. For each antigen assayed and for each participant, the severity indices of the tasks they are involved in may be multiplied by their corresponding duration of exposure and then totaled. To derive a person's dose after, say, three years, their total severity-hours may be divided by the number of work hours in a year. The highest possible exposure then would be "3", meaning a 1,200 ng/M^3 exposure (for rat dander, in our example) full time for 3 years, or 3 severity-years. A lower index, such as 2.0 would indicate a 67% of maximum possible exposure averaged over three years (800 ng/M^3 for 3 years, full-time), or a real dose equivalent to 2 full years at 1,200 ng/M^3. A severity-year system can be easily translated back to real dose by multiplying the index by the "full dose" concentration. Then a table or graph may be set up showing the progress of disease in the sample populations by increments of time (Figure 5A), or by accumulated dose (Figure 5B).

Some confounding factors may be easily corrected for. Suppose that the severity index for an 8-hour TWA for holding room work is 0.50. It is known that in developing the 0.50 index, certain factors were true: 200 to 250 rats occupied the room on the average, the air exchange rate was 15 changes per hour, the room dimensions were about 10 feet by 20 feet, etc. If the individual whose exposure is being investigated worked in a room with 50% more rats, or where the ventilation was substandard, or in a crowded and small room, etc., the 0.50 index may be adjusted accordingly. Exactly how much to adjust is unknown and deserves further study. Also, tasks performed for which there is no severity index may be assigned the same or a similar index to one with similar factors surrounding the exposure, and for which an index is determined.

FIGURE 5A Change In Percent of Studied Populations With Disease Over Time
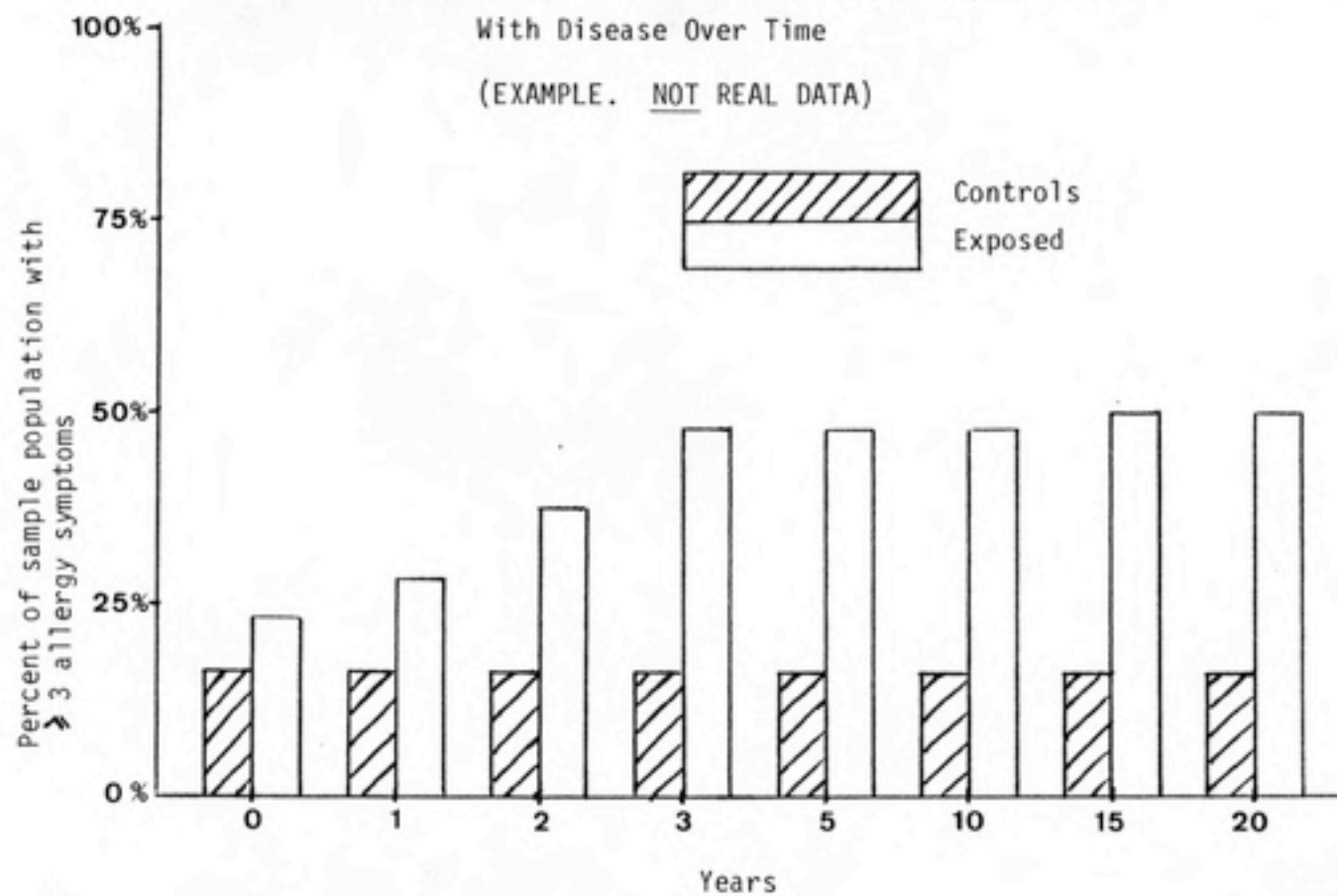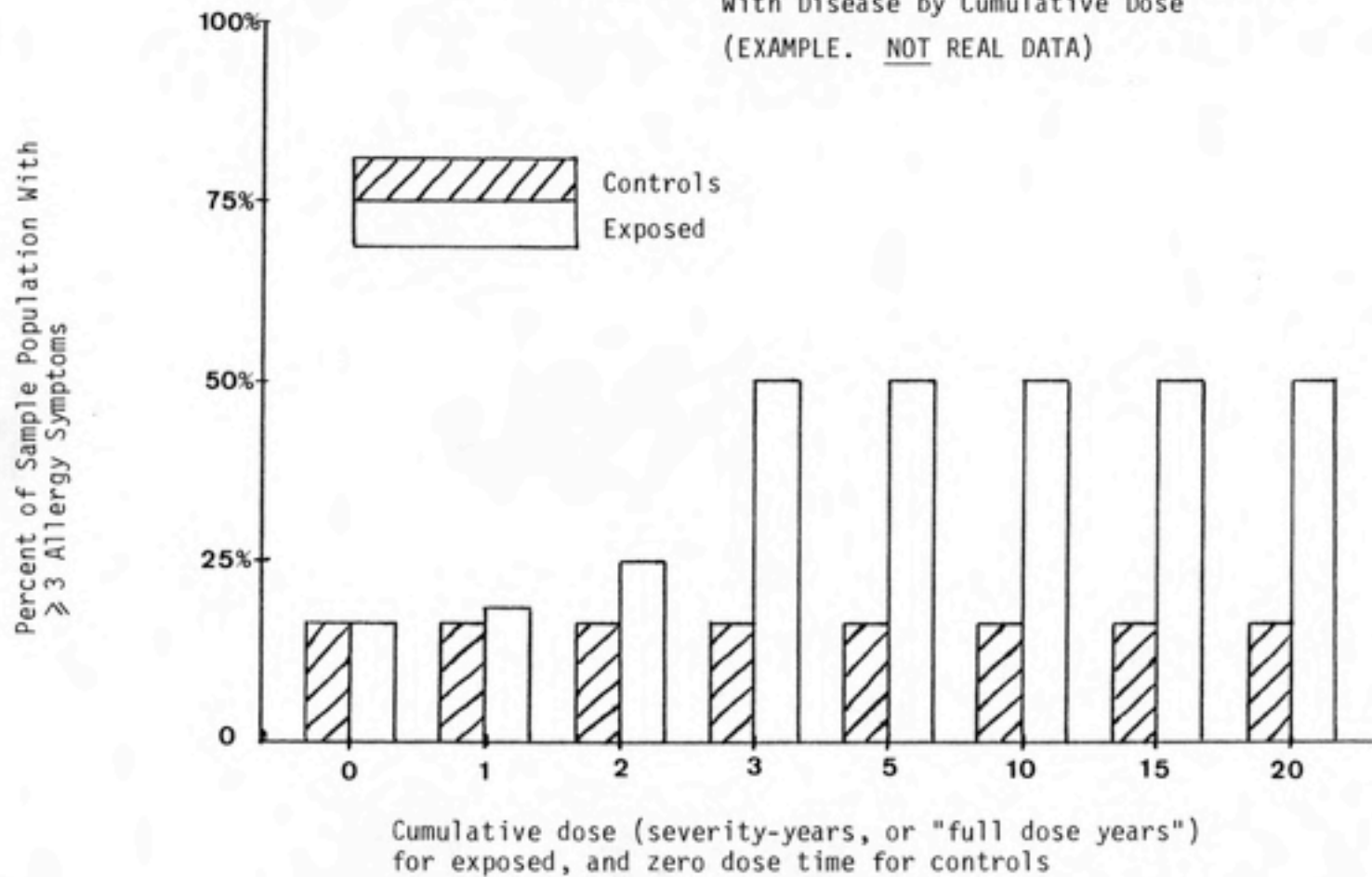
(EXAMPLE. NOT REAL DATA)

FIGURE 5B. Change in Percent of Studied Population With Disease by Cumulative Dose

(EXAMPLE. NOT REAL DATA)

Percent of Sample Population With ≥ 3 Allergy Symptoms

Controls

Exposed

Cumulative dose (severity-years, or "full dose years") for exposed, and zero dose time for controls

Many other confounding factors, such as age, sex, atopy, pre-existing pulmonary disease, smoking, socio-economic strata, etc., may be adjusted for by matching controls or by appropriately stratified analysis. Other important confounding factors may be controlled by selecting a control group of individuals who do similar work in the same facility, but not animal related. This may control for chemical exposures and employee questionnaire bias, and other more subtle factors.

Other interferences must be controlled clinically. Participants who are strongly allergic to non-animal derived allergens found in the atmosphere of their work area might be excluded from the study, since this could mask a progressive allergic reaction to rats. Participants should be tested by skin prick for some common allergens, such as house dust and ragweed as well as other allergens potentially found in laboratory animal-related work environments, such as birch, maple, and beech, the hardwood constituents of bedding at this facility.

To better evaluate the potential for physiological impairment of aerosols generated in laboratory animal work, several of the air samples should be examined by RAST test for house dust, ragweed allergen, the bedding hardwoods, and bacterial endotoxins. Endotoxins are pyrogenic lipopolysaccharide-protein complexes contained in cell walls of gram-negative bacteria, including non-infectious gram-negative bacteria, which are released only on bacterial lysis. Endotoxins have been shown to be airborne in significant concentrations among some poultry workers (9,23).

After accumulated doses are calculated and level of symptomatology of allergy or asthma is known from questionnaires and clinical findings, the incidence or progression of the disease may be evaluated.

CONCLUSIONS

Total and respirable particulate concentrations have now been determined for most common laboratory animal worker exposures using standard industrial hygiene air sampling techniques. These results show that the clean side of the cage and rack washing operation have by far the highest concentrations, due to the generation of dust from falling animal feed and bedding. Wide variability of results is seen in some areas. This may be explained by several factors, such as humidity, smoking in the area, volume of animals in the area and their activity, the degree of worker-animal contact and manipulation, and employee work practices. Improved employee work practices and proper personal protective equipment may be the most pragmatic means of allergy and asthma protection. The RAST test results of the air samples should prove invaluable for following the incidence and progression of laboratory animal allergy and asthma, and determining the relationship of dose to symptoms.

Further air sampling is required to narrow the confidence limits of concentrations corresponding to specific tasks and other samples, and to find particulate concentrations associated with animal species other than the rat.

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