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Mold-induced asthma

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Organic particulate components deposited on aerometric sampling devices include plant pollen, spores from 20,000 to 40,000 species of fungi, numerous actinomycetes, bacteria, mosses, and ferns. All are not equally important causes of allergic symptoms.

Throughout the years, pollen has been widely studied as an aeroallergen, but far less information is known about fungal spores, which are present in the air in concentrations considerably in excess of that of pollen grains. *Cladosporium* spores, for example, outnumber pollen grains by a ratio of approximately a thousand to one. It has long been established that respiratory allergic symptoms can be caused by the inhalation of airborne mold spores. The composition of airborne spore catches is derived mainly from species highly adapted toward wind dispersal. From spring to fall, spore concentrations may range well above 1000 per cubic foot of air. In a clinical sense, four major groups of fungi have become widely known as potential allergens. These include the classes Phycomycetes (subclasses Oomycetes, and Zygomycetes), Ascomycetes, Basidiomycetes, and Deuteromycetes (Fungi Imperfecti). This latter class contains the most recognized fungal aeroallergens that reproduce asexually, plus the asexual stages of a va-

riety of Ascomycetes and Basidiomycetes. Families from the Fungi Imperfecti class have been repeatedly demonstrated to induce respiratory allergic symptoms and sensitize patients, based on skin reactivity and inhalation-challenge testing. However, only a moderate amount of information is available on specific IgE antibody against these fungi. There is also a relative paucity of information on characterization of antigenic fractions of common fungal aeroallergens even through extracts of many species of the Fungi Imperfecti are used routinely in clinical allergy laboratories for skin testing and immunotherapy.¹⁻¹⁵ Large numbers of fungal species remain to be characterized, and most studies of fungi as aeroallergens have been limited to the Fungi Imperfecti, with little information available concerning the possible allergenicity of members of other fungal classes. For example, the Basidiomycete class, the most advanced of all the fungi, which numbers between 20,000 and 25,000 species and contains numerous subclasses, has only recently begun to be studied from the viewpoint of antigen characterization of a few select species.¹⁶ Aerometric sampling studies have, however, shown extremely high atmospheric concentrations of Basidiomycete spores in many areas of the world, including the United States, Canada, and the United Kingdom among others.¹⁷⁻²¹

Role of airborne fungal spores in production of respiratory allergy

The role of fungi in producing respiratory allergy and asthma has long been considered to be established but primarily on anecdotal evidence. In the 1870s,

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Abbreviations used

RAST:	Radioallergosorbent test
CIE:	Crossed immunoelectrophoresis
CRIE:	Crossed radioimmunoelectrophoresis
ELISA:	Enzyme-linked immunosorbent assay

Blackley²² produced "bronchial cattarh" and severe chest tightness after personally inhaling spores from penicillium cultures. Feinberg, Prince, and others²³⁻²⁵ also noted long ago that respiratory allergic reactions to fungi were quite common in their clinic patients. These investigators also pointed out that inhalant fungal allergy was not limited to the effect of mold growth and peculiar indoor home or occupational environments, but rather that ordinary ambient outside air contained huge numbers of fungal spores. These facts notwithstanding, the literature is still inadequate and controversial in many respects with regard to the relationship between inhaled fungal spores and the production of lower respiratory tract allergic disease. This is due to many factors, among which are the following: (1) The choice of fungal extracts for skin testing and provocative inhalation challenge has varied markedly among investigators. (2) The method of preparation of mold extracts as well as their standardization has varied markedly. (3) The quality and potency of mold allergenic extracts have often been poor and variable. (4) Relatively few organisms have been studied in detail, and many common fungi still await initial clinical evaluation and testing. (5) There have been considerable problems with particle viability and selectivity of media when identification of fungi has rested on cultural criteria. (6) The same extraction methods have often been used for fungal antigens as those generally employed for pollens, even though fungi contain primarily chitin and cellulose cell walls in comparison with pollen cell walls, which contain considerable extractable protein. (7) Outside of *Alternaria*, *Aspergillus*, and *Cladosporium* species of the Fungi Imperfecti, there has been a paucity of data on characterization of allergens from the vast number of other fungal species. (8) A single brief well-defined "mold season" usually does not occur, making selection of well defined study subjects with unique seasonal mold allergy difficult. (9) The extent of cross allergenicity among common airborne fungal spores remains to be resolved, and there is some evidence for considerable allergenic uniqueness of fungi as opposed to pollens.

Thus the true clinical impact of airborne fungi on respiratory allergic diseases, particularly on bronchial asthma, is still largely unknown, and the characterization, purification, and standardization of allergens

Fungus aerosols from vaporizers

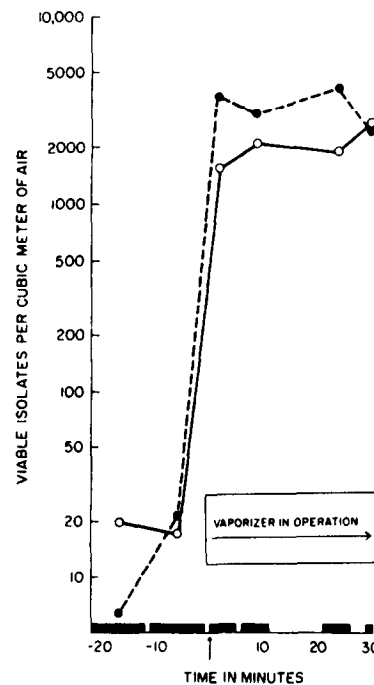


FIG. 1. Levels of fungus particles in bedroom air before and during operation of two vaporizers incriminated in exacerbations of asthma. Black bars along the abscissa indicate the sampling periods employed. (From Solomon WR: J ALLERGY CLIN IMMUNOL 54:222, 1974.)

from a wide range of fungal species will be necessary before this type of information can be obtained.

Biologic and immunochemical characteristics

Structure and distribution in nature. Fungi are distinguished from higher plants by the absence of chlorophyll and from animals by the presence of cell walls. They have true nuclei, as opposed to bacteria, and unlike those of pollen grains, their cell walls consist of chitin or cellulose. They have no stems, roots, or leaves, and they characteristically reproduce by both sexual and asexual means. They may function as either obligate or facultative parasites, but in either case they depend on other organisms for their nutrients because they are unable to synthesize their own food. In spite of their tremendous variation in structure, only two primary morphologic units exist: (1) the common fungi, which are composed of branching hyphae often divided by septa (aggregates of these hyphae are collectively referred to as mycelia) and (2) yeasts, representing a minority of species, which grow as single cells and reproduce by a budding process or by central fission.

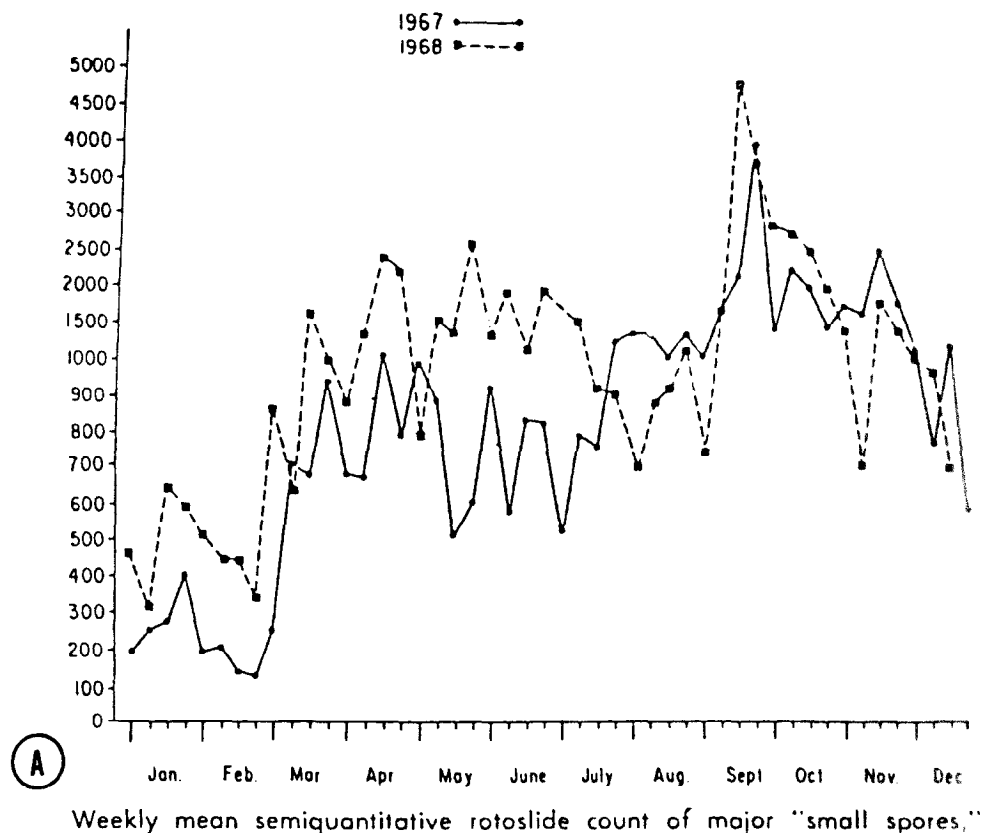


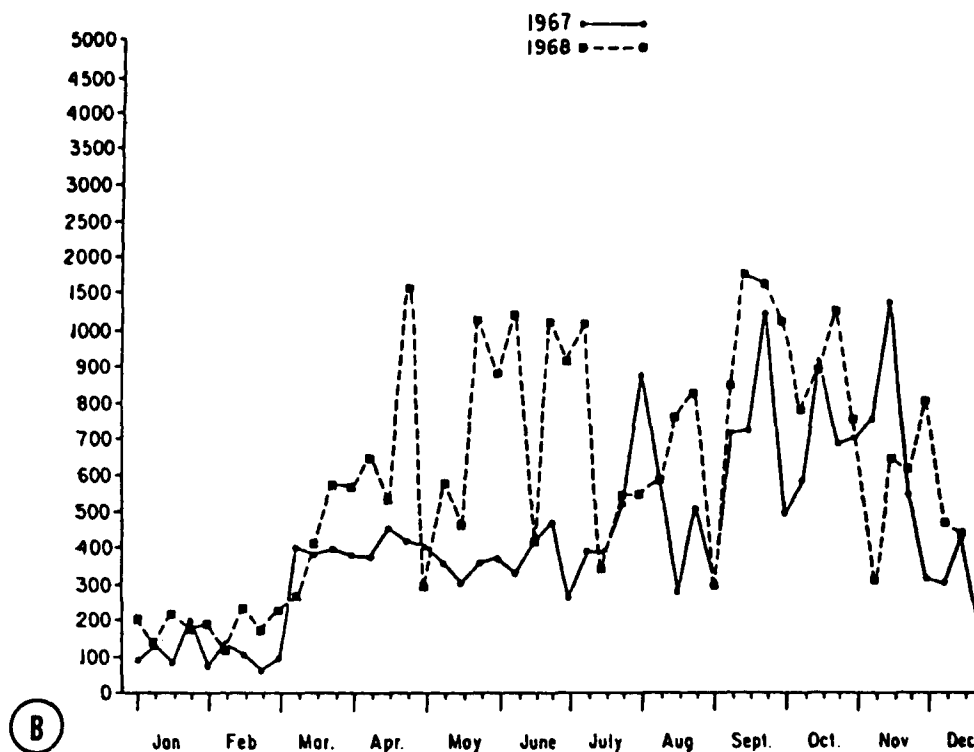
FIG. 2A. Weekly mean semiquantitative roto-slide count of major small spores (1 to 6 by 1 to 12 μ m), 1967 and 1968, city of New Orleans. These included globose, smooth or echinate spores, and ovoid-elliptic clear or greenish spores likely belonging to the genera *Aspergillus* and *Penicillium*. Some identifiable small zygomycete spores and many small basidiomycete-like spores with atypical spicules at one end were also included in this category on the basis of size.

Many fungal spores contain access to free air, but others do not. These spores are virtually always present over large nonarctic land masses and often comprise the greatest portion of suspended biologic materials. The particular species represented in the air at any given time, as well as their concentration, depend on many interacting variables, including temperature, degree of precipitation, prevailing winds, seasonal climatologic factors, circadian patterns of sunlight and darkness, availability of substrates, and degree of both substrate and atmospheric moisture.

For example, the vegetative hyphae of most fungi grow best between 18° and 32° C, whereas other thermotolerant fungi such as several of the *Aspergillus* species grow over a wide temperature range and certain thermophilic forms grow best between 65° and 75° C.²⁶ The dispersal of some spores such as basidiospores as well as their growth are also markedly affected by atmospheric moisture. These spores are propelled into the atmosphere by a process dependent on the presence of free water, and they are known to increase markedly in concentration during

periods of rainfall and dampness.^{16, 27, 28} On the other hand, spores of other fungi such as *Cladosporium*, *Alternaria*, and *Helminthosporium* are blown free by wind, and this type of "dry spore" increases in concentration with diminishing humidity and increasing airflow. Thus these species are often abundant during midday periods with maximal sunlight. On some occasions circadian rhythms in humidity and temperature interact to foster diurnal or nocturnal increases in spore concentrations in which certain basidiospores are increased in numbers.²⁷ Fungi also readily invade and propagate in the interior of homes, and spores from these sources are often a cause of perennial allergic symptoms. Local areas of mold growth in the interior of homes, particularly growth of certain Deuteromycete species such as *Penicillium* and *Aspergillus*, are commonly noted. Other organisms recovered from the interior of homes may have outside air as their source.

Many specific interior areas where growth conditions are particularly favorable are known to be prime sites for domestic mold growth. Among these are gar-



Weekly mean semiquantitative roto-slide count of spores resembling large basidiospores, 1967 and 1968.

FIG. 2B. Weekly mean semiquantitative roto-slide count of spores resembling large basidiospores, 1967 and 1968, city of New Orleans. These included characteristic large orange-brown glabrous, oval rust uridiospores (25 to 20 μ m) and septate teleutospores plus the more abundant smut spores. (Figs. 2A and 2B from Salvaggio J, Seabury J: *J ALLERGY CLIN IMMUNOL* 48:82, 1971.)

bage containers, food storage areas, upholstery, wallpaper, areas of increased moisture such as damp basements, shower curtains, window moldings, and portable window air-conditioning units. Bouts of asthma have also been reported on several occasions in individuals operating cold-mist vaporizers in which high levels of fungus particles are present in their emissions (Fig. 1).²⁹ Particularly high concentrations of yeasts are known to deposit in these devices when they have not been properly cleaned.

Seasonal patterns. Because of the universal presence of molds in the atmosphere and their marked adaptability to environmental conditions, a defined "season" for mold-sensitive patients often does not exist. These patients have perennial symptoms throughout the particular local growth season, often with exacerbation during the midsummer and fall months. Patterns vary slightly in different areas of the country. In the Gulf South area of the U.S., for example, much as in the Great Lakes area, many ascospores and spores of the Fungi Imperfecti appear during the initial warming periods in early spring.²¹ After this initial

peak they persist throughout the summer months, often with a second peak during the fall months, and diminish only slightly during the cooler months of December and January (Fig. 2). In areas that have cold winters, case histories with relief of symptoms after the first snowfall may suggest mold allergies as important causes of asthma. Basidiospores are also present throughout the year, with peaks during the humid, moist, late summer and early fall months. At times these spores appear as "showers" associated with certain climatologic variables.²⁸ Such increases in Basidiospore and other nondescript small spore counts have been associated with epidemics of bronchial asthma during the late summer and fall months,²⁸ although a firmly established cause and effect relationship has not been proved (Figs. 3 and 4). If these observations are ultimately confirmed, recognition of heavy mold spore exposure over a limited period can provide diagnostic clues to the well known increase in asthma attacks after the ragweed season.

Atmospheric sampling for fungal spore detection.

Sampling for airborne fungal spores is important for several reasons. It can allow one to establish airborne spore loads for different geographic areas and environments. It can indirectly aid in disease diagnosis, since spore prevalence and composition may suggest the type of fungal extracts that can be used for skin testing and/or provocative inhalation-challenge testing. Sampling may indirectly aid in the evaluation of the effectiveness of eliminating allergen sources, particularly with regard to indoor spore concentrations. The availability of semiquantitative data on spore counts may also allow correlation between airborne spore loads, climatologic variables, and sudden increases in rhinitis or asthma in particular geographic, occupational, or environmental settings. Such comparisons have been particularly useful in the study of certain forms of epidemic asthma, such as New Orleans asthma.^{28, 30, 31}

Many methods are available to identify or semiquantitate airborne spore loads. The simplest are conventional gravitational devices. In general, spores are measured least accurately by gravitational methods, such as the Durham sampler,³² which tend to obscure the prevalence of smaller particles. The Durham sampler produces crude qualitative data on the presence of spores and can show very gross seasonal trends, particularly for large spores. It also has other drawbacks such as the fact that it is markedly affected by weather conditions and precipitation. Simple gravitation onto Petri plates and related culture-plate techniques have also been widely employed in the past, with recognition of limitation caused by problems with spore viability and media selection. A variety of other devices are available for more accurate semiquantitation of spores, including rotating impactors (rotorod, rotobar, and roto-slide samplers) and suction impactors (Hirst spore trap and its modification, the Burkard sampler).^{26, 27, 33}

Rotating impactor samplers have the advantage of automation and semiquantitation. They combine simplicity and portability with a relatively good sampling efficiency of 50% to 90%. The large sampling volume leading to a rapid uptake of spores is another advantage. However, their efficiency also diminishes with smaller size spores, which may tend to be carried in air currents around the rotating rods because of insufficient mass.

Hirst spore traps and Burkard samplers of the suction impaction variety pull a certain volume of air through an orifice with subsequent impaction of spores on a slide. Again, these samplers are useful for large and small spores (down to 2 or 3 μm in size). Sampling errors can occur if wind speed is not equal

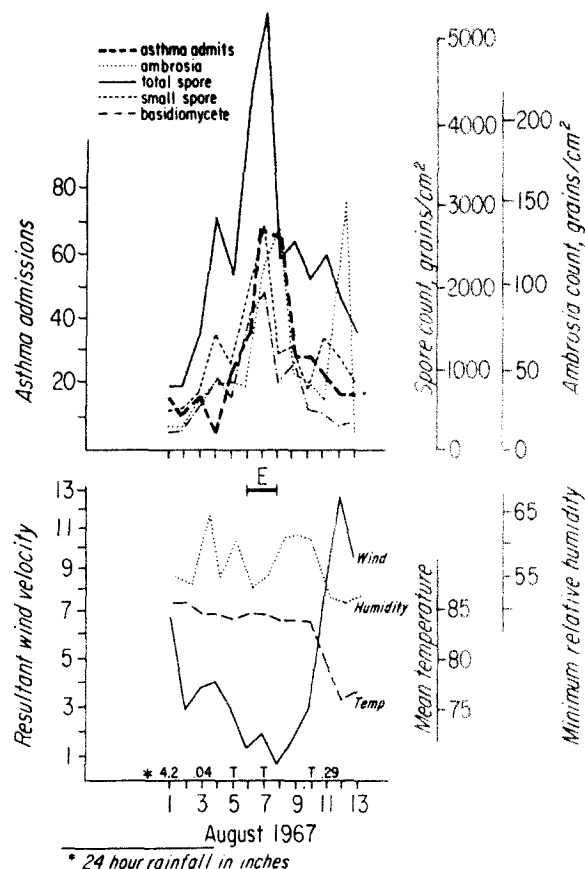


FIG. 3. Relationship between mean temperature, maximum relative humidity, overt precipitation, resultant wind velocity, airborne spore and pollen roto-slide counts, and typical "summer" asthma epidemic, city of New Orleans. Note threefold to fourfold increase in detectable spores during 3-day asthma epidemic, particularly small spores and spores representing large basidiospores.

to sampling speed. With particularly high wind velocities, smaller particles may be deflected, giving falsely low counts; the reverse holds true with extremely low wind velocities.

All of these methods where spores are deposited and become adhesive to a coated slide surface are limited by the pitfalls of identification, especially in quantitating small unicellular spores. Such spores often appear similar in structure and can be confused with one another or with other small inorganic particles. Other samplers, such as the Andersen sampler,^{34, 35} combine suction and impaction methods with a nutrient-agar Petri-dish culture method. In this sampler, a series of Petri dishes are placed on top of one another in close proximity. Air passes through a series of sieves with pores of decreasing diameter located between the plates so that larger spores are deposited on the first plate and smaller size spores on the

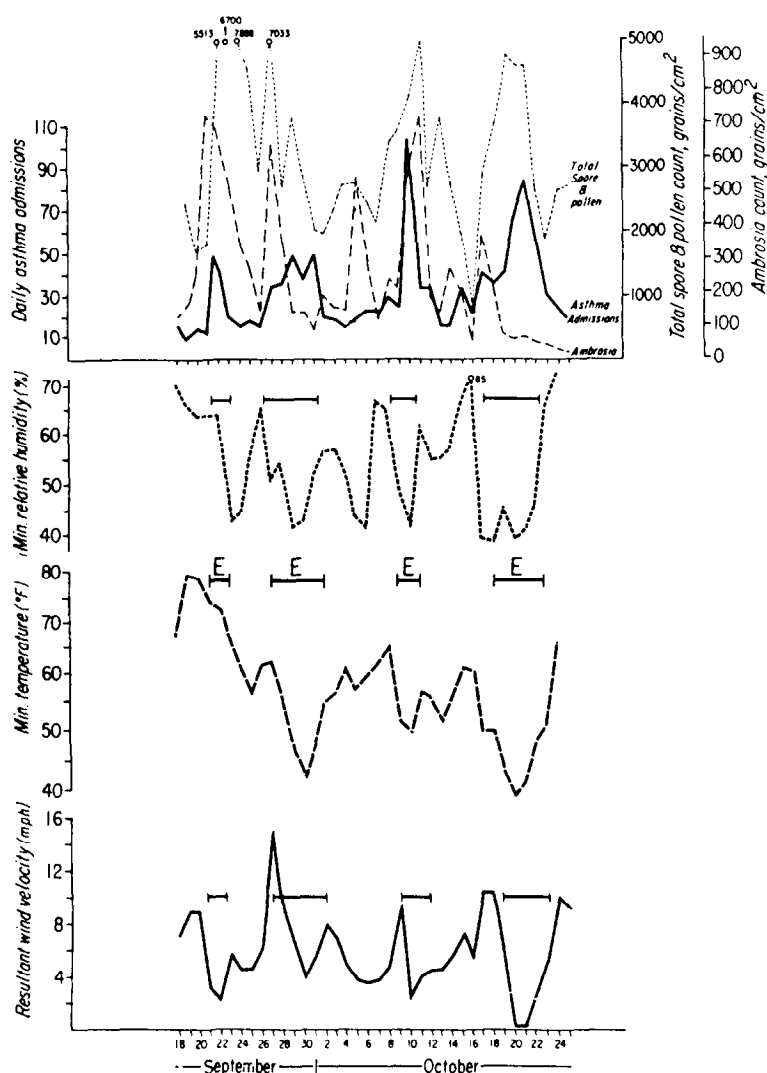


FIG. 4. Relationship between semiquantitative *Ambrosia* counts, total spore and pollen counts, climatologic variables, and major late September-October New Orleans asthma epidemics. Note large increase in total organic particulate counts, especially total spore counts. A decrease in minimum relative humidity and resultant wind velocity occurred with each epidemic. *Ambrosia* counts also increased during or at onset of three of the four epidemics. The largest epidemic involved approximately 200 asthma admissions over a 3-day period in late October and occurred immediately after the peak *Ambrosia* pollination season. A striking rise in total spore count and decrease in relative humidity, wind velocity, and temperature are also noted at this time. Barometric pressure also increased (not illustrated) during these outbreaks. (From Salvaggio J, Seabury J, Schoenhardt E: J ALLERGY CLIN IMMUNOL 48:96, 1967.)

other plates, depending on sieve size. Quantitatively, the Andersen sampler is subject to the same pitfalls noted with the Hirst spore trap and Burkard sampler when wind velocity is different from intake velocity. Andersen samplers must also be operated only for brief periods, such as in 15-sec bursts, so that plates will not become overgrown with colonies.

In essence, simple Durham gravity slide and related gravity culture plate techniques are perhaps the least desirable methods to employ, since they are only grossly quantitative at best and are subject to the

many previously stated limitations. Rotating impactor samplers are simple and portable, quite efficient, and afford a large sampling volume but may be inefficient for sampling smaller particles. Suction impactors, particularly the Burkard spore trap with its large revolving tape, are quite convenient for continuous sampling over long periods but have limitations similar to those of rotating impactors. Ideally one would probably want to combine a rotating impactor or spore trap device with some type of culture plate sampler, such as an Andersen sampler, to aid in specific iden-

TABLE I. Variability of 10 strains of *Cladosporium herbarum* as compared to a reference extract*

Strain no.	RAST (% uptake of ¹²⁵ I-anti-IgE)	Ag-32 (% of reference)	Ag-54 (% of reference)	Protein (% of dry weight)	Carbohydrate hexoses (% of dry weight)	No. of pre- cipitates in CIE
Reference	39.6	100	100	14.5	26	32
1	40.0	1000	65	8.8	85	16
2	39.5	100	50	9.0	83	22
3	35.8	100	50	3.9	42	12
4	34.8	30	60	5.2	36	23
5	33.2	120	30	3.2	54	22
6	32.6	5	45	3.4	68	24
7	32.3	100	40	4.0	36	30
8	27.0	30	30	3.1	42	24
9	21.2	30	1	2.2	89	8
10	7.6	—	—	1.2	74	5

*Note large differences in content of major antigens (32 and 54) as percent of a reference antigen, plus differences in RAST values and number of antigens demonstrable by crossed immunoelectrophoresis.

tification. For the interested reader, details of these and other sampling methods have been well described.²⁶

Classification and taxonomy

The Eumycetes or true fungi are members of the phylum Thallophyta, i.e., plants that lack definite roots, stems, or leaf structures. Some taxonomists however, place them in a kingdom distinct from both plants and animals, although the presence of cell walls reflects a closer association with plants. Unlike the algae, they do not contain chlorophyll. The major classes of the true fungi that predominantly contribute to the airborne spore load are discussed below.

(1) Class Phycomycetes. Of this class, the subclass Oomycetes is generally of little allergenic importance. A second subclass, the Zygomycetes, which generally includes the common sugar and bread molds and contains the important genera *Rhizopus* and *Mucor* of the order Mucorales, is clinically important. The Zygomycetes generally contain broad hyphae with few septae and stalked sporangia. They form thick-walled spinous zygospores after sexual mating of two specialized hyphal branches, and they are characterized asexually by the presence of the sporangia. Many species of this class are prominent in soil, composts, and food residues.

(2) Class Ascomycetes. This term is taken from the Greek word *Ascos* or "sack," and describes a cell in which sexual ascospores develop without any permanent wall attachments. Ascomycetes reproduce sexually by cytoplasmic fusion followed by ascus or sack formation, nuclear fusion, meiosis, and ascospore reproduction. They reproduce asexually forming conidia, either by budding, fragmentation, or

formation of special hyphae (conidiophores). The black molds, yeasts, blue molds, and cup fungi are important groups of this class. *Saccharomyces cerevisiae*, a yeast, and *Chaetomium sp* are species among this class known to produce allergic respiratory symptoms in man.

(3) Class Basidiomycetes. The name of this class is derived from the Greek word *basidion*, meaning a small base. These are the club fungi, so called because after mycelial growth, a fruiting body is formed and club-shaped structures called basidia develop. Basidiospores are produced on basidia. The Basidiomycetes reproduce asexually by means of conidia and fragmentation of hyphae. Sexual reproduction occurs by means of cytoplasmic fusion, formation of the fruiting bodies, development of basidia, and subsequent production of basidiospores at the top of the basidium. This class comprises many diverse forms, including puffballs, common rusts and smuts, mushrooms, and mirror yeasts. The great majority of Basidiomycetes, like most Ascomycetes, do not grow on common laboratory media. They receive their nutrition in nature by existing on decaying organic matter and wood, and as parasites of cereal grains, other plants, and trees.

Abundant small, spiny smut teliospores and rust urediospores are often quite prominent in the atmosphere and may be discharged in bursts during times of high humidity. Although little is known about the relationship between basidiospores and asthma at present, they have recently been shown to be allergenic in man and immunogenic in the rabbit.¹⁶ Since atopic asthmatics may demonstrate IgE-mediated hypersensitivity to Basidiomycete antigens, as evidenced by positive immediate wheal and flare skin

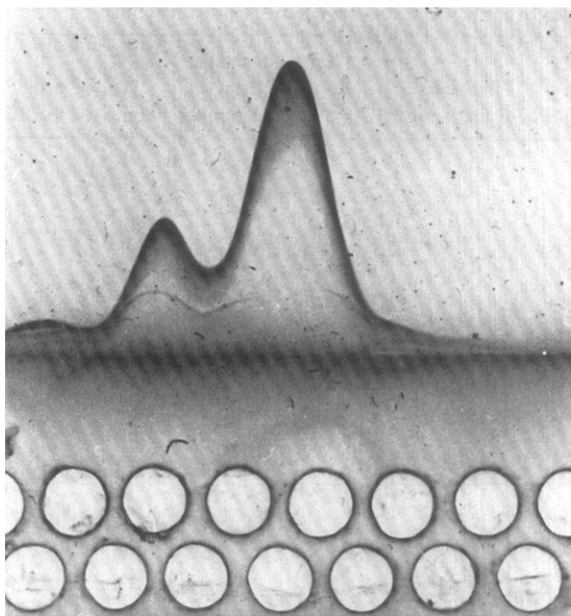


FIG. 5. Microheterogeneity of major individual allergens from *Alternaria alternata* evaluated by fused rocket immunoelectrophoresis. Molecular variants were demonstrated differing in isoelectric points from 4.30 to 4.65. The molecular variants were antigenically identical as demonstrated by the continuous precipitates.

reactivity and/or RAST, awareness of the potential importance of basidiospores as aeroallergens should become more apparent in the future, especially in view of their high atmospheric concentrations during the summer and fall months.¹⁷⁻²¹

(4) Class Deuteromycetes or Fungi Imperfecti. The large majority of fungi allergenic for man are in this class, which contains the asexual stages of a variety of Ascomycetes and Basidiomycetes as well as many other fungi without known perfect stages. In this class, sexual reproduction is extremely rare in nature. The means of common asexual reproduction is by conidia. Their nutrition is either by parasitic means on plants and occasionally animals, or by saprobic means on decaying organic matter. Four orders are commonly distinguished. These include the order Sphaeropsidales, of which the genus *Phoma* is a common allergenic mold; the order Melanoconiales, which contains important plant pathogens but no known human aeroallergens; the order Moniliales, which contains three families accounting for a large number of the fungi causing allergic respiratory disease in man; and the Mycelia Sterilia, a descriptive term for all mycelia that grow on culture plates without spore or fruiting-body production. Many other organisms, however, do not form spores on culture media in spite of excellent growth. These isolates are often derived from basidiospores or ascospores and

should be called Mycelia Sterilia only when the genera of that particular order are indicated.

The problem of variability. The single most important reason for the variable quality of commercial mold extracts is the innate tendency of fungi to vary. Whenever a suitable new ecologic niche is created in nature, fungi are quickly prepared to fill it. They accomplish this partly by existing in a very high number of species and an even higher number of strains. Thus different isolates of the same species will most likely

have different properties and characteristics. As an example, 14 different strains of *Alternaria alternata* and 29 different strains of *Aspergillus fumigatus* may be obtained from the American Type Culture Center (ATCC).³⁶ It has also been suggested that fungi have a high mutation rate. This may explain the observations that they often mutate after lyophilization and when repeatedly subcultured. In the case of *Cladosporium herbarum*, the variability among different strains is evident from Table I.^{9, 37, 38} Variability has also been documented between strains of *Alternaria*³⁹⁻⁴¹ but may be somewhat less pronounced in the case of *Aspergillus*.⁴²⁻⁴⁵ Although some strains are known to produce toxins and may be avoided in the preparation of allergenic extracts, knowledge of which strain is best suited for this purpose is in most cases nonexistent.

Growth factors, such as temperature, time, availability of oxygen, size of inoculum, and the composition of the medium, including pH, nitrogen source and carbon source, are probably important in determining allergen content.^{41, 46-49} Information is practically nonexistent on the ideal culture conditions needed to obtain adequate raw material with optimal allergen content. It may also be difficult or impossible to reproduce identical batches of raw material, even when experimental conditions are kept as constant as possible.⁵⁰ Based on the available literature, in which the variability of fungi has been studied, it is reasonable to assume the postulate, "Regarding molds, everything that can vary, will vary!"

A classic question in mold allergy is whether spores/conidia or mycelium provide the best source of allergens.⁵¹ At present, this question remains largely unanswered because it has been difficult to prepare pure spore preparations.

Furthermore, both mycelium and spore-containing material, as well as growth medium, are possible starting materials for extraction. Growth medium may contain considerable allergenic activity, but it may be difficult to prove that it is qualitatively representative of allergens in the mycelium/spore-containing mat.⁵¹ Because of enzymatic degradation, allergens in the

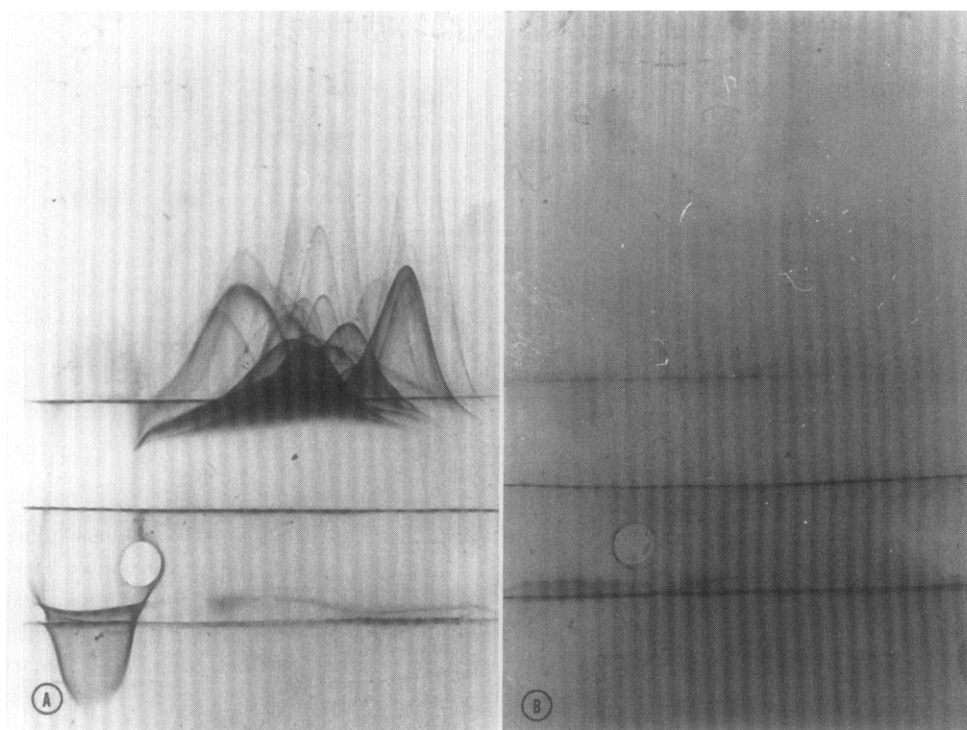


FIG. 6. CIE of crude extracts (10 μ l/l) of **(A)** *Cladosporium herbarum* and **(B)** *Alternaria alternata*, using identical amounts of an antiserum against *C. herbarum*. Note multiple precipitin arcs with *C. herbarum* antigen but only one weakly positive arc when *A. alternata* is electrophoresed into *C. herbarum* antiserum.

medium may be partly degraded and heterogeneous, thus complicating subsequent purification.

Complexity

One of the most notable properties of fungal antigens is their complexity. This is understandable, considering that fungi are whole organisms capable of reproduction as well as of growing under different and often continuously changing nutritional and environmental conditions. The antigenic complexity is well demonstrated in CIE, a two-dimensional electrophoretic technique described later in this text, which employs antiserum against an appropriate crude antigenic extract to obtain a high number of immunoprecipitates. In rabbits immunized with different *C. herbarum* extracts, approximately 60 precipitates have been obtained by CIE.^{8, 9} For comparison, a CIE-pattern of timothy pollen contains 30 precipitates.⁵² High numbers of precipitates have also been observed with other fungi such as *A. fumigatus*^{2, 3, 6, 44} and *A. alternata*.^{3, 53}

The number of distinct allergens present in the crude antigenic material is often high. Allergens may be defined as major, intermediate, and minor, depending on their IgE-binding in CRIE.⁵³ By the addition of the number of different allergens detected in two ex-

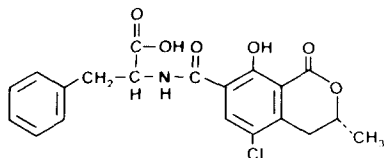
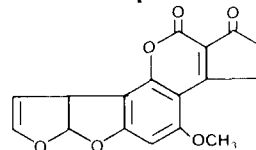
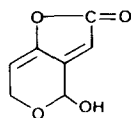
tracts of *C. herbarum*, this species seems to contain at least one major allergen, 10 intermediate allergens, and 25 minor allergens.³⁸

Furthermore, each allergen may also be heterogeneous. This is illustrated by the major allergen in *C. herbarum* (Ag-32), which was shown to exist in five molecular variants differing only in isoelectric point but being antigenically and allergenically identical.^{7, 54} Similar heterogeneity with respect to isoelectric point has also been demonstrated in an isolated allergen from *Alternaria* by Yunginger et al.¹⁵ and Thurston et al.⁴³

Aukrust et al.⁵³ have also recently isolated an allergen from *Alternaria* with similar molecular size and isoelectric points as the one described by Yunginger et al.¹⁵ This allergen gives a three-peak pattern in fused rocket immunoelectrophoresis after isoelectric focusing (Fig. 5), confirming microheterogeneity in *Alternaria* on the molecular level.¹⁵

Cross reactivity

Because of the great number of fungi relevant for the allergic patient, it is important to know the degree of allergenic cross reactivity among various species. In clinical practice, mold-sensitive patients often demonstrate broad patterns of positive skin reactivity

Ochratoxin A**Aflatoxin B₁****Patulin****FIG. 7.** Structures of important mycotoxins.

to fungi of many species. This has led to the assumption that these fungi cross react. A more likely explanation, however, is that mold-allergic individuals often display parallel and independent multiple sensitivity to different species. Furthermore, considering the antigenic and allergenic complexity of molds, the demonstration of some IgE cross reactivity between different strains of a particular species does not necessarily imply similar cross reactivity between members of different species.

Cross reactivity between different fungi may be studied in the IgG system with animal antisera by immunoprecipitating techniques, or directly in the human IgE system by RAST inhibition techniques. Both techniques are useful, but it should be emphasized that antigenic (IgG) and allergenic (IgE) determinants are not necessarily always the same. As an example of these differences, it is known that antigens that are nonidentical in the rabbit IgG system but identical in the human IgE system are present both in codfish^{55, 56} and hazel pollen.⁵⁷ Consequently, cross reactivity studies performed with animal antisera do not necessarily give a correct picture of the degree of cross reactivity in the human IgE system, although in most cases this difference is likely to be negligible.

One suitable immunoprecipitating technique for cross reactivity studies in the IgG system is CIE to assess reactivity of an extract of one fungal species with antiserum against another species. In the IgE system, RAST is an excellent technique. However, it may be difficult to prepare allergosorbents containing all allergens in adequate amounts, as well as to find representative patient serum pools. For thorough investigations on cross reactivity, both types of techniques should probably be employed. Even with the use of such techniques, it may be difficult to prove that an individual extract is fully representative of the clinically relevant allergens of a particular species.

Available data from these techniques suggest that the degree of cross reactivity is negligible between members of different genera. For example, using RAST inhibition it has been shown that some cross reactivity exists between *A. fumigatus*, *A. glaucus*, and *A. flavus*, but not between *Aspergillus* and a member of another genus (*Alternaria* sp).⁵⁸ Similar results with respect to *Aspergillus* were reported by Kim and Chaparas⁴² using immunoelectrophoretic techniques.

There is also little cross reactivity between *C. herbarum* and *A. alternata*. As shown in Fig. 6, a crude *Alternaria* extract gives only one weak precipitate in CIE with a potent antiserum against *C. herbarum*. In similar experiments no antigenic cross reactivity was found between *C. herbarum* and the two basidiomycetes, *Cantharellus cibarius* and *Coprinus comatus*, or between the two Basidiomycetes themselves.

Toxicity

Fungi are known for their ability to produce potent toxins.* Certain fungi have developed an ability, under special environmental conditions, to change their metabolism to the production of metabolites as an alternative to other regulatory mechanisms. That some of the products thus made are toxins may be arbitrary and not necessarily advantageous for the fungus.⁵⁹ Fortunately, most mycotoxins that are potentially important in allergenic extracts are of low molecular weight (<1000 daltons) and are easy to remove together with low molecular weight irritants by methods such as extensive dialysis.

Some mycotoxins such as aflatoxin, ochratoxin, and patulin are well characterized (Fig. 7) and sensitive tests exist for their detection.⁶⁰ It may be adequate to assay for these specific toxins when dealing

*Biochemical and organic compounds for research. Fungal toxins, April, 1980, Sigma Chemical Co.

with fungi that are known to potentially produce them. For example, *A. fumigatus* is known to produce aflatoxin.⁴⁶ Not all mycotoxins are characterized or likely even known, however, and it is mandatory that general toxicity tests be performed on all extracts. Fungi are also potential producers of carcinogens or mutagens. For a crude extract of fungal antigens to be used in man, documentation should be available indicating that the material is negative in a test for mutagenicity, the Ames test for example.^{61, 62} It may be of interest that we have experienced a positive Ames test on one particular batch of *A. fumigatus* raw material.⁴⁴

Clinical relevance of molds in allergic disease

Common fungal allergens. Although positive immediate and delayed skin reactivity is obtained with crude poorly characterized extracts of many fungal species, the clinical relevance of this reactivity is often uncertain because of marked variations in composition of these allergenic extracts and their frequent lack of standardization, characterization, and purity. Since most atopic individuals demonstrate broad patterns of wheal and flare skin reactivity to multiple pollen, fungus, animal dander, and environmental and related allergens simultaneously, the contribution of fungal allergy per se in any given patient is often uncertain. There is little doubt however that IgE-mediated respiratory allergic disease to fungi is very common in atopic individuals, especially to many species of the Fungi Imperfecti such as *Alternaria* and *Cladosporium* sp, which are thought to be responsible for both upper and lower respiratory tract symptoms that persist throughout lengthy growing seasons without particular well-defined peaks.

Other evidence attests to the fact that certain airborne fungi are known to be strong allergens in man. Such evidence is based on skin testing and recovery of certain fungal species from the atmosphere in large numbers. Among the commonly incriminated allergenic fungal species by these skin-test and aerometric sampling criteria are the following: (1) *Cladosporium* sp, which often reach airborne concentrations up to 5000 spores per cubic meter on semiquantitative sampling; (2) *Alternaria* sp, which at times provide up to 1000 spores per cubic meter of air and are known to be highly allergenic on the basis of wheal and flare skin reactivity; (3) *Helminthosporium* sp, which often give rates of wheal and flare skin reactivity equal to those elicited by *Alternaria*; (4) *Epicoccum* sp, which in certain surveys have been, along with *Cladosporium* and *Alternaria*, the most important source of spores isolated from the atmosphere²⁶;

(5) *Fusarium* sp, *Penicillium* sp, and *Geotrichum* sp; (6) several of the Zygomycetes such as *Rhizopus* and *Mucor*, spores of which are generally not prominent in ambient air but are often abundant in damp basement areas; (7) several members of the Ascomycetes, airborne concentrations of which often reach several thousand per cubic meter of air; (8) *Aspergillus* sp, which are often more abundant in the interior of homes than in free ambient air and which are the prime etiologic agents in allergic bronchopulmonary aspergillosis; and (9) many of the Basidiomycetes (smuts and rusts), which are being found with increasing prevalence in the atmosphere in worldwide studies. These spores often occur in "showers" during periods of high humidity and are conspicuous in extensive areas of cultivation or agricultural settings.

Gastrointestinal and other nonrespiratory symptoms. The role of fungal allergens in production of urticaria, eczema, gastrointestinal symptoms, or other nonrespiratory symptoms is highly speculative. Fungi are also used in the production of many foods, such as the various yeasts used in manufacture of vinegar products, beer, wine, and baked goods. *Aspergillus* species are employed in the manufacture of soy and steak sauces, and *Penicillium* species are used for fermentation and preparation of certain cheeses, such as Roquefort. Other stored and canned food supplies have been demonstrated to be contaminated with fungi. Nonetheless, there is no hard evidence that ingested fungi are important offending agents in production of allergic symptoms, except perhaps in the case of large amounts of ingested yeast-containing foods such as wine or cheese, which occasionally on an anecdotal basis have been known to precipitate sudden symptoms.

Rhinitis and asthma

RESPIRATORY TRACT PENETRATION. Fungi such as *Candida albicans* and other species have often been cultured from normal sputum. It has also been established, based on experimental inhalation studies with dry and aerosolized particles, that a small fraction of large pollen grains and smaller particles containing pollen antigen can penetrate into the respiratory tract.^{63, 64} Such studies have shown that pollen grains are normally present in the lower respiratory tract of humans in concentrations above 10 grains/ml of tracheal bronchial secretion. Thus it is safe to assume that if pollen grains ranging from 15 to 40 μ m in diameter are present in these locations, many more fungal spores that are generally smaller in size are also present in higher concentrations. It is possible that such organic particles or their products can directly interact with specifically sensitized mast cells known to be present in tracheobronchial secretions.

TABLE II. Results of skin prick tests with various mold extracts from different manufacturers (A, S, C, D, F) and of RAST with Phadebas material in 90 patients with suspected allergy to molds

	SPT (% positive)					RAST (%)
	A	S	C	D	F	
<i>Alternaria</i>	—	—	10% (8/80)	14% (11/80)	35% (14/40)	59
<i>Aspergillus</i>	—	—	10% (8/80)	—	42.5% (17/40)	62.5
<i>Cladosporium</i>	49% (39/80)	60% (24/40)	12.5% (10/80)	30% (24/80)	65% (26/40)	67.5
<i>Mucor</i>	—	—	1% (1/80)	9% (7/80)	27.5% (11/40)	40
<i>Penicillium</i>	—	—	2.5% (2/80)	9% (7/80)	32.5% (13/40)	56

— = not done; SPT = skin prick test.

*Note marked difference in degree of skin reactivity and RAST activity for different commercial extracts of each species employed. (Slightly modified from Aas et al: *Allergy* 35:443, 1980.)

RESPIRATORY SYMPTOMS. There is unanimity amongst clinicians that inhaled fungi are responsible for production of upper and lower respiratory tract symptoms. Yet there is a paucity of hard data on the actual incidence of asthma and rhinitis induced by inhaled fungal spores. Most efforts attempting to correlate various respiratory symptom patterns with specific fungal spore types have been inconclusive because of the lack of extensive inhalation challenge study data and the complexity of simultaneous and prolonged exposure to many species of different classes in nature. Thus clinical impressions are based primarily on patterns of skin reactivity to a wide range of fungal extracts that have usually been poorly characterized and standardized, plus anecdotal clinical evidence relating to the development of wheezing, cough, and rhinitis in areas where heavy atmospheric concentrations of fungal spores are known to be present. Even the type of respiratory symptoms produced by fungi are thought by some to be different, i.e., either delayed in onset or smoldering and chronic in nature, as opposed to the more acute symptoms induced by pollen. By means of challenge tests with inhaled fungal extracts, however, these peculiar symptoms after prolonged asymptomatic latent periods have generally not been produced. It is possible that such delayed chronic smoldering or infectious type symptoms might be caused by other constituents of fungi, such as toxins. Provocative inhalation challenge testing has, however, demonstrated that clear-cut "late" decreases in timed vital capacity can occur after immediate asthmatic reactions, particularly with inhalation of *Aspergillus* and *Candida* allergens.⁶⁵⁻⁶⁷ In such challenge studies, both the immediate and subsequent late reactions can be blocked with cromolyn sodium and adrenergic agonists, whereas only the late responses can be abrogated with corticosteroids.⁶⁷

INHALATION CHALLENGE TESTING WITH FUNGI. There is great variation in the relative frequency of

positive skin reactivity to fungal extract in patients with asthma and rhinitis in different countries of the world, ranging between 3% and 4% in studies from Sweden and Switzerland to above 80% in certain studies from the United States.⁶⁸⁻⁷¹ Some investigators have compared results of skin testing with clinical symptoms, and data of this type suggest that fungal hypersensitivity plays only a minor role in production of asthmatic symptoms, even though the frequency of positive skin reactivity, particularly with intradermal testing, is comparatively high.⁷² In contradistinction, however, other studies have shown a greater than 50% correlation between symptoms and skin reactivity.⁷³ There are many potential reasons for these varying results, including the general lack of standardization and characterization of extracts plus the fact that neither wheal and flare skin reactivity to fungal antigens or highly suggestive perennial symptoms alone provide definite proof that clinically relevant fungal allergy is present. In such cases, inhalation provocation testing, if properly performed with appropriate controls and nonirritant antigen concentrations, can be of great benefit. Although it provides little information about mechanisms, inhalation challenge testing can establish a definite cause-and-effect relationship and make possible more objective evaluation of the significance of positive skin reactions. However, there have been relatively few studies of provocation challenge tests with mold extracts, and the results have often been ambiguous because of the lack of standardized antigens and inhalation challenge techniques. In patients with allergic bronchopulmonary aspergillosis, Pepys and co-workers^{65, 67} have repeatedly produced positive dual immediate and late bronchospastic asthmatic responses on provocative inhalation challenge. These same investigators have also produced asthmatic reactions, often with systemic features of fever, malaise, leukocytosis, and eosinophilia, after inhalation provocative challenge testing of patients with

asthma who exhibited immediate wheal and flare reactivity and/or precipitating antibody to *Candida albicans*. Although these consisted mostly of immediate asthmatic reactions, some subjects demonstrated only late asthmatic responses. Similar results were obtained by Itkin and Dennis,⁶⁶ who noted a rough correlation between positive bronchial reactivity and skin reactivity to *Candida* antigens. The potential diagnostic value of provocation tests in asthma associated with suspected fungal hypersensitivity has also been emphasized in other areas of the world.⁷⁴⁻⁷⁶ In an extensive study of 601 patients with fungal hypersensitivity, many of whom underwent inhalation challenge testing, Ripe⁷⁷ showed a high incidence of bronchial reactivity. There was a significant increase in frequency of bronchial reactivity with both increased intensity of skin sensitivity and the number of extracts to which the patient reacted on skin testing.

Serologic studies and skin tests in diagnosis of fungal allergy

Skin reactivity. Wheal and flare skin reactions to crude allergenic extracts of fungi have long been documented to occur in asthmatic subjects, and skin testing with fungal allergens is routinely employed in most allergy clinics. Nonetheless, skin-test results continue to be highly variable because of the lack of characterization, purification, and standardization of fungal allergens.

In view of the crude nature of uncharacterized ground-extracted fungal growth, it is not surprising that the quality of fungal extracts commercially employed for skin testing and serologic diagnosis is variable and low. This is illustrated by recent results of Aas et al.,⁷⁸ who skin tested 80 patients with suspected mold allergy with various commercial extracts and found that the incidence of positive tests with *C. herbarum* varied from 12% to 65%, depending on the extract employed (Table II). It is also known that the same strains of an organism grown under identical culture conditions and for the same time period can produce extracts that vary markedly in potency.^{9, 45} Because of this fact, some have suggested that standardized pools of a wide variety of strains be employed for skin testing to ensure the presence of a sufficient number of determinants from a representative group of strains of a particular species.

RAST and ELISA. The RAST technique, although employed routinely to detect IgE antibodies against various pollens, danders, mites, and other inhalants, is still not used with great confidence to identify antifungal IgE antibody. The use of better characterized antigens has given higher RAST values. For example, studies by Yunginger et al.^{15, 40, 79} with crude *Alter-*

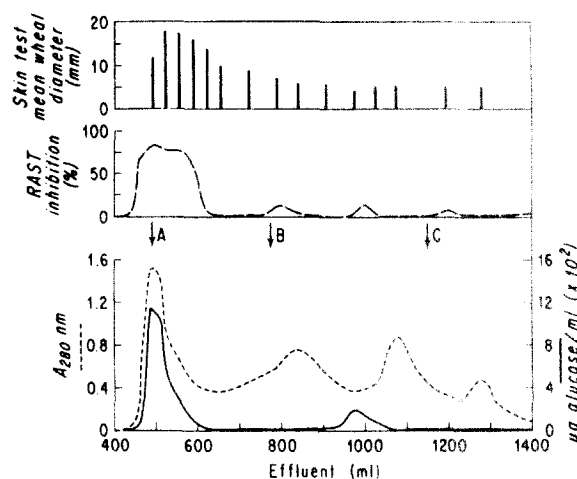
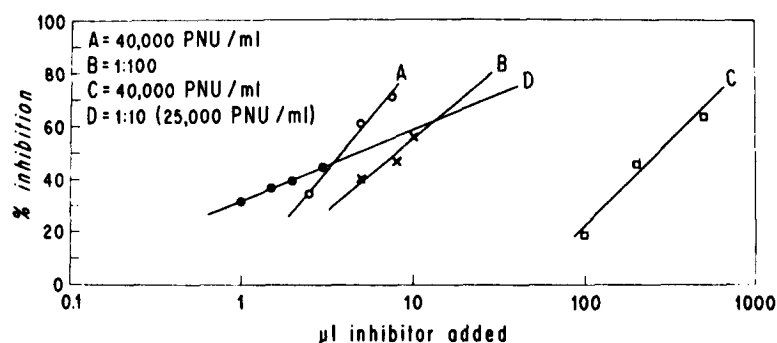


FIG. 8. Sephadex G-25 chromatogram of extract prepared from commercial dry *Alternaria* powder. Elution buffer was 0.05M Tris-HCl and 0.1M NaCl (pH 7.8). Elution volumes of the markers blue dextran (M.W. 1,000,000), bacitracin (M.W. 1411), and glycyl-L-tyrosine (M.W. 232) are indicated by arrows A, B, and C, respectively. Note that only those peaks appearing at the void volume produced strongly positive skin tests and significant activity in the RAST inhibition procedure. (From Yunginger J, Roberts G, Gleich G: J ALLERGY CLIN IMMUNOL 57:293, 1976.)

naria extracts have indicated that major allergenic fractions with molecular weight in excess of 5000 elute in the void volume of Sephadex G-25. Employing this protein- and carbohydrate-containing peak, these investigators were able to produce satisfactory and strongly positive RAST results with the sera of *Alternaria*-sensitive subjects. Potency of these *Alternaria* extracts, as measured both by RAST inhibition and direct RAST, was also strongly correlated to potency as measured by skin testing (Fig. 8). In contrast, however, neither weight per volume nor protein nitrogen content of crude extracts were related to allergenic potency as measured by skin testing or by RAST, and many crude commercial extracts of *Alternaria* were shown to differ by as much as 3000-fold in allergen content (Fig. 9).^{15, 40, 79} In a similar manner, Aukrust has shown that better allergosorbents can be obtained with a partially purified preparation of *C. herbarum* than with a crude extract.^{9, 80}

The overall results with these *Alternaria* and *Cladosporium* antigens show that RAST can be used to detect IgE antifungal antibody, particularly when partially characterized and purified allergen preparations are employed. These findings clearly illustrate the real need for isolation, characterization, and purification of individual allergenic components of fungal extracts for better serologic and skin-test diagnosis.

In the RAST procedure, the presence of solid phase-coupled allergens involves the activation of



Measurement of the potency of 4 commercial *Alternaria* extracts (A, B, C, D) by RAST inhibition.

FIG. 9. Measurement of the potency of four commercial *Alternaria* extracts (A to D) by RAST inhibition using a pool composed of serum from 10 *Alternaria*-sensitive subjects. Note a nearly 100-fold difference in allergen potency as measured by 50% inhibition points between extracts A and C, both of which were labeled as 40,000 PNU. (From Yunginger J, Roberts G, Gleich G: J ALLERGY CLIN IMMUNOL 57:293, 1976.)

polysaccharide polymers of cellulose discs by cyanogen bromide with the formation of a reactive amino group. Soluble protein allergens may then be covalently bound to the reactivated polymer through an amino group on the allergen. Although results of RAST with *Alternaria* and related fungal species suggest that these allergens contain free amino groups, many fungal allergens may also be polysaccharides or glycoproteins that cannot be readily coupled to cellulose discs. Indeed, some authors have been able to eliminate over 95% of extractable nitrogen from crude fungal extracts or to alter their protein content by other means, with no subsequent loss in skin test potency.⁸¹⁻⁸³ In view of this fact, other techniques such as the ELISA technique have been successfully employed for detecting IgE antibody as well as antibody of other immunoglobulin classes against fungal antigens of predominantly polysaccharide nature.^{84, 85} It is possible that such in vitro assays may ultimately prove to be simpler and of lower cost, since they use relatively unsophisticated equipment and nonradioiodinated reagents.

Many other techniques may be of future clinical relevance in the diagnosis of mold allergy. For example, the polystyrene tube technique, as employed for detection of immunoglobulin class-specific antibody against *A. fumigatus*, has proved to be a particular value in bronchopulmonary allergic aspergillosis. This technique allows quantitation of anti-*Aspergillus* antibody of specific immunoglobulin classes and establishment of correlations with clinical parameters.⁸⁶

CIE and CRIE. Until recently, manufacturers of commercial fungal extracts have done little to limit their batch-to-batch variation and to make each extract as representative as possible for the species in

question. Today, however, the combination of modern biochemical and immunologic techniques such as RAST and RAST inhibition are excellent in vitro methods for standardization and comparing "allergenic" content of fungal allergens without regard to purity. Another useful technique is CRIE,⁸⁷ which allows identification of individual allergens in crude extracts. A detailed description of CRIE has recently been given by Weeke and Lowenstein.⁸⁷

Initially, standard two-dimensional CIE is performed by migrating crude fungal antigen horizontally in 1% agarose gel containing Tris buffer at pH 8.6, followed by vertical migration in gel sections containing the appropriate antifungal serum. After performance of CIE, a tracing of the precipitate pattern can be made and arbitrary numbers are assigned to the antigen-antibody precipitate arcs or cones (Fig. 10). The antigens can later be referred to by their numbers in the reference pattern. Immunoplates are incubated with patient serum, and the specific IgE antibodies bind to the allergen-containing precipitates. These precipitates are visualized by a second incubation with ¹²⁵I-anti-IgE and subsequent autoradiography on X-ray films. A semiquantification of specific IgE binding may be obtained with a CRIE reference system.⁸⁸ According to this system, CRIE classes are assigned to the precipitates, depending on the degree of radiostaining obtained. Examples of CRIE films are shown in Fig. 11. When CRIE is performed with sera from "mold-sensitive" atopic patients, results may be summarized as in Fig. 12. From the patterns of IgE binding in CRIE it is possible to draw conclusions regarding the importance of the individual allergens in the extract as contrasted with the many nonallergenic antigens. By means of such CRIE analysis one can gain information on the

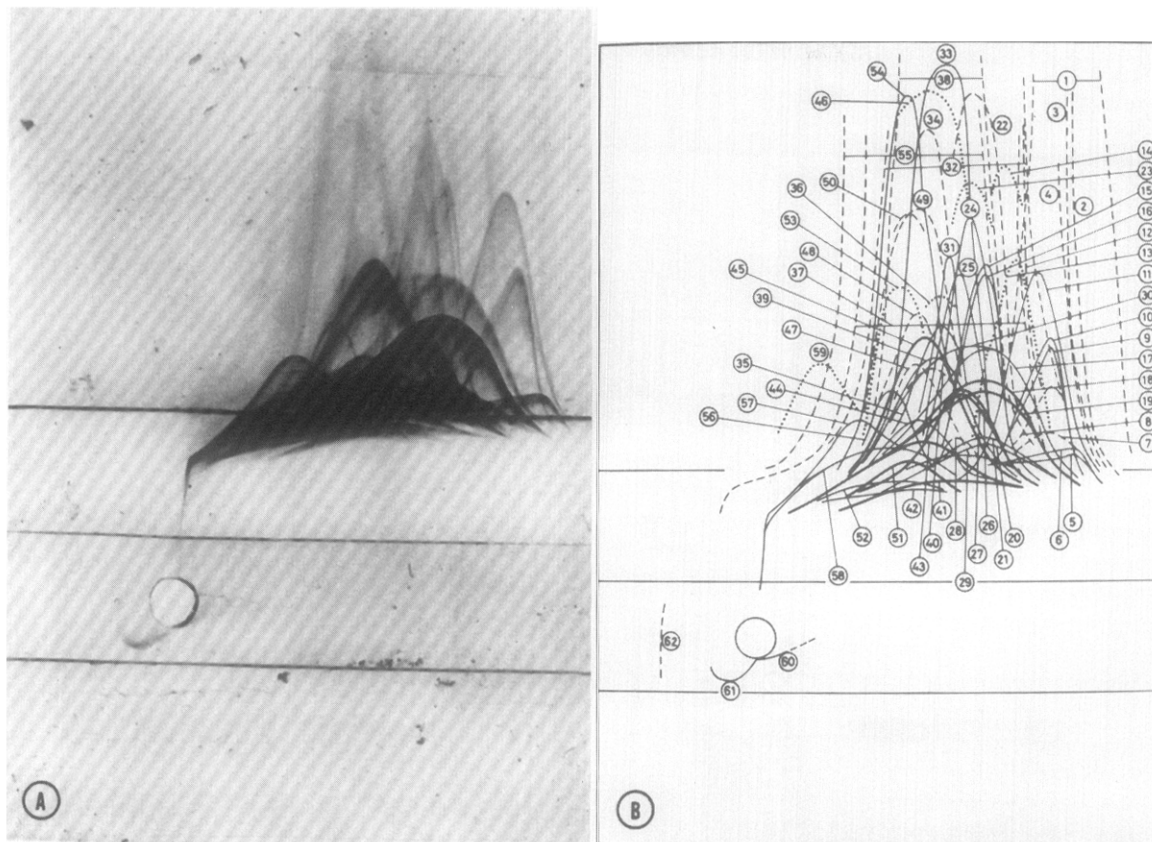


FIG. 10. CIE of a reference extract of *Cladosporium herbarum*. **A**, Photograph; **B**, drawing. Sixty-two antigens, some illustrating partial identity, can be identified in this single extract. (From Aukrust L: Int Arch Allergy Appl Immunol, S. Karger AG, Basel 58:371, 1979.)

frequency of patients having specific IgE against a particular fungal allergen and, in a semiquantitative way, on the relative amounts of specific IgE against individual allergens. Precise definitions of major, minor, and intermediate allergens have been based on IgE binding in CRIE.^{88, 89} Through the use of CRIE, tracer systems can be developed to allow the monitoring of each important fungal allergen during purification and to quantitate them when comparing different extracts and fractions.^{7, 38, 87-89} This facilitates both the comparison of different raw materials in a meaningful manner and the controlled purification of extracts without loss of important allergens. Further improvement of fungal extracts can be obtained if they are lyophilized for maximum stability prior to reconstitution. For commercial use, stability of the extract when reconstituted should be stated and documented.

Likewise, the potency of fungal extracts for diagnostic serologic and skin-test use should be stated in a meaningful way, abandoning terms like "protein nitrogen units." Biologic standardization procedures based on skin testing have been developed to find suitable and approximately identical levels of potency

for each extract.^{90, 91} The potency of different batches of the same extract may also be adjusted to a constant level by means of RAST inhibition.⁹⁰⁻⁹² It is possible that through isolation of individual allergens, mono-specific antisera could be produced to be ultimately used for quantitation of individual fungal allergens by simpler immunodiffusion techniques.

In spite of development of these promising *in vitro* procedures, our present state of knowledge suggests that the simple skin test, if properly performed and with properly standardized fungal allergens, remains the best procedure for routine office clinical diagnosis. In view of the paucity of information concerning characterization of fungal allergens, their current lack of standardization, and the marked variation in potency of different commercial extracts, there remains an urgent need to develop good fungal antigens for both *in vivo* and *in vitro* diagnostic testing and therapy.

Therapeutic considerations

Because of the ubiquitous occurrence of fungal spores in both indoor and outdoor environments, it is obvious that their elimination is a virtually impossible

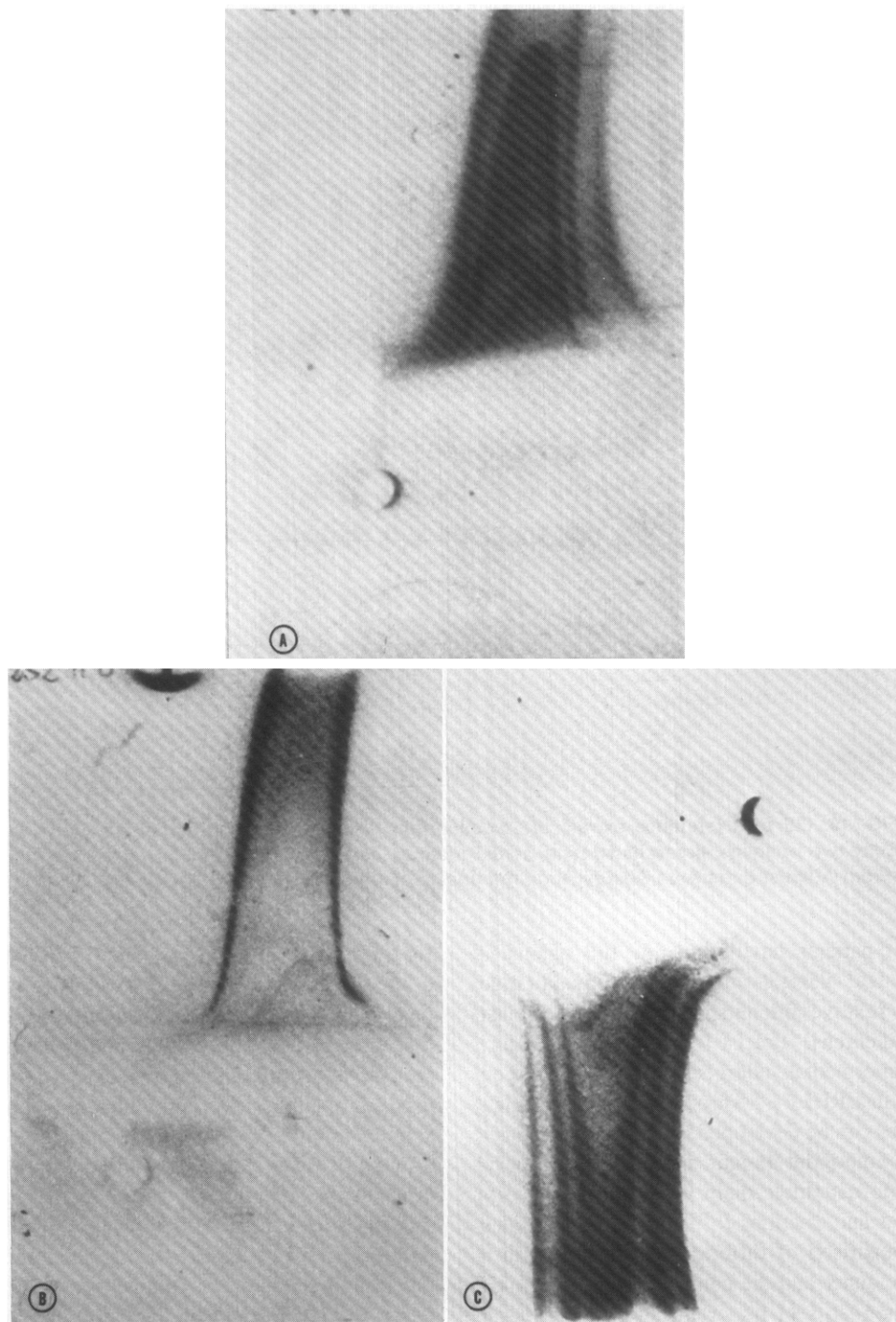


FIG. 11. Examples of CRIE photographic films from experiments with sera of three patients, using CIE plates of *Cladosporium herbarum* reference extract employed in Fig. 10 plates. Note presence of IgE antibody against only a few of the 62 demonstrable antigens shown in Fig. 10.

task, except in polar or other snow-covered regions of the earth. Exposure can, however, be lessened or minimized in certain industrial, environmental, occupational, or peculiar geographic situations where particularly high concentrations of fungal spores or yeasts are present.

Pharmacotherapy for mold-related asthma and rhinitis is the same as that for both intrinsic asthma and extrinsic asthma caused by other inhalant allergens such as pollens. These include the use of conventional bronchodilators such as β_2 stimulants and theophylline derivatives, the prophylactic use of cromo-

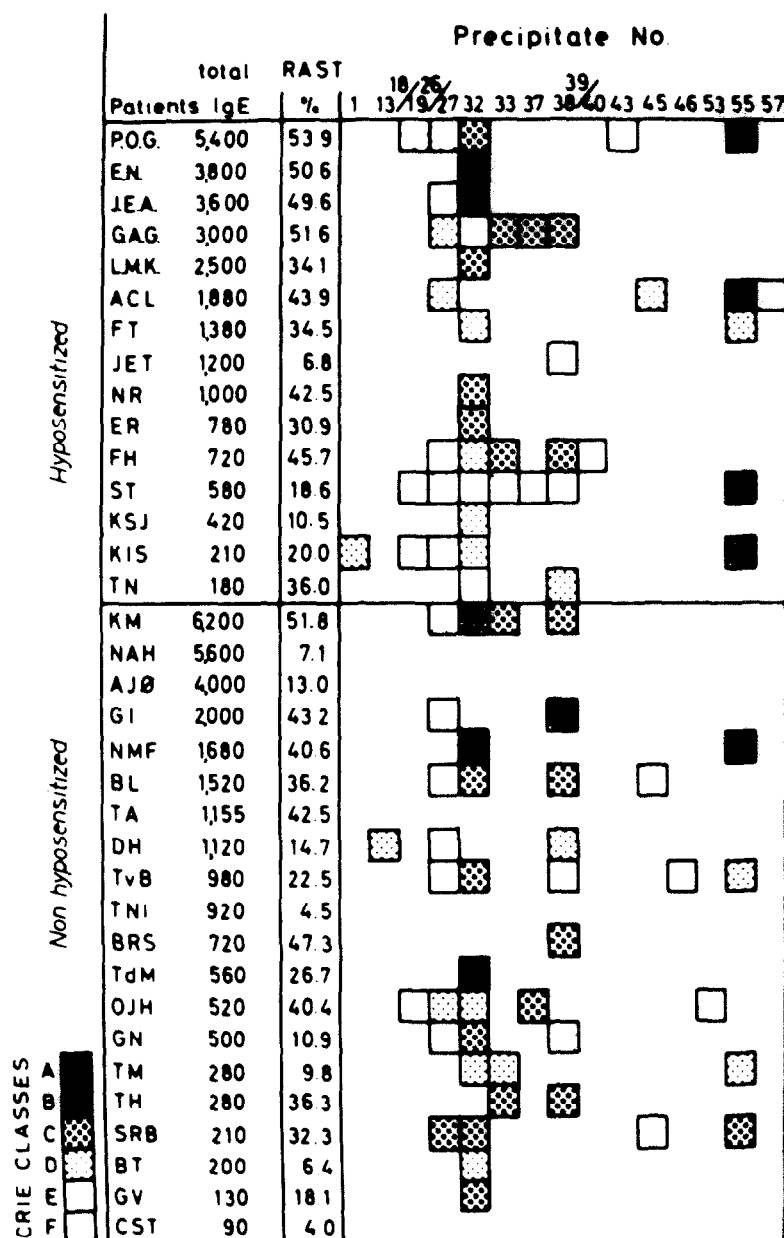


FIG. 12. CRIE results from experiments with 35 patient sera, showing degree of radiostaining corresponding to the various precipitates. Intensity of radiostaining is shown as shaded squares symbolizing CRIE classes from A (dense) to F (faint). (From Aukrust L: *Int Arch Allergy Appl Immunol*, S. Karger AG, Basel 58:371, 1979.)

lyn sodium where indicated, and if necessary, corticosteroids applied topically to the respiratory mucous membranes or used systemically.

Although the efficacy of immunotherapy for pollen-induced rhinitis has been clearly established,⁹³⁻⁹⁵ the role of immunotherapy in mold-sensitive asthma is highly controversial and there is a virtual absence of published data to permit firm conclusions in this regard.⁹⁶

Indeed, there are few if any studies showing that immunotherapy for extrinsic asthma per se is effi-

cacious with any allergen. The question of immunotherapy for inhalant fungal hypersensitivity in general clearly requires study, since it is likely that fungal spores account for a major cause of perennial rhinitis and extrinsic bronchial asthma in certain areas of the world. It will be extremely difficult to carry out controlled double-blind studies on mold-sensitive asthma for many reasons, not the least of which is the fact that there is little agreement on the definition of asthma itself.

There are several major problems in considering

studies of immunotherapy with mold extracts. Their variable and low quality makes it difficult to pinpoint the particular species that are clinically important. In addition, an extract of a particular fungal species will likely not contain all important individual allergens in adequate amounts. Considering the apparent low degree of cross reactivity among different fungi and the high frequency of multiple concomitant mold allergies, it is thus probable that immunotherapy with currently available fungal extracts will not include a sufficiently broad spectrum of appropriate allergens. The complexity of fungal extracts will likely further compound these problems. As compared to immunotherapy with other allergenic extracts, that with fungal extracts is considerably more complex. Thus the number of different antigenic determinants that the patient's immune system has to handle simultaneously is extraordinarily high. This may lead to a lesser immune response than would have been obtained if a single allergenic fraction had been injected. This problem may be increased because fungi seem to contain considerable high molecular weight materials (above 100,000 daltons), which are nonallergenic. High molecular weight components are known to be particularly good antigens in the IgG system. Besides being the most probable candidates for undesirable immune complex formation, these antigens may reduce the desired immune response to more low molecular weight allergens. Although these considerations are speculative, they are in accordance with the general concept of antigenic competition.⁹⁷ Support for this theory has also been presented in immunotherapy studies with pollen extracts, where better effects were obtained with refined preparations than with crude extracts.^{98, 99}

In spite of these limitations, clinical and anecdotal evidence does attest to the fact that many patients with broad patterns of wheal and flare skin reactivity to crude fungal extracts and bronchial asthma improve after empirical immunotherapy with grossly crude fungal allergenic extracts. The practice of medicine will, to be sure, remain primarily an art rather than a science, and physicians will of necessity continue to use clinical judgment and weigh benefit/risk ratios in prescribing a large number of therapeutic procedures that have not been proved to be efficacious by controlled studies. Indeed, one could fill several pages with a list of commonly employed therapeutic procedures in all fields of medicine that have not been proved to be efficacious. Undoubtedly, the busy practitioner, when faced with mold-sensitive patients who often constitute a large number of his clinic population, will continue to use immunotherapy with fungal allergens as a modality of therapy, and today's

allergist, who is well versed in the concepts of modern clinical immunology, mediator release, and pulmonary function, will continue to care for mold-sensitive asthmatic patients in this fashion. These facts notwithstanding, it must again be emphasized that appropriately controlled immunotherapy studies with mold extracts have simply not been carried out. The characterization and standardization of many species of fungal allergens will be necessary prerequisites to the undertaking of such clinical trials, and it is incumbent on those of us who use these procedures to carry out the necessary antigen characterization studies and controlled therapeutic trials.

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