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History of Medical Mycology in the United States

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INTRODUCTION

Medical mycology is concerned with the study of medically important fungi and fungal diseases in humans and lower animals. Fungal infections occur throughout the world, but some of them are more predominant or endemic in certain geographic areas. Although fungal diseases are not considered to be as common as bacterial and viral diseases, they are frequently associated with severe morbidity and mortality. Among the superficial fungal infections, tinea pedis (athlete's foot) is one of the world's most common diseases. During the last 15 years, the incidence of fungal infections has increased as a result of the AIDS pandemic and the rapidly expanding number of patients with chemically induced immunosuppression, transplants, and cancer. As a result of improved management protocols, these patients are able to live longer, thereby becoming highly susceptible to life-threatening opportunistic fungal infections. Since health care professionals are not required to report mycotic diseases, their true incidence and prevalence are unknown.

Even though the history of medical mycology involving humans began between 1837 and 1841, when Gruby and Remak discovered the first human mycosis (tinea favosa), it was the Italian lawyer and farmer Agostino Bassi who in 1835 discovered the mycotic nature of an epidemic disease of silkworms called muscardine (1, 9, 117). He was the first individual who demonstrated that a microorganism, a fungus, could cause an infectious disease. The recognition by the Europeans of the relationship between fungi and disease in humans was the basis for the development of medical mycology (61, 323). By the end of the 1890s, Raimond Jacques Sabouraud had crystallized and organized the scattered observations regarding the role of pathogenic fungi in dermatophytic infections, and his work marked the transition from the study of the dermatophytoses to that of systemic mycoses in the United States during the 1890s. This transition initiated the development of medical mycology in this country.

The purpose of this historical study was to examine the scientific development of the discipline in the United States from 1894 to 1994. This 100-year period was conceptualized into five areas of development: the era of discovery (1894 to 1919), the formative years (1920 to 1949), the advent of antifungal and immunosuppressive therapies (1950 to 1959), the years of expansion (the 1970s), and the era of transition (1980 to 1994) (Table 1). A chronological description of the scientific development of medical mycology in relation to the crisis in training resulting from the decline of important training centers since the early 1980s is provided. Data collection focused on the selection of significant scientific publications as well as important, related educational and technological events in this country. The narrative description of the sequence of important historical events is integrated with analyses and explanations given for human behavior. The scope of the paper does not include two important fungal pathogens, *Paracoccidioides brasiliensis* and *Pneumocystis carinii*. The former is important in areas of Latin America where it is endemic, and the latter was considered phylogenetically closely related to fungi only in the late 1980s.

THE ERA OF DISCOVERY, 1894 TO 1919

Overview, 1894 to 1900

Major contributions to the development of medical mycology in the United States can be highlighted by the discovery of *Blastomyces dermatitidis* in 1894 and *Sporothrix schenckii* in 1896 as two new etiologic agents of disease. The newly established medical center at Johns Hopkins coupled with the creation of its bacteriological research laboratory enabled Caspar Gilchrist and Benjamin R. Schenck to conduct a thorough investigation of these mycotic diseases. Also in 1896, reports of the first two cases of *Coccidioides immitis* infection in the United States were published from California. The incidence of dermatophytic infections (reported as ringworm) in the Boston area and the first report of a mycetoma were published in 1898 and 1899, respectively (Table 1). Most of these early American contributors were physicians who followed the bacteriological standards set by Louis Pasteur in France and Robert Koch in Germany. The publications on fungal diseases coming from Europe and South America gave American investigators comparative studies and were another contributory factor in the origin of the discipline. During this era, most publications were illustrated with numerous drawings and photographs and consisted of lengthy and detailed descriptions of lesions, tissue reactions, culture results, and experimental infections dealing with the etiologic agents.

Scientific Contributions, 1894 to 1900

Blastomycosis. The first American report of a fungus as an etiologic agent of human disease appeared in 1894, following Gilchrist's presentation of a case of blastomycosis at the meeting of the American Dermatological Society held in Washington, D.C., in June 1894 (148). In 1896, Gilchrist published an extensive and detailed account of this case (149), and the fungus was named *Blastomyces dermatitidis* in 1898 by Gilchrist and Stokes (151). This etiologic agent was seen when tissue sections of the hand from a patient who lived in Philadelphia were examined by Gilchrist for the presence of *Mycobacterium tuberculosis* (149). The chief pathological features were numerous, well-defined, miliary abscesses of different sizes containing polymorphonuclear cells (PMNs), nuclear debris, a few epithelial cells, scarce giant cells, and some budding yeasts. The walls of the abscesses consisted of epithelial cells; large collections of endothelial cells and PMNs were seen around the blood vessels.

B. dermatitidis yeast cells in tissues were described by Gilchrist as deeply stained, homogeneous, oval or roundish cells or bodies (6 to 16 μm) with a well-marked spherical nucleus (3.5 to 4 μm) which stained more deeply than the rest of the yeast cell (149). He also described what he thought was a capsule in the peripheral zone or the space between the protoplasmic contents and the membrane (149). The developmental stages from single cell to budding cell and the separation of "daughter" cell from "parent" cell were studied. Each definition was accompanied by drawings, photographs, or both. *B. dermatitidis* was initially considered a protozoan by Gilchrist in 1894 (148), but the presence of budding cells suggested to him in 1896 (149) that he was actually dealing with a yeast. However, he believed erroneously that this yeast was *Cryptococcus neoformans*, which had been described previously by Busse,

TABLE 1. Developmental eras in the history of medical mycology in the United States, 1894 to 1994

Era	Major events
Discovery (1894–1919)	Discovery of the dimorphic fungi and establishment of their dimorphic nature
	Recognition of fungi as etiologic agents of disease, including systemic diseases
Formative years (1920–1949)	Establishment of the first medical mycology training and research center at Columbia University (1926) and other higher-education institutions and federal agencies
	Development of laboratory diagnostic tests and classification systems
	First epidemiologic studies and subsequent recognition that fungal diseases were prevalent
	First ecological studies
Advent of antifungal and immunosuppressive therapies (1950–1969)	Discovery of first antifungal agents (e.g., nystatin and amphotericin B)
	Establishment of the relationship of severe opportunistic fungal infections with antibiotic therapy (1950s) and immunosuppressive therapy (1960s)
	Awareness that fungal diseases cause morbidity and mortality among cancer patients and transplant recipients
	Development of rapid diagnostic tests, e.g., immunodiagnostic tests for coccidioidomycosis and histoplasmosis and the detection of fungi in tissues by fluorescent antibodies and selective fungal stains
	Transition from biological to cellular studies of fungi, e.g., antigenic composition of *C. neoformans*, dimorphism mechanisms, virulence factors, host-parasite interactions
	Founding of the Medical Mycology Society of the Americas in 1961
	Creation of new training and research centers
Expansion (1970–1979)	Expansion and improvement of laboratory diagnosis, e.g., commercial yeast identification systems, detection of fungal antigens and metabolites, exoantigen test development, and standardization of methodology
	Establishment of the regimen of choice for treatment of cryptococcal meningitis
	Expansion of basic research studies, including descriptions of the perfect states of certain fungi
	Increased awareness of the importance of systemic mycoses and subsequent targeting of Brown-Hazen grant support for new or existing medical mycology training and research centers and an overall expansion of training, e.g., postdoctoral training, workshops, and short courses
	Formation of the Mycoses Study Group
Transition (1980–1994)	Increased incidence of fungal infections among AIDS patients and larger numbers of cancer patients and transplant recipients
	Major transitions and conflicting events in the field:
	Depletion and termination of federal and foundation support led to a crisis in training and the decline and closure of established training programs
	Leaders retired or died but were not replaced at their institutions
	Researchers became more specialized, focusing on specific aspects of fungal diseases
	Leadership transition to new and collaborative groups formed with scientists from other medical disciplines
	Initiation and expansion of basic research in molecular biology and genetics and expansion of other studies funded mostly by NIH
	Application of DNA-based methods to more accurate and rapid diagnostic laboratory tests and reliable epidemiologic tools
	Provision of standards for antifungal susceptibility testing and establishment of better therapeutic regimens for certain systemic diseases in patients with and without AIDS supported by pharmaceutical and corporate funds

Buschke, and Sanfelice in Europe between 1894 and 1895 as *Saccharomyces neoformans* (117).

In 1896, a 31-year-old man with multiple facial skin lesions was referred to Halstead for evaluation at the Johns Hopkins Hospital. Gilchrist and Stokes then had the opportunity to study a second case of blastomycosis more closely (150). The infection had begun 11 years earlier as a small pimple located on the back of the patient's left ear, which became pustular. The disease process extended forward very slowly (3 to 5 cm in 4 to 5 years), and gradually several lesions covered almost the entire face. The central portion of each lesion presented as an atrophic cicatricial condition, and the whole area was enclosed by a distinctive and irregular border or demarcation. The planned management was to curette the active cutaneous lesions and then apply silver nitrate to them, but the patient left and later reported the spontaneous healing of these painless lesions without treatment (150).

From the miliary abscesses, Gilchrist and Stokes obtained pure cultures within 1 week, which later developed profuse mycelium with conidia arising from hyphae on short conidiophores (150, 151). The hanging-drop culture technique was used to observe the development of hyphae and conidia (151). Dogs, guinea pigs, a horse, and a sheep were successfully inoculated with hyphal cells, and the tissue reaction in the experimental animals was identical to that seen in the human patients. The disease was named blastomycetic dermatitidis by Gilchrist and Stokes, who recommended examining tuberculous-type lesions of the skin for "blastomycetes" (151). Be-

cause of general unfamiliarity with the fungus and poor staining, the yeast cells could be missed in tissues when stained with hematoxylin and eosin.

Coccidioidomycosis. In 1896, Rixford and Gilchrist reported two fatal cases of coccidioidomycosis or what they thought were unquestionably genuine parasitic infections distinctive from "the doubtful, so-called blastomycetes" (345). This disease had been reported by Posadas and Wernicke in Buenos Aires in 1892 (345). The patients were two Portuguese men who had lived in California in the San Joaquin Valley for several years. One was admitted to a San Francisco hospital in July 1893, and the other was admitted to St. Mary's Hospital in August 1894. The latter case was presented to the California Academy of Medicine on 15 September 1894. Both male patients had similar cutaneous lesions involving the face, neck, and extremities that were "horribly destructive." The autopsy of the first patient revealed involvement of the lungs, adrenals, genitals, liver, spleen, and bone. Tissue sections revealed "close resemblance, amounting to histological identity, between many of the nodules and a genuine tuberculous process with caseation or coagulative necrosis" (345).

Rixford and Gilchrist described the tissue form of *C. immitis* in 1896 as spherical, unicellular bodies, varying from 7 to 27 μm in diameter and consisting in the encapsulated condition of a thick, doubly countered capsule which encloses a finely granular protoplasm. As many as 100 small endospores were noted to be set free by the bursting of the capsule (345). Lesions of experimentally infected animals showed caseation and large numbers of fungal cells. The attempted cultures were assumed to be negative because they were mistakenly discarded as contaminated by a filamentous fungus. They gave the name protozoan or coccidioidal pseudo-tuberculosis to the disease and designated the organism from the first case as *C. immitis* and that from the second case as *Coccidioides pyogenes*. They were not certain if the two fungi were the same, for one patient had a chronic process and the other had an acute disease (345).

Sporotrichosis. Schenck reported that on November 30, 1896, a patient presented himself at the Surgical Clinic of the Johns Hopkins Hospital with an infection of the right arm (361). The primary infection involved the index finger, with the infection extending up the radial side of the arm, following the lymph system, and giving rise to several uncircumscribed, ulcerated indurations. Schenck's examination of skin tissue revealed the characteristics of a chronic abscess, consisting of inflammatory and cicatricial tissues, a zone of leukocytes, and newly formed connective tissue in which were present several minute secondary abscesses (361). In contrast to Gilchrist's fungus (151), the etiologic agent was reported to stain well and to be gram positive. Cultures of the gelatinous, puriform material and of small pieces of tissue gave rise to the abundant growth of an "organism, not resembling bacteria" (361). Experimental infections were successful in a dog and in mice. Schenck used the hanging-drop technique to study the development of the fungus in culture, and then E. F. Smith from the U.S. Department of Agriculture described the characteristic morphologic features of the mycelial form of *Sporothrix schenckii* (361). Smith placed this fungus in the genus *Sporotrichum*, but he stated that he could not identify this fungus to a species level based on Saccardo's limited descriptions of more than 100 species of *Sporotrichum* (361).

Mycotic mycetoma. The first well-documented case of mycetoma in the United States was provided by Wright in 1898 (467). Wright's patient was a 26-year-old Italian woman who was admitted to Massachusetts General Hospital on 29 December 1897 for evaluation of a possible 6-month-old mycetoma. The foot, which was amputated, was described as swollen, with desquamation of the epithelium and with a small sinus tract that exuded on pressure a dirty, greyish fluid, containing some black, hard, irregular granules like grains of gunpowder (467). The granules were treated with KOH and crushed under a coverglass to reveal ovoid or rounded translucent bodies cut in various planes and of various sizes, typical septate hyphae, and atypical connective tissue. The fungus grew on various solid media in 4 to 5 days and appeared first as a "tuft of delicate, whitish filaments," which increased in number and length and did not bear conidia (467). On potato agar, the colonies became dark brown and moist. The fungus was considered to be a hyphomycete.

Dermatophytosis. The last major medical mycology paper of the 1800s was published by White in 1899. He was a dermatologist who reported his personal observations of 279 cases of ringworm that he treated in Boston from October 1895 to July 1898 (454). Of the 279 cases, 139 (50%) were caused by *Microsporum audouinii* in children under 13 years of age and 127 by a mold that he called megalospora, and 13 were not diagnosed. The treatment for these infections consisted of thorough surgical measures for mild cases or epilation followed by application of either sulfur, naphthol, carbolic acid, or mercury or curetting of the entire area and later skin grafts for severe cases. White concluded that the infections in America were milder than the European cases and required less severe treatment. Microscopically, clinically, and culturally, these ringworm infections resembled the ones reported in London and Paris, especially in the former city (454).

Overview, 1900 to 1919

During the early 1900s, medical mycology research expanded rapidly, findings were published and presented at scientific meetings, and fungal infections began to be considered in the differential diagnosis of various diseases. As a consequence, *Histoplasma capsulatum*, *Phialophora verrucosa*, and *Absidia corymbifera* (as *Mucor corymbifera*) were recognized as etiologic agents of disease. Other significant contributions to the development of medical mycology in the United States were achieved between 1900 and 1919. The dimorphic nature of *C. immitis*, *S. schenckii*, and *B. dermatitidis* was established between 1900 and 1907. Studies on the biology, epidemiology, and serology of *C. immitis* were begun. *B. dermatitidis* and *C. immitis* were considered distinctive etiologic entities of invasive disease, and it was noted that their clinical features had been confused with those of tuberculosis. In addition, the first two cases of cryptococcal meningitis were reported from New York in 1916 (Table 1). Kwon-Chung stated in 1994 that while medical mycology in Europe was still centered around cutaneous pathogens, medical mycology in the United States expanded the studies of the deep mycoses (237). However, although the discipline was established, there was much confusion, and organized training was not available.

Scientific Contributions, 1900 to 1919

Coccidioidomycosis. The concept of dimorphism was introduced for the first time by the physicians Ophüls and Moffitt in 1900 (316). They isolated at autopsy a mold from the lungs of a farm laborer who had entered the City and County Hospital (San Francisco) on 26 January 1900 and died on 6 February 1900. The patient had a painful and inflamed joint, irregular fever ("up to 104°C"), cough with mucopurulent and occasionally bloodstained expectorations, and swelling over the left eye. The lungs showed signs of irregular nodular consolidation with necrotic centers (1 cm), several abscesses, and pneumonia. The disease had spread to the spleen, kidneys, liver, bones, and

lymph nodes. The fungus was seen in all infected tissues and grew on agar as a white to slightly yellowish mold that was visible at 48 h. The infection was reproduced in guinea pigs by intravenous inoculation of the mold. Although the animals did not die, the parasitic form of the fungus and typical tubercle-like lesions were found in their lungs (316). Therefore, Ophüls and Moffitt suggested that the protozoan-like bodies and the mold were different stages in the development of the same fungus. In 1905, Ophüls investigated further the etiology of coccidioidomycosis (317), a disease that was then thought to be caused by the two species designated by Rixford and Gilchrist as *C. immitis* and *C. pyogenes* (345). Ophüls reviewed the six reported cases and conducted studies in experimentally infected animals and on media (317). He reported that in the infected rabbits, the parasitic form or spherules arose directly from the arthroconidia (saprophytic infectious cells) by a process of enlargement. His overall conclusion was that the infection was caused by a pathogenic fungus, and he suggested the name *Odium coccidioides* for the fungus and coccidioidal granuloma for the disease and its lesions.

By 1915, Dickson reported that 35 of the 40 known patients with coccidioidomycosis had been residents of California, 3 had visited the state, and 27 had spent some time in the San Joaquin Valley (88). Most of the patients, 37, were adult male laborers. The review of these 40 cases prompted Dickson to conclude that coccidioidal granuloma was a clinical entity; the disease was almost identical to tuberculosis; coccidioidal granuloma and blastomycosis were etiologically distinctive diseases; coccidioidal granuloma may be treated by radical removal of the infected organ; and some patients may recover spontaneously. Therefore, physicians began to consider mycoses in the differential diagnosis of disease with the aid of laboratory data. During the same year, Cooke described the first immunological studies with *C. immitis* (66). He detected precipitin antibodies (titer of 16) in the serum of a patient by using an antigenic extract (as the "precipitinogen") of dried cultures. The serological reaction was specific, since the precipitin antibodies did not cross-react with *B. dermatitidis* antigen. Although he failed to demonstrate both complement-fixing (CF) and agglutinating antibodies, he suggested that the use of the CF test was a potential diagnostic tool. In 1919, Bowman was the first to describe the X-ray findings for five patients afflicted with this disease while he was at the Los Angeles County Hospital (36).

Sporotrichosis. While at Rush Medical Center, Hektoen and Perkins diagnosed a third case of *S. schenckii* infection between 1900 and 1901 (178) and, most importantly, compared the characteristics of their isolate with those of the organisms recovered by Schenck (361) and Brayton (178). Hektoen and Perkins demonstrated that the three infections were caused by the same fungus and concluded that the yeast was the only parasitic form found in tissues of humans and experimentally infected animals. They named this fungus *Sporothrix schenckii* (178). Their paper contains photographs that clearly depict the morphological characteristics of the saphrophytic and parasitic forms of this species. By 1913, Davis had confirmed Hektoen and Perkins' (178) earlier suggestion concerning the dimorphism of *S. schenckii* when he studied the clinical isolates obtained by Hektoen, Gougerot (Paris), and others from patients with sporotrichosis (75). Langeron, Gougerot, and other French investigators had studied numerous cases of infection caused by *S. schenckii* at the Pasteur Institute. A number of these isolates were probably *Exophiala jeanselmei* and not *S. schenckii* (281).

Blastomycosis. In 1902, Walker and Montgomery, a surgeon and a dermatologist, respectively, at Rush Medical Center were the first to report that *B. dermatitidis* could cause invasive disease in humans (436). They originally believed that their patient had died of miliary tuberculosis, but tuberculosis was the patient's secondary infection, not the cause of death. Only a few *Mycobacterium tuberculosis* bacilli were found in several hundred skin sections, in contrast to the large numbers of yeast cells that had been missed by the pathologists. The lungs also were full of typical yeast cells (as blastomycetes), which clearly demonstrated that this was a systemic infection. The patient was curetted, and the clean granulating areas were covered with Thiersch's grafts, but death occurred 33 days after surgery. Although it was believed that curettage hastened a patient's demise, it was the treatment indicated when large doses of potassium iodide were not effective. During the ensuing 6 years, the prevalence of systemic blastomycosis was confirmed by Montgomery and Ormsby (294). They "collected" and reviewed 22 American and European cases. The common and most pronounced feature of these cases was the formation of multiple abscesses in various organs of the body (294). The cultural characteristics of *B. dermatitidis* were first described by Hamburger in 1907, when he reported that *B. dermatitidis* grew well and rapidly on routine laboratory media and even faster on faintly acid glucose-agar (171). However, his most significant contribution was his description of the dimorphic nature of this pathogen. Hamburger recommended the duplication of cultures at 37°C and ambient temperature, a practice still used in many clinical laboratories.

Histoplasmosis. In 1906, Darling, while he was working as a pathologist in Panama, found numerous small, oval to round bodies while examining autopsy smears of the lungs, spleen, and bone marrow from a patient from Martinique who was suspected of having pulmonary miliary tuberculosis (73). Most of these bodies were within alveolar epithelial cells in the pseudotubercular areas, while others appeared to be free in the spleen and rib marrow. PMNs were rare, a few mononuclear cells were seen, and *M. tuberculosis* bacilli were absent. Darling described the organisms as bodies surrounded by a clear refractive nonstaining rim, about one-sixth the diameter of the parasite (73). He believed erroneously that this clear area was a capsule and thought the fungus was similar to the parasite *Leishmania donovani*. Although Darling named this microorganism *Histoplasma capsulatum*, it was the Brazilian da Rocha-Lima who concluded in 1913 that this organism was a fungus (73a). The discovery of another fungus as an etiologic agent of invasive disease was thus accomplished.

Chromoblastomycosis. The first case of a *Phialophora verrucosa* infection in the United States was reported simultaneously in 1915 by physicians in the departments of pathology (283) and dermatology (241) of Boston City Hospital. Lane's patient was a 19-year-old Italian immigrant who for a year had had a painless lesion on the right buttock (241). The lesion had started as a purulent pimple that gradually increased in size to 2.5 cm. A painless lump developed near the lesion 6 months later. A diagnosis of tuberculosis verrucosa was considered, and the lump was excised. The initial laboratory diagnosis was blastomycosis based on the "bodies seen in the smears." However, because the lesion appeared to be different from the ones known to be caused by either *B. dermatitidis* or *S. schenckii*, a biopsy was performed (241) and then sent to Medlar for further study (283). The biopsy revealed the same cellular reaction seen in blastomycosis, but the parasitic cells were different and were described by Medlar either as brownish-black sclerotic cells (8 to 15 μm) or as budding yeasts. The sclerotic cells enlarged during the septation process, up to 25 μm (283). The microscopic observations of the black mold which was initially discarded as a "contaminant" showed mycelium composed of

septate branched hyphae supporting conidial formation on lateral branches by sequential budding, as well as by phialides (283). Two important diagnostic observations were made by Medlar: laboratory diagnosis is needed for certain types of chronic skin infections, and not all fungi that appear as yeast cells in tissue are necessarily yeasts (283). Medlar gave his fungus to the mycologist Thaxter, who concluded that it should be described as a new taxon. He followed Thaxter's recommendation, used Saccardo's classification system, and proposed the new name *P. verrucosa*, which was added to the list of fungi pathogenic for humans.

Zygomycosis. A brief and inconclusive report of an *Absidia corymbifera* (as *Mucor corymbifer*) infection of the vocal cords was reported in 1918 by Ernst (115). This seems to be the first report of zygomycosis by this genus in the United States. The disease was reproduced experimentally in guinea pigs, and it was found that this fungus did not grow well at temperatures above ambient temperature in vitro. It is noteworthy that such an unusual cause of zygomycosis was the first to be described.

THE FORMATIVE YEARS, 1920 TO 1949

Overview, 1920 to 1929

The course of medical mycology was changed by the occurrence of a number of scientific events during the 1920s (Table 1). The first medical mycology laboratory was established in 1926 by the dermatologist J. Gardner Hopkins under the direction of Rhoda Benham at Columbia University College of Physicians and Surgeons (380, 382), now known as the Columbia-Presbyterian Medical Center. Until then, medical mycologic studies had been conducted by physicians and bacteriologists at bacteriology laboratories. During this decade, serological and skin test studies were performed with crude antigens of *C. immitis* and *Cryptococcus neoformans*; diagnostic laboratory tests such as India ink preparations were used to visualize the capsule of *C. neoformans*, carbohydrate fermentations were used to aid in the identification of *Candida albicans* and *C. neoformans*, and chlamydospore production for *C. albicans* began to be developed; a new formulation for Sabouraud's medium was designed; *Pseudallescheria boydii* was identified as an etiologic agent of mycetoma; revisions of reported cases and isolates were conducted as a means of classifying different isolates within a species or genus; better descriptions of histoplasmosis and its etiologic agent were published based on observations of the first antemortem case; and sporotrichosis was declared an occupational disease.

Scientific Contributions, 1920 to 1929

Mycotic mycetoma. Boyd and Crutchfield described the first case of mycetoma caused by *Pseudallescheria boydii* (as *Allescheria boydii*) at the University of Texas Medical Branch in Galveston in 1921 (37). The patient's foot had been infected following injury by a thorn, and 3 months later, the ankle became swollen and the lesion finally ruptured, discharging bloody pus. The lesions healed temporarily but continued to develop for 12 years (37). The treatment of the era for mycetomas was solely surgical and involved either removal of small areas of tissue or amputation of the extremity. But Boyd gave his patient X-ray treatment (37), which had been proven beneficial for certain fungal infections during those years. The patient improved but did not return after the fourth treatment. In 1922, Shear subcultured this isolate on cornmeal and glycerine agar to study the formation of conidiogenous cells, conidia, and fruiting structures (376). He observed that the fungus produced conidia, ascospores in cleistothecia, and synnemata. Saccardo had classified a similar fungus, identified by Constantin in 1893, under the generic name *Allescheria*. Shear named this patient's isolate *Allescheria boydii* in honor of Boyd (376). *P. boydii* was the first fungal pathogen known to reproduce sexually.

Cryptococcosis. In 1923, Freeman and Weidman at the University of Pennsylvania reported a case of "cystic infiltration" of the brain, which they believed was caused by *C. neoformans* (as *Torula histolytica*) (129). The name *T. histolytica* had been given by Stoddard and Cutler in 1916 (409) to a yeast that they found in two American patients, and it had been reported from Europe in three patients with cryptococcal meningitis (as *Torula* infections). However, Freeman and Weidman (129) dismissed the two American cases as unacceptable infections caused by *C. neoformans*, recognizing only the three European cases and the yeast recovered by Frothingham in 1902 (132) from the lung of a horse. They conducted an extensive study of their own case (129), which is the first well-documented report of cryptococcal meningitis in the United States. The patient, aged 39, was admitted to the hospital with symptoms of severe paroxysmal headaches of 1-month duration, vomiting, poor vision, rigidity of the neck, and difficulty in writing and talking. He had a prior diagnosis of Hodgkin's disease in 1916. The patient worsened during the following 2 months and then died. The yeasts were easily seen in the cerebrospinal fluid (CSF), and their capsules were demonstrated by making an "emulsion of yeasts" in India ink. Stained tissue sections showed edema, fibrous hyperplasia, and cellular infiltrates with plasma cells and lymphocytes (without PMNs). The yeasts were found both intra- and extracellularly, with only a few budding cells (129). Although physiologic studies were performed, no conclusive results were reported.

By 1926, the pathologist Rappaport and the bacteriologist Kaplan reviewed 13 reported cases of cryptococcosis (2 from Germany and 11 from America) and concluded that cryptococcal meningitis was acquired through the respiratory system by inhalation of the fungus, which disseminated from the lungs to the other organs (332). They also noted that although intravenous iodides, tartar emetic solution, and intraspinal injections of immunized rabbit serum and colloidal silver were used as therapy, the infection was always fatal. Rappaport and Kaplan were the first to perform serological testing (CF and agglutination) of sera and CSF from a patient as well as sera from immunized rabbits and guinea pigs (332).

Histoplasmosis. Riley and Watson, at the University of Minnesota, reported in 1926 the fourth case of histoplasmosis and the first in the United States (339). Their patient was a German woman who had been living in the United States for 23 years and whose clinical symptoms and postmortem findings were similar to those in Darling's report (73). Using the Giemsa stain, Riley and Watson recognized that the microorganism did not have the characteristic features of *Leishmania* spp. (339); however, like Darling (73) before them, they thought that it was encapsulated. Therefore, they concluded erroneously that histoplasmosis was caused by two related species of *Cryptococcus*, one causing systemic infections and the other causing superficial infections (339). Histoplasmosis was then considered a fungal disease, but because no culture was obtained, the real identity of the etiologic agent of this case remains unknown.

Sporotrichosis, an occupational disease. Foerster reported the observation of 18 cases of sporotrichosis during a 5-year period from 1921 to 1926 (127). Since most of his patients worked at a plant nursery and the disease was acquired in at least 10 of the 18 patients following inoculation by thorns of

the barberry shrub, he concluded that sporotrichosis was an occupational disease among individuals engaged in farming and horticulture. From that time, American courts of law began to award compensation for the cost of medical care for this disease as an employment-connected disability (1).

Laboratory diagnosis. Fineman stated in 1921 that bacteriological textbooks of the era had meager descriptions of the etiologic agent of candidiasis (as thrush) (125). The fungal classification systems had described *C. albicans* by at least 10 different names since its discovery by Robin in the 1800s. Because carbohydrate fermentation and agglutination tests were useful in the 1920s to classify bacteria, Fineman adopted this system to study what she called the "thrush parasite" (125). She performed cultures on several differential media (e.g., milk, gelatin, and carrots), tested 17 carbohydrates, and adopted the medium developed in Europe by Linossier and Roux for chlamydospore formation. Three important conclusions were made by Fineman concerning *C. albicans* (as *Oidium albicans*): carbohydrates are useful for yeast identification, isolates of this species produce chlamydospores and no ascospores, and this species tends to be filamentous in liquid media at low oxygen and surface tension and unicellular on solid media (125).

Two important contributions were made in 1928 at the University of Alabama (182) and the University of Pennsylvania (440) concerning the selection of a suitable medium for studying dermatophytes. Difco's (Detroit, Mich.) peptone was unacceptable as a substitute for the imported French peptone. The morphological characteristics that are distinctive for each dermatophyte species were not evident with Difco's peptone. This created a problem, because the identification of dermatophytes relied heavily on Sabouraud's descriptions and illustrations of the dermatophytes grown on agar containing the French peptone. After numerous experiments were performed with a variety of medium formulations (peptones and carbohydrates) by Weidman and Spring (440), a suitable medium was selected by Hodges (182). In its final formulation, this medium contained a peptone from America (1%, Fairchild), dextrose (4%), and 1.8% agar (final pH, 5.0). This formulation was named Sabouraud medium (liquid and agar) and is the basic routine culture medium used to grow fungi in clinical laboratories.

Skin test development. In 1927, Hirsch and Benson were the first to successfully obtain specific skin reactions in patients with coccidioidomycosis by using heat-stable (80°C), soluble filtrate antigens extracted from 14-day-old peptone broth cultures of *C. immitis* (181). The skin reactions resembled those seen with tuberculin, and the immediate reaction was similar to the "wheal" produced by specific pollen extracts in patients with hay fever. Prior investigations of skin test responses by Cooke in 1915 (66) and by others in animals or in patients had lacked specificity. The report by Hirsch and Benson may be considered the forerunner of the more intensive studies of skin testing performed during the 1940s.

First training and research center. While bacteriology laboratories were abundant around the country, there were no laboratories devoted to the etiologic diagnosis of fungal infections. At Columbia University, J. Gardner Hopkins began doing his own culture work from skin lesions in 1926, while B. O. Dodge, a professor of botany, helped him with the fungal identifications (117, 380, 382). They recognized the need to have a full-time mycologist and offered the position to Rhoda Benham. At the time, she was conducting research for her doctoral degree under the supervision of the classical mycologist Richards (117, 380, 382). The Rockefeller Foundation awarded a 5-year $50,000 grant for the support of research in medical mycology to this center on 11 March 1929 (349). This was the first grant awarded for training and research in medical mycology, and Benham's laboratory became the first nucleus for training and research in the field.

Overview, 1930 to 1939

Prior to 1930, there was no clear distinction among the three important systemic fungal diseases, blastomycosis, cryptococcosis, and coccidioidomycosis. Also, the classification system used to identify *C. albicans*, the etiologic agent of thrush, was inadequate and failed to distinguish this fungus from other yeasts isolated from the intestinal tract and stools of healthy individuals. In addition, the dermatophytes were classified according to Sabouraud's system, which was primarily based on the clinical presentation of the infections. Rhoda Benham and Chester Emmons at Columbia University provided more logical classification systems for the identification of these fungi by utilizing morphological characteristics, which greatly improved the scientific study of these organisms (Table 1). Although they had little knowledge of the fungi pathogenic to humans, they had learned the biology of plant fungal pathogens under leading classical mycologists. Between 1935 and 1936, two additional training and research centers were established, one at the National Institutes of Health (NIH) under Emmons and another at Duke University under Norman Conant. By the end of the 1930s, the frequency of fungal diseases involving the central nervous system (CNS) and the occurrence of coccidioidomycosis as a mild but common disease in the San Joaquin Valley had been reported. The first report of a *Fonsecaea pedrosoi* infection in the United States, the isolation of *C. immitis* from the soil, and the establishment of *H. capsulatum* as a dimorphic fungus appeared in the literature between 1932 and 1936 (Table 1).

Scientific Contributions, 1930 to 1939

Coccidioidomycosis. In 1932, a short report appeared in the literature describing the first isolation of *C. immitis* from "earth" collected around the sleeping quarters at a ranch near Delano, Kern County, California (406). The "fungus spores" were set free from the soil by using a column of brine. Between 1937 and 1938, three papers were published by Dickson and Gifford at Stanford University (89–91). These publications provided significant information concerning *C. immitis* because the authors had the excellent opportunity of studying five patients with the common infection known as valley fever (of unknown etiology then), which was characterized by colds or bronchopneumonia, high temperature, and painful erythema nodosum or skin lesions. One of these patients was a medical student who had acquired the infection in the laboratory when he inadvertently opened an old "petri dish culture" of *C. immitis*. He stated later that he had noticed a light-brown cloud arising from the dish, a cloud of arthroconidia (as chlamydospores) (89). The other patients had acquired the infection in the San Joaquin Valley, where they were exposed to a great deal of dust. Although some of these patients were diagnosed at first by X-ray as having pulmonary tuberculosis, the diagnosis of coccidioidomycosis was established by the demonstration of the typical spherules in sputa, with subsequent culture confirmation (90). It became evident then that the first manifestation of the fungal disease caused by *C. immitis* was either an acute "cold," bronchopneumonia, erythema nodosum, or all three. It was also confirmed that the inhalation of arthroconidia was followed by a primary infection and in some cases by secondary progressive disease (90, 91). Dickson and Gifford suggested in 1938 that the name coccidioidomycosis should

include the different clinical manifestations of this infection (91). The fact that valley fever was actually a manifestation of coccidioidomycosis demonstrated that this fungal disease was not a rare infection.

Meningeal infections. The incidence of mycotic meningeal infections was reported in New York City and Washington, D.C., by Freeman (130) and Smith and Sano (384) in 1933. Freeman studied tissue sections from 13 cases of *C. neoformans* meningitis and one case each of a CNS infection caused by *Aspergillus* spp., *B. dermatitidis*, *C. immitis*, *H. capsulatum*, and *S. schenckii* (130). He concluded that these were chronic meningeal infections without invasion of other organs and that the finding of the fungus in the CSF established the diagnosis. Smith and Sano, on the other hand, reported what they considered to be the first case of *C. albicans* meningitis (384). Their patient was a 22-month-old infant who died 9 days after admission at the Willard Parker Hospital. The same yeast was isolated from the CSF and oral lesions, which was identified by Benham as *C. albicans* (as *Monilia albicans*).

Histoplasmosis. Three significant publications appeared in the literature in 1934 regarding *H. capsulatum* (77, 97, 172). Dodd and Tompkins described the third case of histoplasmosis in the United States and the sixth world-wide (97). This was the first antemortem report describing the occurrence of this fungal disease in a 6-month-old, white, male infant. The clinical, pathological, and morphological findings were similar to those found in previous cases in 1906 (73) and 1926 (339). DeMonbreun cultured material from the child on various media and was surprised when a mold was recovered, because only a yeast was seen in tissue (77). However, further studies demonstrated that the yeast form grew well in serum and blood agar cultures incubated between 34 and 37°C and that the mold form grew in duplicate cultures incubated at ambient temperature (77). The dimorphic nature of *H. capsulatum* was then established. DeMonbreun also provided excellent descriptions and photographs of the diagnostic macroconidia of *H. capsulatum*, which he referred to as tuberculated spores (77).

Hansmann and Schencken reported a case of chronic infection caused by "a yeast-like organism in tissue," which they erroneously thought belonged to the genus *Sepedonium*. The organism was characterized by "large spiculated chlamydospores" (172). Although they saw *H. capsulatum* cells similar to those described by Darling (73), they did not believe that this was a case of histoplasmosis because there was no involvement of the spleen in their patient. Furthermore, the culture had been studied by Emmons, and he had identified it as a *Sepedonium* sp., based on the presence of the characteristic thick-walled "tuberculated chlamydospores" of that genus. Emmons stated during his 1968 lecture at the annual meeting of the Medical Mycological Society of the Americas (MMSA) that "since the hyphal form of *H. capsulatum* was unknown to me at the time, I made the mycologically reasonable identification of *Sepedonium*. It was fortunate that DeMonbreun was able to establish the dimorphism of *H. capsulatum*" (114).

Chromoblastomycosis. The first case of a *Fonsecaea pedrosoi* (as *Hormodendrum pedrosoi*) infection in the United States was reported in 1936 from Duke University (272). This case of chromoblastomycosis (as dermatitis verrucosa, a name coined by early Brazilian investigators) had begun 4 years earlier on the left hand and fingers of the patient, as a series of pruritic vesicles and putsules, which progressed to elevated, scaling, verrucous nodules with itching and burning and a tremendous amount of swelling (the patient needed a sling). Examination of the slide cultures demonstrated three types of conidiogenous development: the *Cladosporium*-like and *Phialophora*-like types and the sympodial form. Arturo Carrion and Emmons, from the University of Puerto Rico, previously had reported the presence of the *Phialophora* type of conidiogenous cells in cultures of *Fonsecaea* spp. Also, it was reported from Duke University that staining with lactophenol and cotton blue facilitated the finding of fungi in clinical materials, thus avoiding the various confusing artifacts found when using only KOH (414).

Taxonomy and classification. Benham's doctoral dissertation on yeast identification was published in 1931 (19) and shed much light on this confusing subject (380, 382). The confusion was due to the lack of a generally accepted yeast classification system as well as recognized criteria for the determination of the species that were pathogenic to humans. The "rudimentary" morphology of these fungal pathogens and their varied appearance under different cultural conditions complicated matters. In order to clarify this confusion, Benham performed a systematic investigation of the morphological characteristics, fermentation profiles, and serological reactions of 30 different yeasts recovered from thrush lesions, various body sites, and plants (19). Data from these studies permitted her to conclude that the majority of the fermenting, yeast-like fungi isolated from thrush lesions were identical and belonged to the well-defined species *C. albicans* (as *Monilia albicans*), which had distinctive morphological and cultural characteristics. The other species of yeasts had "definite morphological differences" by the Dalmau technique, which were depicted in her excellent drawings. Six years after Benham's first paper (19), a survey of the "mycologic flora of 1,000 adults' normal mouths and throats" was undertaken (421) as a means of determining the role of the pathogenic yeasts found in thrush infections. It was found that *C. albicans* was present in 7% of "normal" individuals and that serum samples from 22.5% of them had agglutinins against this yeast.

Benham's paper concerning the classification of *C. albicans* was followed in 1935 by an important contribution on the classification of *C. neoformans* (21). She studied more than 40 strains of *Cryptococcus* spp., which included the original cultures from Busse, Buschke, and Curtis as well as strains recovered from the skin and intestinal tract of both normal individuals and patients suffering digestive disease. Her most significant conclusion was that all the pathogenic isolates belonged to only one species, *C. neoformans* (as *Cryptococcus hominis*) (21). Benham's third important scientific contribution was published in 1934 (20). This time, she studied three different mycotic infections: cryptococcosis, blastomycosis, and coccidioidomycosis, which had questionably been called yeast infections. Benham stated definitely that each infection was an etiologically distinct disease that could be diagnosed by the characteristic morphology of their etiologic agents in tissue and culture (20).

Benham's studies were not confined to these genera. She became interested in the nutrition of fungi when she discovered that *Malassezia furfur* (as *Pityrosporum ovale*) required oleic acid to grow in vitro (22). Benham studied the nutritional requirements of *M. furfur* and thus introduced the study of the nutrition of fungi, a field in which several other investigators were interested at that time. Benham's publications on the nutrition of dermatophytes, many of them in collaboration with her student, Lucille K. Georg, and with Margarita Silva-Hutner, expanded the knowledge of medical mycology in this country.

In 1929, Emmons was offered a job by Hopkins as associate in mycology at Columbia University, where Emmons was a doctoral candidate under the direction of the classical mycologist Robert Harper (51, 117, 235). Emmons expanded his knowledge of fungal pathogens while he managed the Depart-

ment of Dermatology's culture collection. At that time, the collection consisted mainly of dermatophytes, a few yeasts, and a couple of strains of *S. schenckii*. He collected other dermatophyte isolates from skin, hair, and nails at the Vanderbilt Clinic, which were eventually used for his classical taxonomic study of the dermatophytes. Emmons realized in 1934 that medical mycology needed a more logical and usable classification of the dermatophytes according to the botanical rules of nomenclature (107). He followed the development of conidiophores and conidia (as spores) by using Henrici's modified slide culture, in which agar was run under a coverslip fastened to a slide; he substituted sterile shavings of cow horns for the agar (107). On the basis of morphological characteristics, Emmons proposed to retain the generic names *Trichophyton*, *Microsporum*, and *Epidermophyton* and declared that the genera *Achorion* and *Endodermophyton* were unnecessary (107). He believed that such a natural mycological classification followed in a general way Sabouraud's clinical classification but avoided its inconsistencies. His proposed classification system remains in use today.

Fungal endotoxins. In 1939, Henrici reported for the first time that *Aspergillus fumigatus* produced two endotoxins, a hemolytic toxin and a potent pyrogenic toxin (179). The importance of Henrici's discovery was recognized during the well known "turkey" epidemic in England in the early 1960s. The turkeys, swine, poultry, and cattle affected with severe systemic disease had consumed particular foodstuffs that contained a metabolite, aflatoxin, of *Aspergillus flavus* (344).

Training and research centers. In 1935, Norman Conant was invited to join the staff at Duke University (known as Trinity College until 1930), where he reported the third case of chromoblastomycosis caused by *F. pedrosoi* (64). Conant, who trained under William Weston at Harvard University, became well known as a teacher and leader because of his famous summer courses and medical mycology textbook (9, 52, 281). A very important contribution to the development of medical mycology was the recognition by the federal government of the need to establish a mycology section at the NIH. This was accomplished with the appointment of Chester Emmons as the senior mycologist on 15 February 1936. At the time, the NIH was located in Washington, D.C., and with President F. D. Roosevelt's support, it was moved to Bethesda in 1939 (428). By 1936, in addition to Benham's laboratory, these two medical mycology centers had also been established on the east coast.

Overview, 1940 to 1949

The outbreak of World War II was another defining watermark for medical mycology. The military training of troops in the arid areas of California and Arizona created problems for the Army and Air Force because many trainees acquired coccidioidomycosis. The Armed Forces asked Charles E. Smith to investigate this problem, which led to studies on the epidemiology of *C. immitis* and later of *H. capsulatum* by skin test reactions (Table 1). Smith's studies on coccidioidomycosis provided a wealth of information on the epidemiology and laboratory diagnosis of this mycosis, which improved patient management and clinical research. Medical mycology became a respected discipline within the scientific community. The early epidemiological findings prompted Emmons to become involved in fungal ecological studies, which led to his isolation of *H. capsulatum* from soil in Virginia. As Norman Goodman stated in 1994 (155), the development of skin testing facilitated the distinction between infection and disease, the study of fungal epidemiology, and the understanding of the natural history of the systemic mycoses. Reports of *Candida* spp. endocarditis and meningitis as well as meningitis caused by other opportunistic fungi began to appear in the literature between 1940 and 1948. The isolation and identification of fungi in the laboratory were advanced by the development of a fungal stain by Gomori and the descriptions of fungal tissue reactions by Roger Baker. During the war, the Army also experienced a major problem with athlete's foot, which was common among servicemen because of poor hygiene and a lack of proper bedding and facilities (9). The increased incidence of fungal infections during World War II prompted the establishment of other training and research centers and created a demand for medical mycology training for a varied group, including clinical laboratory personnel and physicians (Table 1).

Scientific Contributions, 1940 to 1949

Systemic candidiasis. The first case of *Candida* endocarditis was reported in 1940 by Joachim and Polayes from New York City (200). The patient was a 48-year-old white male who had been addicted to morphine and heroin for 20 years. For 18 months, he had given himself intravenous injections without aseptic or antiseptic precautions. Although this case was attributed to a *Candida* sp. (as *Monilia*), the identification of the yeast was not given. Another case of *Candida* endocarditis and meningitis was reported in 1946 following massive treatments (4 weeks each) with penicillin for two episodes of bacterial endocarditis (140). The yeast was identified as *C. albicans* (as *M. albicans*) by Emmons. These are the first reports of the incidence of severe opportunistic fungal diseases as a sequel to broad-spectrum antibiotics as well as the association of *C. albicans* with intravenous drug use.

Zygomycosis. Strikingly similar cases of rhinocerebral zygomycosis (as mucormycosis) of the meninges and brain were diagnosed in three patients with severe diabetes mellitus at the Johns Hopkins School of Medicine in 1943 (163). The other well-documented case of rhinocerebral zygomycosis was that described from Germany by Paltauf in 1885 (320a). Upon admission, the patients were unable to talk and developed increasing drowsiness, which progressed to coma. In the next few hours, their faces became edematous, particularly about the eyes, until the whole face was involved, with the patients then dying during the first to the fifth hospital day. Cultures were not obtained. Tissue sections from these cases were sent to Emmons, who described the large diameter of the hyphae, the manner of branching, and the coenocytic structure as characteristics of the *Mucoraceae*, probably a *Mucor* sp. (163). Rhinocerebral zygomycosis has been associated with acidotic diabetes since this well-documented report in 1943. Bauer and coworkers were the first to isolate the prime etiologic agent of rhinocerebral zygomycosis, *Rhizopus arrhizus* (as *R. oryzae*) (15a).

Mycotic mycetoma. Emmons described the first case of mycetoma of the hand caused by the black fungus *Exophiala jeanselmei* (as *Phialophora jeanselmei*) in 1945 (110). This fungus had been described by the French mycologist Langeron as *Torula jeanselmei*. Because Emmons erroneously thought that it resembled the members of the genus *Phialophora*, he transferred it to *Phialophora* as a new species.

Pseudallescheriasis. Benham and Georg reported in 1948 the first case of meningitis due to *P. boydii* (23), and Creitz and Harris described the presence of this fungal pathogen in a cavitary lung lesion 7 years later (70). In 1944, Emmons demonstrated that the fungus Shear described as *Pseudallescheria boydii* (376) and the fungus from numerous mycetoma cases outside the United States described as *Monosporium apiosper-*

mum were in reality the same fungus (109). *Pseudallescheria boydii* was the ascocarpic stage of *M. apiospermum.*

Epidemiology and ecology. In 1940, Smith reported the first cooperative field and laboratory study of San Joaquin fever (valley fever) in Kern and Tulare counties, California, where there was a high incidence of this infection (385). From 7 December 1937 to 12 May 1939, 432 patients who recovered without sequelae were enrolled in the study. In addition to the clinical picture and the presence of erythema nodosum or erythema multiforme, a positive coccidioidin skin test also served as a criterion for enrolling patients. Many facts were learned. Seasonal incidence corresponded to the climate, with the peak in the dusty fall and an ebb in the wet winter; benign valley fever was more common among white females and severe granuloma was more common among "dark-skinned" males; and eventually, most individuals in the region experienced the infection—an estimated 8,000 to 10,000 infections in the two counties—but only 5% of them developed erythema nodosum (385). Smith's study made a significant contribution to the development of medical mycology, because it expanded and confirmed the results obtained by Dickson and Gifford (91) concerning the high incidence of benign coccidioidomycosis in certain portions of the United States. Smith and coworkers conducted a detailed investigation on coccidioidomycosis at four army field camps in the San Joaquin Valley from July 1941 until February 1946. They emphasized the importance of dust control by planting grass, paving roads and airfield runways, and, ultimately, the use of highly refined oil on athletic areas and demonstrated that coccidioidin reactors were immune to the disease (386). The method for the preparation of coccidiodin was published in 1948 by Smith et al. (387).

During the summer of 1941, *C. immitis* was isolated by Emmons from 5 of 150 soil samples collected in the desert and from 25 of 105 rodents trapped near the village of San Carlos, Ariz. (108). The high percentage of Indian schoolchildren in this area who were sensitive to coccidioidin and had demonstrable pulmonary nodes (11) prompted Emmons' ecological studies. It also confirmed previous findings made during the 1930s that failure to react with tuberculin in patients having calcified pulmonary nodes (as nodules) was associated with a fungal infection and that *C. immitis* could be recovered from the soil in areas where coccidioidomycosis was endemic (90, 406).

The first intradermal injections of *H. capsulatum* soluble filtrate antigens (histoplasmin) were given to a patient and experimentally infected animals in 1941 by Van Pernis et al. (429). The injections produced specific, immediate and delayed, cutaneous reactions and led to Christie and Peterson's (59) and Palmer's (320) epidemiological studies in 1945. Emmons prepared the histoplasmin for Palmer by growing two strains of *H. capsulatum* in the medium used by C. E. Smith for producing coccidioidin. Palmer injected histoplasmin and tuberculin into approximately 3,000 student nurses living in Detroit, Kansas City (Missouri and Kansas), Minneapolis, and Philadelphia (320). From their skin test reactions and chest X-rays, Palmer confirmed Christie and Peterson's results (59) and concluded that mild, probably subclinical infection with *H. capsulatum* was widely prevalent in certain states and relatively infrequent in others, and that the incidence of *H. capsulatum* infection corresponded with the presence of pulmonary calcification in most tuberculin reaction-negative individuals (320). Although the incidence of tuberculosis-like lesions in individuals who had a negative tuberculin reaction had been reported since 1926, it was Gass et al. in Williamson County, Tenn. (139), who in 1938 conclusively demonstrated this unexpected phenomenon in 24.7% of 1,291 cases. These epidemiologic studies also led to other ecological studies by Emmons in 1949 (111). He isolated *H. capsulatum* from 2 of 387 soil samples collected between 18 December 1946 and 18 December 1948, from farms in Loudoun County, Va. Emmons previously had found that dogs and other animals trapped in the area had histoplasmosis. Two fatal histoplasmosis cases in human siblings had also been diagnosed in the area.

Laboratory diagnosis. Several scientific advances made between 1946 and 1948 facilitated the diagnosis of fungal infections in the laboratory. A stain for the microscopic observation of fungal cells in tissue was first described by Gomori in 1946 (153). This histochemical test was based on the demonstration of fungal elements which were stained black by a reduction of a silver solution. This stain was modified by Grocott in 1955 (165) and later by Huppert et al. in 1978 (195). Using other tissue stains, Baker made extensive observations of the tissue reactions of a series of fungal infections and attempted to catalog them based on these distinctive reactions (14). The observation of asteroid formation, an important tissue reaction to *S. schenckii*, was reported for the first time in the United States in 1946 (295).

Wickerham and Burton demonstrated the usefulness of assimilation tests in the classification of yeasts in 1948 (455). The senior author had spent several years evaluating fermentation and nitrogen assimilation tests to improve the techniques used in the classification of yeasts. They employed a better basal medium as well as more and different carbon compounds than previously evaluated by other investigators. The technique used was Beijerinck's modified assay, in which small amounts of the carbon compounds were placed on the inoculated agar. This simple procedure, which was based on the development of growth, was modified and became an invaluable tool in the clinical microbiology laboratory for many years. It led to the development of commercial kits during the 1970s.

During the late 1940s, several investigators concentrated on determining the nutritional requirements for growth and conidia production by the dermatophytes (175, 346). Georg, while at Benham's laboratory, studied several species of *Trichophyton* and the nutritional "deficiencies" of these species (144), which led to her significant discoveries in the next decade. Georg also provided the first description of the macroconidia of *Trichophyton violaceum*. In 1947, Campbell reported that Francis' glucose-cystine blood agar luxuriantly supported growth of the yeast form of *S. schenckii* and that a modified formulation of this medium supported the growth of *H. capsulatum*'s yeast form (50). Conversion of these molds to the yeast form establishes their definite laboratory identification.

Antifungal agents. Reports on the antifungal activity of three different compounds, benzimidazole (466), propamidine (106), and cycloheximide (as actidione) (242), appeared in the literature between 1944 and 1947. Benzimidazole was the first reported imidazole that inhibited the growth of the saprophytic yeast *Saccharomyces cerevisiae*. Low concentrations of propamidine were found to be fungistatic against *B. dermatitidis*, *S. schenckii*, and *Aspergillus fumigatus*. Although only propamidine was used briefly as a therapeutic agent in humans, these reports were forerunners of the intense search for active and safe antifungal agents during the ensuing years.

Training and research centers. Charles E. Smith was recruited in 1934 by Ernest Dickson, a professor of public health at the Stanford School of Medicine, to work on *C. immitis*. From Stanford, Smith moved to the University of California School of Public Health at Berkeley, where he founded another center for medical mycology research and training (117). Between 1940 and 1945, the Armed Forces maintained a my-

cology laboratory at the Walter Reed Army Medical Center in Washington. In this laboratory, Charlotte Campbell, S. Saslaw, and G. Hill performed thousands of serologic tests for the diagnosis of histoplasmosis, coccidioidomycosis, and blastomycosis. In 1944, A. Wadsworth, director of the New York State Laboratory, appointed Elizabeth L. Hazen, who had joined the state laboratory as a bacteriologist in 1931 and received her training in medical mycology at Benham's laboratory (117), as its first diagnostic and research mycologist (13, 160). The increased incidence of fungal diseases during World War II had stimulated the creation of this position. Following the epidemiologic studies with *H. capsulatum*, the federal government established the Kansas City Field Station as part of the Centers for Disease Control (CDC) to study non-tuberculosis-related calcified pulmonary disease in the Mississippi-Ohio river valley area. Under the direction of the physician Michael L. Furcolow, this center became a source of knowledge about histoplasmosis between 1945 and 1964 (64, 154). As a result, other groups became involved in the study of fungal infections.

The recognition that fungal diseases were not rare also prompted the CDC to place an ad in *Science* for a medical mycologist in 1946 (9, 57). Libero Ajello, who had finished his doctoral training at Columbia University under J. S. Karling, a specialist in aquatic chytrids, competed for and won the appointment. Ajello was sent first to Duke University to establish a medical mycology unit there with Conant, but 2 months later, the federal government decided that this unit (which became the Division of Mycotic Diseases of the CDC) should remain in Atlanta (9). Ajello developed an excellent team of mycologists at the CDC. Under his guidance, Morris Gordon, Lucille K. Georg, Leo Kaufman, William Kaplan, and Arvind Padhye worked on all aspects of medical mycology, including laboratory diagnosis, training, and research (205, 281). In 1947, Jack Utz began his clinical studies and training program at the NIH (117), and Howard W. Larsh began his graduate program at the University of Oklahoma at Norman (156). By the end of 1949, medical mycology training and research were also being conducted at Michigan State University by Everett Beneke and at the University of Cincinnati by Jan Schwarz.

THE ADVENT OF ANTIFUNGAL AND IMMUNOSUPPRESSIVE THERAPIES, 1950 TO 1969

Overview, 1950 to 1959

When the 1950s began, drastic changes were needed in medical mycology because the mortality rate for patients with invasive mycoses stood between 50 and 100%, depending on the severity of the disease. The 1950s experienced the discovery of nystatin, the first active antifungal drug to be used in humans, followed by the development of stilbamidine and, more importantly, amphotericin B (Table 1). Although the azoles have been great additions, the use of amphotericin B remains the gold standard for current physicians (126, 326), and its discovery opened the field of antifungal therapy. Other important contributions were made during this decade. Serological tests for the diagnosis of coccidioidomycosis and histoplasmosis and practical nutritional tests for the identification of the dermatophytes were developed between 1950 and 1958. The ability to identify dermatophytes by their nutritional requirements and morphology made possible accurate reporting by many laboratories (169). The antigenic composition of *C. neoformans* was determined, and the detection of fungi in tissues was improved by the use of fluorescent-antibody conjugants and selective fungal stains. The relationship of opportunistic fungal infections to antibiotic and cytotoxic or immunosuppressive therapies, certain malignancies, and diabetes began to be evaluated during this decade. The preliminary transition from studies of the biology of the fungi to studies at the cellular level were made between 1953 and 1959 (Table 1), mostly at Rutgers University and the NIH. The Veterans Administration Armed Forces Cooperative Study Group on Coccidioidomycosis was formed in 1955. This group, currently known as the Coccidioidomycosis Study Group, has met annually for 37 years to discuss the state of coccidioidomycosis research.

Scientific Contributions, 1950 to 1959

Opportunistic fungal infections in compromised patients. The association of a high incidence of severe fungal infections with antibiotics and steroid therapies, drug addiction, and leukemia or lymphoma, especially Hodgkin's disease, was first documented in the 1950s. Zimmerman reported three cases of *Candida* and *Aspergillus* endocarditis in 1950, "apparently stimulated" by the constant administration of penicillin (472). Torack in 1957 noted that 13 cases of fungal disease were observed at necropsy in patients who had been treated with either bacterial antibiotics, steroids, or both (423). An increase from three fungal eye infections between 1933 and 1952 to 13 cases between 1952 and 1956 was documented at the Registry of Ophthalmic Pathology (167). These 13 patients had received topical applications of steroid-antibiotic combinations.

Since 1894, various individuals had believed that cancer was caused by a yeast. However, in 1902, Nichols concluded that there was no evidence that yeasts have anything to do with human cancer (311). In 1954, Zimmerman and Rappaport (473) evaluated the divergent views concerning the relationship observed, since 1932, between cryptococcal infections and malignant lymphoma, particularly Hodgkin's disease. They reviewed 60 *C. neoformans* cases at the Armed Forces Institutes of Pathology and grouped them according to the extension of the infection. They found that dissemination occurred in patients with malignant lymphoma or leukemia (18 of 24 disseminated cases), and clearly negated the prevalent but erroneous concept that *C. neoformans* was responsible for Hodgkin's disease.

Keye and Magee reviewed the data for 88 cases of mycoses among 15,845 autopsies performed between 1919 and 1955 (213). Although the incidence of primary fungal infections had not increased after 1947, there was a higher volume of secondary mycoses. In general, cryptococcosis and zygomycosis occurred in patients who had lymphomas or leukemia; histoplasmosis, candidiasis, and aspergillosis complicated other diseases as well. The higher incidence of fungal infections observed after 1947 was attributed to the frequent use of multiple antibiotics, cytotoxic chemotherapy, and cortisone (213). These early reports alerted the scientific community to the reality that various immunologic and nonimmunologic factors can predispose individuals to opportunistic fungal infections.

During the 1950s, attempts were made to understand the mechanisms by which diabetes, antibiotics, and cortisone increased the susceptibility of the host to fungal infections. In 1955, Bauer et al. experimentally reproduced the lesions of cerebral zygomycosis (as mucormycosis) in diabetic and nondiabetic rabbits (15). Because the animals were infected by intranasal instillation of conidial suspensions, the infection began in the nasal mucosa and rapidly extended to adjacent tissues, bone, and finally the brain in the diabetic rabbits. In contrast, the nondiabetic rabbits had fewer and smaller lesions that were surrounded by many neutrophils, which did not show the same degenerative changes. Sidransky and Friedman determined that the combination of antibiotics plus cortisone or

cortisone alone rendered animals that inhaled *A. flavus* conidia highly susceptible to developing fatal pulmonary infections (377).

Cerebral phaeohyphomycosis. In 1952, Binford et al. recovered *Cladophialophora bantiana* (as *Cladosporium trichoides*) from a brain abscess of a 22-year-old black male who had complained of frontal headaches for 2 weeks. He was sleepy and drowsy by the time of hospital admission (31). Emmons proposed the name *Cladosporium trichoides*, signifying its hyphal growth in human brain tissue (31). This fungus was transferred to the genus *Xylohypha* in 1986 (280) and to the genus *Cladophialophora* in 1995 by de Hoog et al. (76a). A new species was added to the list of fungi that could cause opportunistic infections in humans.

Dermatophytosis. A Kligman concluded in 1952 that "little basic knowledge has accumulated on ringworm of the scalp since Sabouraud's *Les Teignes* in 1910" (220, 354b). Epidemics of tinea capitis due to *Microsporum audouinii* in the early 1950s prompted Kligman's important, although currently questionable, investigations on the pathogenicity of *M. audouinii* and *Microsporum canis* (220, 221). This epidemic, which was known as the Atlantic City Board Epidemic, was so severe that schoolchildren were required to wear hats in order to go to school (117). Kligman's data were derived from observations on experimentally infected humans that were carried out in a state institution for congenital "mental defectives," where tinea capitis was endemic. He found that only 60% of children and adults were susceptible to experimental infection. He divided the natural course of the infection into four phases: (i) first few days of incubation, (ii) 3-month enlargement of original lesions and development of new ones, (iii) refractory or static infection, and (iv) involution.

Portal of entry for *C. immitis* and *B. dermatitidis*. Wilson et al. followed the course of a case of coccidioidomycosis involving the skin in 1953 (459). The patient was an embalmer who had severely abraded the skin of his finger against a casket. During the embalming of a body, bloody and seropurulent material from the visceral organs containing *C. immitis* cells had come in contact with the abraded skin. From the clinical and mycologic data, it was concluded that the disease originates very rarely as the result of primary cutaneous inoculation (459) and that skin lesions are nearly always the result of dissemination by hematogenous means. A similar conclusion was reached concerning blastomycosis in 1951 by Schwarz and Baum, who reviewed 154 cases of blastomycosis, 58 from their institution (367). It was established that blastomycosis and coccidioidomycosis initiated in the respiratory tract.

Antifungal therapy. The awareness that fungal diseases were common and prevalent emphasized the need for new antifungal agents. Toxicity and other undesirable pharmacologic properties of earlier compounds made them unsuitable for use in humans. Hazen, a bacteriologist, and Brown, a chemist, announced the discovery of nystatin (as fungicidin) at the autumn meeting of the National Academy of Sciences in Schenectady, N.Y., in 1950 (176). They were able to identify, characterize and purify nystatin (named for New York State) following its detection in cultures of *Streptomyces noursei* at the New York State Department of Health. They demonstrated its in vitro and in vivo activity in experimental animal studies. Brown and Hazen donated the royalties of their discovery, which amounted to more than $13,000,000 by 1976 (patent expired), to a special fund designated the Brown-Hazen Program. Rachel Brown and Elizabeth Hazen were inducted posthumously into the National Inventors Hall of Fame. They are the second and third women, respectively, to be so honored (160, 336).

Until 1950, blastomycosis was treated with iodides, X-ray therapy, and surgical procedures. Nevertheless, a 92% mortality rate occurred in patients with disseminated *B. dermatitidis* infections. Following Elson's report in 1945 (106) that propamidine was effective in vitro against *B. dermatitidis*, three patients were treated with this agent and a related compound, the aromatic diamidine stilbamidine, in 1951 (365). These patients were suffering from systemic blastomycosis and showed improvement; unfortunately, the toxicity of stilbamidine necessitated brief and intermittent therapy. In 1953, a derivative of stilbamidine, 2-hydroxystilbamidine, was given to another three patients with blastomycosis for a prolonged and extensive course without evidence of toxicity (389).

In the meantime, the search for more effective antifungal agents continued. The two antifungal "materials," amphotericins A and B, were isolated from a broth culture of a *Streptomyces* sp. at the Squibb Institute (152). Broth dilution assays for in vitro susceptibility demonstrated that amphotericin B was more active but less soluble than amphotericin A against the yeasts and filamentous fungi tested. Both amphotericins A and B demonstrated antifungal activity in vivo when administered subcutaneously to experimentally infected animals. Steinberg et al. demonstrated between 1955 and 1956 that the oral administration of amphotericin B was not as effective as intravenous therapy (401). In 1957, Harrell and Curtis were successful in treating four "stilbamidine-failure male patients" with intravenous amphotericin B (173). These four patients had blastomycosis and had been treated for 6 to 8 h with 50 mg of amphotericin B suspended in 1,000 ml of 5% dextrose in water. A few months earlier, Fiese had treated a patient who had disseminated coccidioidomycosis with oral amphotericin B and obtained a "beneficial clinical response" (124).

Beginning in 1956, investigators at the NIH studied the in vitro and in vivo efficacy of amphotericin B in experimental cryptococcosis and histoplasmosis (258), as well as the efficacy, safety, and pharmacokinetics of oral and intravenous amphotericin B given to patients with severe fungal infections (259, 424). Their data indicated that oral or intraperitoneal amphotericin B was highly protective against usually fatal murine cryptococcosis and histoplasmosis, but in humans oral amphotericin B was poorly absorbed, and only low serum and CSF levels were attained. In contrast, intravenously administered amphotericin B was excreted slowly, and it produced fungistatic concentrations in blood for prolonged periods after the medication was discontinued. Louria concluded in 1958 that the intravenous dosages used were beneficial for systemic infections not involving the central nervous system (259).

Several investigators used a new formulation of amphotericin B (E. R. Squibb & Sons), which had became available in 1956 under the trade name Fungizone, between 1958 and 1959 (252, 253, 368, 425). When dissolved, it formed a golden colloidal suspension (368). Fungizone was very effective against *B. dermatitidis*, *H. capsulatum*, and *S. schenckii* as an intravenous solution. Unfortunately, the results were inconclusive for patients with disseminated *C. immitis* infections and *C. neoformans* meningitis. Also, toxic effects which disappeared after therapy were reported. In 1958, Chick et al. determined the effect of this drug in an experimental rat model with the opportunistic zygomycete *Rhizopus arrhizus* (as *R. oryzae*) and concluded that amphotericin B appeared promising as a therapeutic agent for human zygomycosis (58).

A third antifungal agent, griseofulvin, a metabolite produced by *Penicillium janczewskii* (as *P. griseofulvum*) (319), was developed in Scotland. It was demonstrated in 1958 that griseofulvin was effective against *Trichophyton mentagrophytes* infec-

tions in guinea pigs (142). This was the first oral antifungal compound known to be effective against a dermatophyte.

Laboratory diagnosis. In the 1950s, C. E. Smith and coworkers discovered that the mixture of coccidioidin with serum from infected guinea pigs yielded a precipitate (388). Smith collaborated with two serologists, K. Meyer and B. Eddie (a coauthor), who were experts on CF testing. Together, they performed 21,000 precipitin and CF tests for coccidioidomycosis from 1940 to 1949. It was demonstrated that the combined CF and precipitin tests detected less than 10% of asymptomatic coccidioidomycosis cases, more than 90% of clinically apparent cases, 60% of pulmonary cavitation lesions, and 99% of disseminated cases. Each test alone detected from 22% (CF) to 44% (precipitin) of these cases. However, there were cross-reactions with histoplasmosis. From the results of both skin reaction and serology tests, it was discovered that the skin reaction is positive first, followed by the detection of precipitins and CF antibodies in primary noninvasive disease. The CF antibodies persisted, and an increase in titer corresponded to the severity of the infection; a decrease correlated with patient improvement (388).

Coons and M. H. Kaplan developed in 1950 a specific histochemical technique for detecting antigenic material in tissues by using fluorescein-labeled antibodies (67). This method was applied initially to demonstrate the capsules of bacteria and later by Eveland and coworkers for the study of *C. neoformans* in tissues (122). In 1958, Vogel and Padula used the fluorescent stain method to demonstrate antibodies in sera from patients who had various systemic diseases (431), and Gordon applied this method to the differentiation of yeasts in the clinical laboratory (158).

Ouchterlony's bacteriologic agar-precipitin technique (318) was adapted by Heiner for the immunodiagnosis of histoplasmosis in 1958 (177). He noted that CF tests were disadvantageous because of cross-reactions and the insuitability of anticomplementary sera. He evaluated more than 2,000 sera from patients with culturally proven blastomycosis, coccidioidomycosis and histoplasmosis; patients suspected of having these mycoses; patients suffering from other diseases; and "normal" volunteers. Heiner's examination of the precipitin bands demonstrated that histoplasmin contained at least six distinct soluble antigens and that the absence of a histoplasmin precipitin band ruled out active histoplasmosis. He concluded that sera from proven cases of histoplasmosis showed h and m bands which correlated with CF titers. M bands were also found following a histoplasmin skin test (177). This easy-to-perform test has been useful for many years for the presumptive diagnosis of histoplasmosis.

Special staining techniques for the specific demonstration of fungi in tissues were developed from 1951 to 1953. Examination of tissue sections was difficult because most pathologists did not have sufficient mycologic experience to adequately examine these specimens. The adaptation of Hotchkiss' (186) and McManus' (282) stains for fungi provided a solution to the problem. The procedure was based on the fact that the cell wall of fungi is composed of chitin, which is absent in higher animals. Tissues were hydrolyzed with periodic acid to release the aldehydes, which were then combined with the Schiff reagent (219). The light green counterstain in tissue offered a good contrast to the deep rose-colored fungi. The periodic acid-Schiff (PAS) stain was modified further by Gridley in 1953, who replaced periodic acid with chromic acid in order to demonstrate both hyphal and yeast cells in tissues (164).

Cycloheximide was added to the mycologic medium, mycobiotic agar or Mycosel, which was developed originally as a selective isolation medium for *C. immitis* in 1951 by Georg et al. (145). This medium inhibits the growth of *C. neoformans* as well as most saprophytic fungi when used at low concentrations. The description of the usefulness of cycloheximide had a great impact. The nutritional requirements of the dermatophytes continued to be studied by Benham and coworkers (25, 378, 379, 381) and by Ajello, Georg, and Camp (3, 146). Georg and Camp simplified the procedures for studying the dermatophytes in the clinical laboratory from a nutritional point of view in 1957 (146). Georg's doctoral dissertation, which was supervised by Benham, was the basis of her nutritional studies. Their differential medium formulations have been used to prepare seven commercially available *Trichophyton* agars, which have been an invaluable laboratory diagnostic tool. In the same year, Ajello and Georg (3) further investigated Davidson and Gregory's (74) discovery in Canada of the formation of wedge-shaped perforations caused by organized groups of hyphae in hair filaments exposed to *T. mentagrophytes*. Ajello and Georg systematically studied the morphology, pigmentation, and hair penetration of 40 isolates of *T. mentagrophytes* and *T. rubrum*. They concluded that the different manner in which these two species attack hair in vitro is a diagnostic aid for their identification when correlated with morphology (3).

Taxonomy and classification. A taxonomic issue was clarified by Benham in 1950, when she reviewed the different names applied to the yeast *C. neoformans*. The generic name *Cryptococcus* was adopted by Vuillemin in 1901 for pathogenic yeast-like fungi that did not develop ascospores (24). Vuillemin gave the name *C. hominis*, a fungus isolated originally in Europe by Busse and Buschke in 1894, what is known today as *C. neoformans*. Lodder compared Sanfelice's *Saccharomyces neoformans* isolated from a peach with Busse and Buschke's isolate and found them to be identical (24). Therefore, Benham concluded that the fungi described by Stoddard and Cutler (409) and others (129) from meningeal infections were actually the same fungus, that is, *C. neoformans*. She also stated that although yeast cells are found in tissues in both blastomycosis and cryptococcosis, blastomycosis is an entirely different disease, caused by the mycelium-producing fungus *B. dermatitidis* (24).

At the CDC in 1951, Gordon was able to culture "spherical forms" of *Malassezia furfur* (*Pityrosporum ovale*) by adding olive oil or saturated fatty acids to Sabouraud dextrose agar (157). Because the spherical forms grown had different morphological and physiological characteristics than those of *P. ovale*, Gordon named this fungus *P. orbiculare*. Burke described pityriasis versicolor in individuals experimentally infected with *M. furfur* (as *P. orbiculare*) in 1961 (46), and 2 years later, Keddie and S. Shadomy studied the morphologic and antigenic relationships between *P. orbiculare* and *M. furfur* (210). Everyone now agrees that *P. ovale* and *P. orbiculare* are forms of *M. furfur.*

Ecology. The lack of a specific distribution pattern of *C. neoformans* suggested that this fungus occurred widely in nature as a saprophyte. In 1951, Emmons, while he was looking for *H. capsulatum*, isolated four strains of *C. neoformans* from soil in Loudoun County, Virginia (112). Further studies in 1955 permitted Emmons to observe that *C. neoformans* was present in pigeon feces and that feces provided a good medium for the fungus (113). At that time, Emmons was not able to appreciate the significance of his findings because of the rarity of cryptococcosis. The association of *C. neoformans* infections with immunosuppression was not recognized until later.

Ajello and Zeidberg isolated *P. boydii* from soil while they were searching for *H. capsulatum* in Williamson County, Tennessee, in 1951 (2). Emmons had previously isolated *H. capsulatum* from soil in 1949 (111); Zeidberg et al. found that *H.*

capsulatum was isolated more frequently from soil that was enriched with chicken feces in 1952 (471). Therefore, chicken coops and chicken yards were shown to be potential point sources of infection for the development of histoplasmosis.

Immunochemistry. Evans investigated the antigenic composition of *C. neoformans* from 1949 to 1953 (118–121). It was difficult to perform serologic investigations of *C. neoformans* because the antibody response in experimentally immunized animals was poor. However, Evans successfully developed high-titer antisera by injecting encapsulated Formalin-killed *C. neoformans* cells into rabbits (118). Evans and Kessel reported that *C. neoformans* may be divided into three serologic types, A, B, and C, on the basis of antigenic differences revealed by agglutination reactions (118–120). Isolation of the capsular polysaccharide of types A, B, and C provided similar results by precipitin reactions. Further experiments demonstrated that the capsular materials of type B had two carbohydrate fractions, called SB1 and SB2 (121). Seventeen years later, Vogel identified the fourth serotype, D (as R) (432).

Physiology and nutrition. The fact that most fungal pathogens are dimorphic indicated the importance of studying the phenomenon of their conversion from a mycelium form to the corresponding tissue form. In 1953, Nickerson initiated basic studies on the mechanisms of the cellular division and morphogenesis of *C. albicans* (312). Nickerson and Mankowski found that the replacement of glucose by soluble starch, glycogen, or dextrin diminished the filamentous growth of *C. albicans* (313). The growth of budding yeast cells was dependent on glucose as the source of carbon. Later, Nickerson was able to identify for the first time a cellular oxidation mechanism at the flavoprotein locus, which was essential for cellular division by budding but not for growth (314). He demonstrated this metabolic locus by the development of a filamentous mutant of *C. albicans*. This morphological mutant differed from the parent only in its impairment of a cellular oxidation mechanism at a flavoprotein locus (314). In 1959, Kessler and Nickerson isolated "clean cell walls" of three *C. albicans* strains and determined that they contained a glucan-protein and two distinct glucomannan-proteins (212). Salvin discovered that *C. albicans* was the second pathogenic fungus shown to produce a toxic effect after inoculation into laboratory animals (358).

Two important papers appeared in the literature between 1957 and 1958 concerning the nutritional requirements of the in vivo forms of *C. immitis* and *H. capsulatum* (65, 329). Converse grew the spherules of *C. immitis* in a medium composed of glucose, ammonium acetate, and inorganic salts in 1957 (65). The addition of an anionic surface-active agent to the medium maintained the tissue form through serial transfers. In 1954, Pine conducted studies at the NIH regarding the conversion of *H. capsulatum* to its yeast form, which resulted in the development of a three-amino-acid medium for yeast conversion (329). Salvin's finding in 1949 that sulfhydryl groups affected by temperature determined the growth form of *H. capsulatum* (357) was confirmed by Pine and Peacock in 1958 (329).

Training and research centers. In 1950, Lucille K. Georg was recruited by Libero Ajello to continue her work with the dermatophytes and actinomycetes at the CDC. She retired in 1972 and died on 17 June 1980 (8). A new species, *Trichophyton georgiae*, was named in her honor in 1964. In addition, the Lucille K. Georg medallion was created as a scientific award by the International Society for Human and Animal Mycology (8). Margarita Silva-Hutner, who trained under William Weston at Harvard University, replaced Georg at Columbia University. Benham died on January 17, 1957, and Silva-Hutner became director of the laboratory in 1956 (117). Early in 1955, the physician Morris F. Shaffer, who had arrived at Tulane University in 1943, traveled to Berkeley and recruited Lorraine Friedman to develop a medical mycology unit at Tulane University (374). Friedman had obtained her doctoral degree in 1951 under Conant at Duke University (117). In addition to diagnostic work, Friedman's laboratory became a training and research nucleus as she led the field in training the first generation of medical mycologists to use molecular techniques (223). The first NIH medical mycology training grant was awarded to Friedman in 1958 and was renewed continuously for more than 30 years (100, 223).

Dexter Howard began his work at the University of California at Los Angeles in 1956 as an instructor of mycology. He is recognized for his numerous basic research contributions on dimorphism and host-parasite interactions in histoplasmosis and for the training of numerous master's and doctoral degree students (117). In 1959, H. Levine was appointed chief of the Mycology Division at the Naval Biological Laboratory in Oakland, California (248). Hazen retired from the New York State Laboratory in 1959 and was succeeded by Gordon, who had been working at the CDC (13, 160).

Overview, 1960 to 1969

Evaluations of large numbers of patients in cancer, leukemia, and transplantation service centers alerted the medical community to the fact that fungal infections were one of the important causes of morbidity and mortality among these patients (Table 1). The response of the government and the pharmaceutical community was immediate and provided the financial, technological, and human resources that brought rapid advancement in the field during the 1960s. The NIH, the CDC, and other government-sponsored centers initiated evaluations of antifungal therapies and developed improved and faster diagnostic tests. Others within the discipline expanded academic education and training.

The perfect states of some fungi were discovered during this decade. Other basic research contributions included achieving a better understanding of the mechanisms of the mycelium-to-yeast conversion by *H. capsulatum* var. capsulatum and *S. schenckii* as well as the role of host defenses in chronic histoplasmosis and other opportunistic mycoses. The role of the capsule of *C. neoformans* as a virulence factor was also investigated. In applied research, additional adaptations and the development of serological procedures contributed new dimensions to the rapid diagnosis of fungal diseases by laboratorians (Table 1). Another antifungal agent effective against yeast infections, 5-fluorocytosine, was developed and tested. In clinical research, tuberculosis began to be managed effectively by 1955, and the Veterans Administration and armed forces increased their support for the study of fungal diseases. As a result, multicenter investigations began to study mycoses in large numbers of patients. A leader of this approach was J. Buse, who gathered and analyzed a great deal of data while at the VA Medical Center in Jackson, Miss. His group initiated the first cooperative study for the evaluation of the effectiveness of amphotericin B and stilbamidine (117).

On November 6, 1961, Friedman and D. Schneidau from Tulane University requested the creation of a Medical Mycology Division within the American Society for Microbiology (ASM) from ASM President John E. Blair. The purpose of this division was to arrange a program centered in medical mycology during the annual ASM meetings and provide a focal point to the activities of medical mycologists (189). In 1972, microbiologist groups that gained the affiliation (votes) of at least 150 members were given the status of ASM divisions. The

Medical Mycology Division was then established by the votes of 181 members (10).

In 1965, a steering committee, which included Ajello, Campbell, Friedman, Milton Huppert, Levine, and Silva-Hutner, organized the Medical Mycological Society of the Americas. They met in Phoenix, Arizona, during the Second Coccidioidomycosis Symposium and planned an organizational meeting to be held during the ASM Annual Meeting in 1966 (5, 6). Ajello was the first president. In 1969, the society established the Rhoda Benham Award to be given to individuals who contributed significantly to the development of medical mycology.

Scientific Contributions, 1960 to 1969

Opportunistic fungal infections in immunocompromised patients. Human renal transplantation and immunosuppressive therapy began in the early 1960s. The incidence of infectious diseases, including fungal infections, was similar to that observed in patients with various malignancies. Rifkind et al. found systemic fungal infections at autopsy in 23 of 51 patients (45%) treated by means of renal transplantation and immunosuppressive agents between 1962 and 1965 (338). Antemortem diagnosis and amphotericin B therapy were possible for only two of these patients. These diagnoses were confirmed at autopsy.

The increased incidence of mycoses in cancer and leukemia patients began to be reported by 1964. Aspergillosis was found to be a contributing factor in the cause of death in 37% of cancer patients (196). Bodey found 189 fungal infections among 161 patients with acute leukemia at the National Cancer Institute (NCI) (61% mortality rate), with a significant increase in their incidence between 1959 and 1964 (35). The patients had more prolonged granulocytopenia prior to infection than patients enrolled in the control group. Bodey concluded that cryptococcosis and histoplasmosis were less closely associated with adrenal cortical steroid therapy than other mycoses. This conclusion correlated well with the results from experimentally infected animals (260) and in vitro studies (56). A review of 3,374 autopsies also conducted at the NCI from July 1953, when the NCI opened, until January 1968 disclosed 98 cases of fatal aspergillosis (469). The most perplexing feature was the difficulty in isolating the etiologic agent antemortem (only 34%). These studies emphasized the need for improvement in the laboratory diagnosis of mycoses.

Antifungal therapy and clinical studies. Successful therapy of the mycoses with amphotericin B was followed by earnest efforts to improve diagnosis, treatment, and the understanding of the different aspects of fungal disease. By 1963, Winn stated that systematization of coccidioidomycosis was needed because this infection had many variable manifestations (462). He developed a system of classification based on radiologic changes in the lungs using his 20 years of experience in dealing with the pathologic manifestations of this infection (462). Winn reviewed data from 100 patients who had been favorably treated with amphotericin B and divided coccidioidomycosis into primary pulmonary coccidioidomycosis, acute and chronic disseminated coccidioidomycosis (respiratory to meningeal diseases), and primary extrapulmonary disease by cutaneous inoculation (462). The value of intrathecal amphotericin B therapy rather than of long-term intravenous therapy for coccidioidal meningitis was emphasized in this publication.

The need for cooperative studies regarding blastomycosis and other systemic diseases was recognized by members of the Cooperative Study Group on the Chemotherapy of Tuberculosis of the Veterans Administration-Armed Forces in the 1950s (48). As a result of the success of that program, committees were established in the mid-1950s to study coccidioidomycosis and histoplasmosis and later in 1957 to study blastomycosis. The first of these studies was led by Busey in 1963, who synthesized clinical information through a retrospective study of 198 cases of blastomycosis diagnosed over a 12-year period at 26 Veterans Administration hospitals (48). Blastomycosis was confirmed to be prevalent in the Mid-Atlantic, South Central, and Ohio-Mississippi river valley states. The latter information was confirmed by Furcolow et al. in 1970, when they reviewed all proven cases of human (1,476 cases) and canine (384 cases) blastomycosis (134).

The study to compare the effectiveness of 2-hydroxystilbamidine and amphotericin B began in 1955 and revealed that amphotericin B was more effective (78% successfully treated) than the former drug (58%) in managing this mycotic infection (254). Furcolow and the CDC Cooperative Mycoses Study Group members in Kansas City reported the results of their first large cooperative study on long-term follow-up evaluation of amphotericin B therapy for histoplasmosis in 1963 (133). Comparison of data from 194 treated and 115 untreated patients indicated that the dose should be at least 25 mg/kg (23% mortality). Inadequately treated patients (<25 mg/kg) showed little difference from untreated patients (85% mortality).

Despite the introduction of amphotericin B and its successful use for the treatment of fungal infections, its toxicity limited its use. Drutz et al. investigated a new therapeutic approach to reduce the dose-related toxicity of amphotericin B (103) when Drutz was doing his postdoctoral training at the NIH. The drug was administered daily for a 10-week period in doses large enough to achieve peak serum levels that were at least twice those necessary for inhibition of the fungus. These lower total doses were sufficient to control 15 systemic cryptococcosis, histoplasmosis, and blastomycosis infections in patients with impaired renal function. This antifungal regimen was used in the 1970s by Bennett and his coworkers for their prospective study on the treatment of cryptococcal meningitis (26).

The new oral agent 5-fluorocytosine (flucytosine [5-FC]) was developed in 1964 by Hoffmann-La Roche in Switzerland and found to be effective in the treatment of two patients and mice infected experimentally with *C. albicans* and *C. neoformans* (419). The first patient had *Candida* sepsis and received oral 5-FC for 21 days (2 to 4 g daily). The second patient had cryptococcal meningitis and was treated with 2.25 to 9 g of 5-FC daily for 50 days (419). Also, Utz and his associates treated 15 patients who had cryptococcosis with oral 5-FC for 14 to 42 days in 1968 (426). They noted improvement in 9 of the 11 patients with meningeal disease. However, relapse occurred in four of those nine patients. By the 1970s, the development of drug-resistant *C. neoformans* isolates during therapy with 5-FC was reported (33). Nevertheless, the utility of this drug was fully recognized in the late 1970s, when it was used in conjunction with amphotericin B for managing cryptococcal meningitis. Synergism between these two antifungal agents was demonstrated in the 1970s, which provided new alternatives for therapy. S. Shadomy reported the first systematic study of the in vitro susceptibilities of 5-FC and amphotericin B against *C. neoformans* in 1969 (373). The procedures that he developed became the basis for preclinical in vitro screening of new compounds and as testing procedures in clinical laboratories throughout the world.

Laboratory diagnosis. Rapid, reliable, and reproducible serological methods for the diagnosis of fungal infections were developed in the 1960s. The earlier adaptation of the fluorescent-antibody technique (67) by several investigators (122, 158, 431) demonstrated the potential value of this method for fungi. Data from these earlier studies led to further adaptations of

this method at the CDC for the rapid detection and identification of *S. schenckii* (203), *H. capsulatum* (208), and *B. dermatitidis*, *C. immitis*, *C. neoformans*, *H. capsulatum*, and *S. schenckii* (204, 205) in Formalin-fixed and paraffin-embedded tissues. This application of the fluorescent-antibody technique provided a faster laboratory diagnostic method than culture.

The first demonstration of the "occurrence" of cryptococcal antigen (as serologically reactive substances) in body fluids from a patient infected with *C. neoformans* was reported in 1951 (308). This discovery led to the development of the latex agglutination test for the diagnosis of systemic cryptococcosis by Bloomfield et al. in 1963 (34). These investigators detected *C. neoformans* antigens in serum and CSF samples from nine patients with cryptococcal meningitis. This was an important breakthrough, because existing diagnostic assays to detect the production of antibody to *C. neoformans* were negative for 60% of serum specimens from patients with this infection. Even today, tests for antibody detection give false-negative results. On the other hand, by 1974, high cryptococcal antigen titers had been identified as valuable predictors of the outcome of cryptococcal meningitis (83). Other investigators attempted to develop latex and immunodiffusion tests for *C. immitis* to replace the CF test (193, 194). However, the CF test remains the gold standard among the serologic tests for coccidioidomycosis. Lurie and Still demonstrated by electron microscopy that the halo surrounding *S. schenckii* cells in tissue was not a capsule but an artifact formed by an antigen-antibody complex (265).

A new culture medium called dermatophyte test medium (DTM) was devised by Taplin et al. (418) for the simplified diagnosis of dermatophytosis during the Vietnam conflict, when skin lesions were heavily contaminated with soil organisms. This medium contained a mold inhibitor, antibiotics, and a pH indicator. Growth of a dermatophyte raised the medium pH, as evidenced by a color change from yellow to red.

Taxonomy and classification. The perfect states of several fungal pathogens were described between 1962 and 1967. Georg et al. described *Microsporum vanbreuseghemii* and its perfect state in 1962 (147). They proposed that this dermatophyte be classified in the genus *Nannizia* as *N. grubyia*. In 1967, Ajello and Cheng demonstrated that the granular form of *Trichophyton mentagrophytes* was heterothallic and named its perfect state, *Arthroderma benhamiae*, in honor of Benham (4). Weitzman clarified several taxonomic issues concerning the sexual state of *Microsporum gypseum* in 1964 (445, 446). She confirmed Stockdale's (407) conclusion that *M. gypseum* was the imperfect state of at least two cleistothecium-forming species, *Nannizzia incurvata* Stockdale and *Gymnoascus gypseum* Nannizzi emend Griffin (445). From the latter findings, Weitzman also concluded that pleomorphism in *M. gypseum* was the result of gene mutation and therefore the term pleomorphism was to be discarded in favor of the term mutation (446). These results represented a portion of Irene Weitzman's doctoral dissertation, written under the guidance of L. Olive at Columbia University (117, 448). While Kyune-Joo Kwon-Chung was doing her doctoral training with K. Raper at the University of Wisconsin, Kwon et al. reported the first heterothallic species of *Aspergillus* (*A. nidulans* group) in 1964 (230). The sexual stage of *B. dermatitidis* was discovered by McDonough and Lewis in 1967 (275). The discovery was made during the study of the growth characteristics of paired strains of *B. dermatitidis*, which was A. Lewis' dissertation research project. The teleomorph was described as *Ajellomyces dermatitidis* in honor of Ajello by McGinnis and Katz (277).

Ecology. The first report of the isolation of *B. dermatitidis* from nature was reported in 1961 by Denton and coworkers (81). Arthur Di Salvo stated in 1994 that prior to this report, the ecologic niche occupied by this fungus was only presumed to be soil (92). It required 15 years to show that *B. dermatitidis* could be cultured from natural substrates (soil specimens) associated with an outbreak of human disease.

Dimorphism mechanisms. The transformation from the mycelial form to the yeast form of *H. capsulatum* and *B. dermatitidis* had been studied by Howard since the late 1950s (187). He thought that the conversion process of the dimorphic fungus *S. schenckii* was similar to that observed in these two other fungi. Howard demonstrated that *S. schenckii* had two different morphologic transformations during its transition from the mycelial to the yeast phase, the formation of budding structures at the tip of the hyphae and the formation of chains of conidia and fragmentation of those chains (187). The latter transformation was similar to those of *H. capsulatum* and *B. dermatitidis*. Therefore, Howard nullified the prevalent concept that yeasts were formed only directly from conidial cells. Pine and Webster (330) in 1962 used the synthetic medium developed earlier by Pine and Peacock (329) in 1958 to study the conversion of *H. capsulatum*. Pine and Webster observed that the formation of yeast cells could occur either by direct conversion of microconidia, by budding of hyphal cells, or by "monilial" chain formation. Beneke et al. studied the enzymological basis of conversion in *B. dermatitidis* in 1969 and showed that this fungus produced acid and alkaline phosphatases in the yeast form (37°C) but little or none of these enzymes in the mycelial form (18).

Host defenses against *C. albicans*. The capacity of neutrophils to phagocytize *C. albicans* was also investigated in the 1960s. *C. albicans* cells were visualized within neutrophils after their intravenous inoculation into mice. Louria and Brayton found that *C. albicans* cells were phagocytosed and survived within the host cells in 1964 (261). The *Candida* hyphae penetrated viable neutrophils in vitro. These results led to the studies conducted in 1969 by Lehrer and Cline (245), who measured quantitatively the candidacidal activity of human leukocytes and serum from normal individuals and patients with fungal and other infections. They concluded that the neutrophil played an important role in resistance to *C. albicans* infections and that the lysosomal enzyme myeloperoxidase and its oxidant substrate, hydrogen peroxide, were the major participants in lethal neutrophil activity. Although serum factors were necessary for optimal phagocytosis, they lacked direct candidacidal activity. Two decades later, it was confirmed that neutrophils are the most effective killers of most fungal pathogens.

Host defenses against *H. capsulatum*. In 1965, Howard studied the effect of environmental conditions on the intracellular generation time of *H. capsulatum* (188), which he previously had shown to be constant within mammalian histiocytes. He demonstrated that monocytes from immunized mice did not restrict intracellular proliferation and that hyperimmune serum did not alter this outcome. Howard concluded that it was difficult to accept any proposed mechanism of immunity which involved the activity of specific humoral antibodies (188). In 1968, Newberry et al. studied the stimulation or transformation by histoplasmin of peripheral blood lymphocytes from both histoplasmin-negative and -positive individuals as well as patients with either acute or chronic histoplasmosis (310). Using a more "sensitive indicator" of lymphocyte transformation, the uptake of a radioactive DNA precursor, they observed that lymphocyte transformation was depressed in chronic patients. These results suggested that cellular immunity was a major aspect in chronic histoplasmosis.

Host defenses against *C. neoformans*. Since the 1950s, conflicting results had been obtained regarding the capsule of *C. neoformans* and phagocytosis. Bulmer et al. isolated seven non-

encapsulated mutants of *C. neoformans* and compared their virulence with that of their encapsulated parent in 1967 (44). They observed that the mutant strains were initially avirulent for mice but became virulent to various degrees after reversion to the encapsulated state. Histopathological examination of tissue from human cases of cryptococcosis have shown for many years that giant cells contain *C. neoformans*. Because the capsule seemed to be a virulence factor, Bulmer and Sans investigated capsular mutants and phagocytosis (45). Phagocytosis was approximately three times more effective when nonencapsulated mutants were used, which indicated that the polysaccharide capsule inhibited phagocytosis of *C. neoformans.*

DNA studies. Fungal DNA studies began to be reported in the 1960s. Storck (410) in the United States and Stenderup and Bak (402) in Denmark studied the nucleotide composition of DNA from usually nonpathogenic filamentous fungi and 18 species of the genus *Candida*, including the opportunistic pathogens *C. albicans* and *C. tropicalis*. They found that the mean base composition, most frequently expressed as the percent guanine plus cytosine content, varied among the species studied and that this kind of analysis appeared to have taxonomic and phylogenetic significance.

Physiology and nutrition. Rippon stated in 1967 that although the mechanisms of disease induction had been investigated for bacteria, the factors involved in fungal infections had been neglected (341). He conducted important enzymologic studies between 1967 and 1969 and associated the production of elastase and collagenase with the more virulent forms of dermatophytoses (341–343). The production of enzymes also correlated with mating types (343).

An important contribution to the development of medical mycology was the delineation of the characteristics that separate fungi from other eucaryotic organisms. Fungi biosynthetically synthesize lysine by using L-α-aminoadipic acid, and they have chitin in their cell walls. These characteristics delineated the uniqueness of fungi (344). Whittaker proposed in 1969 that fungi should be grouped as a separate kingdom (453), which was a fundamental step in the development of medical mycology as a science.

Training and research centers. At the Medical College of Virginia (MCV), the history of medical mycology began when Jack Utz moved there from the NIH in 1966 as the first chairman of the Division of Infectious Diseases (117, 372, 428). He brought with him Jean and Smith Shadomy. J. Shadomy was trained by Orda Plunkett at the University of California, Los Angeles (117, 168, 372). These three investigators and Richard Duma, who succeeded Utz as chairman in 1974, established a medical mycology training and research program at MCV. Also in 1966, Kyune-Joo Kwon-Chung came to Emmons' laboratory at NIH as a visiting fellow to work on the genus *Aspergillus* as well as the genetics of the dermatophytes (117, 237). In that same year, John Bennett, who received his mycology training under Emmons, became head of the Infectious Diseases Section at NIH (27, 117). The demand for more medical mycology training led to the appointment of Leanor Haley at the CDC as chief of the newly founded Medical Mycology Training Branch in 1968. By 1969, medical mycology training and research was being conducted at seven additional centers. The CDC Kansas City Medical Mycology Unit was closed in 1968 following Furcolow's departure from this center in 1964. C. E. Smith, who provided vital information on the epidemiology and laboratory diagnosis of coccidioidomycosis during the 1940s, died on 18 April 1967.

THE YEARS OF EXPANSION, 1970 TO 1979

Overview

During the years of expansion, the science of medical mycology flourished at all levels of research and training (Table 1) as major scientific events changed and enlarged the role of fungal diseases in clinical medicine. There was an increase in the spectrum of available antibiotics, immunosuppressive and cytotoxic therapeutic agents, and their use. The latter two targeted the hematopoietic cells of the neutrophil lineage in the host and rendered patients highly susceptible to opportunistic fungal infections. The increased incidence and prevalence of these infections led to the development of rapid and commercial tests for the detection of fungi in clinical specimens and the identification of fungi in the clinical laboratory, e.g., improved techniques for the direct demonstration of *Candida* cells in blood samples and the API 20C yeast identification system for the differentiation of medically important yeasts. The latter test was the first commercial product that permitted rapid and accurate identification of the majority of pathogenic yeasts (356). When it was evident that the detection of antibodies was not a reliable laboratory tool for the diagnosis of invasive aspergillosis and candidiasis, alternative approaches, such as the detection of fungal antigens and metabolites, began to be investigated. The identification of the dimorphic fungi in vitro was also simplified by the development of exoantigen tests. The availability of these tests stimulated interest in medical mycology among microbiologists and physicians, and the simplification of diagnosis improved patient care. As a consequence, there was further demand for advanced formal and informal medical mycology training as well as research. Particularly acute was the demand for trained clinical laboratory personnel and the need to provide physicians with concise, up-to-date information on the fungal pathogens. Several postdoctoral and graduate programs were developed and supported with NIH and Brown-Hazen Program funds. The latter funds were devoted exclusively to medical mycology training and research.

During the 1970s, data from large, cooperative therapeutic trials established that combined 5-FC and amphotericin B was the regimen of choice for the treatment of cryptococcal meningitis. Also, ketoconazole was developed by Janssen Pharmaceuticals in Belgium. In the late 1970s, the Mycoses Study Group, later known as the National Institute of Allergy and Infectious Diseases Mycoses Study Group (NIAID-MSG), was formed under the sponsorship of NIAID by a group of physicians who saw the need to conduct systematic studies of fungal diseases and their therapy by developing collaborative protocols. John Bennett, Richard Duma, William Dismukes, and Gerald Medoff met informally at O'Hare Airport in the late 1970s and selected Dismukes, at the University of Alabama–Birmingham, as the chair of the group (95). The first NIH grant for these studies was awarded in 1978. The effect of the NIAID-MSG contributions to the development of the discipline of medical mycology was dual, medical and global (95, 354). From the medical point of view, the main contribution was the evaluation of oral antifungal agents for the treatment of systemic mycoses. From the global point of view, NIAID-MSG crystallized thinking about the importance of systemic mycoses and brought attention to the field. The management and understanding of fungal diseases have been improved by this group.

Pathogenesis and host-parasite interaction studies continued in the 1970s with the development of animal models. The understanding of cell defense and other immunologic mechanisms and virulence was viewed as the means to develop new

strategies for the control and prevention of fungal diseases. In the pursuit of a complete understanding of fungal pathogens, investigations on the immunochemistry and taxonomy of the fungi were intensified. The perfect states of other fungi were also described.

Scientific Contributions

Opportunistic fungal infections in immunocompromised patients. Meyer et el. found in 1973 that cases of aspergillosis doubled between 1969 and 1970 at the Memorial Sloan-Kettering Cancer Center in New York City (288). Kauffman et al. reported 58 histoplasmosis cases in immunosuppressed patients between September 1953 and December 1957 at the Indiana University Medical Center (206). A higher incidence of disseminated *C. tropicalis* infections, which previously had been associated with only 20 to 25% of candidiasis cases, began to appear in the literature (287). Wingard et al. reported in 1979 that 15 of 18 candidiasis cases were caused by *C. tropicalis* during a 12-month period at the Johns Hopkins Oncology Center (460). Wingard et al. reported in 1991 that the replacement of intravenous miconazole with oral fluconazole for antifungal prophylaxis in marrow transplant recipients had created an increase in both colonization and disseminated infections caused by *Candida krusei* at the same cancer center in the spring of 1990 (461).

Phaeohyphomycosis. The new species *Phialophora parasitica* was isolated from a subcutaneous infection in a kidney transplant patient by Ajello et al. in 1974 (7). The patient, who was on immunosuppressive maintenance therapy at Stanford University Hospital, developed an abscess on the left arm that drained small amounts of yellow exudate. The lesion increased in size, and the entire granulomatous mass (1 cm) was surgically removed, after which the patient was free of further infection. Biopsy material revealed numerous granulomata in the dermis, with a central core of necrosis that contained septate, branched, phaeoid hyphae. Microscopic examination of the colonies revealed the presence of numerous hyaline, oxoid to cylindroid conidia and simple phialides without collarettes (7). The fungus was named *P. parasitica* because it resembled other members of the genus *Phialophora*. The term phaeohyphomycosis was proposed by these authors as a collective name for mycoses caused by several species of phaeoid fungi when phaeoid hyphal tissue-form cells were seen, in contrast to the muriform tissue cells of chromoblastomycosis. Historically, chromoblastomycosis and subcutaneous infections caused by phaeoid fungi would have been described under the single name chromomycosis.

Antifungal therapy. In the beginning of the 1970s, two antifungal drugs were available for the systemic treatment of fungal diseases, amphotericin B and 5-FC. Amphotericin B had toxic side effects, and 5-FC was effective only against certain yeasts. Improvement of antibacterial therapy through the synergistic effect of the two antibiotics had been shown. At Washington University, Medoff et al. discovered in 1971 that the combination of the two antifungal agents against yeasts in vitro was 10 times more active than either drug alone (284). The synergistic mechanism was the result of increased entry of 5-FC into yeast cells potentiated by the amphotericin B (285). The synergistic effect of amphotericin B and 5-FC was also demonstrated in experimentally infected mice in 1973 (420).

From the reports originating from Washington University regarding the synergistic activity of 5-FC and amphotericin B (284, 285), two collaborative clinical studies were designed to evaluate this combination of antifungal agents in the treatment of human cryptococcosis. The first study was an uncontrolled prospective evaluation conducted from May 1971 to November 1973 at MCV in Richmond and the University of Virginia in Charlottesville by Utz et al. (427). Of the 15 patients with *C. neoformans* meningitis, 12 patients were cured and no patients relapsed. These encouraging results led to a second prospective collaborative study involving 10 institutions to compare 6 weeks of combined amphotericin B and 5-FC therapy with 6 weeks of amphotericin B therapy alone in 50 patients with cryptococcal meningitis (26). Since the combined therapy produced fewer failures and relapses (3 versus 11), sterilized the CSF more rapidly, and produced less nephrotoxicity than amphotericin B alone, they concluded that amphotericin B plus 5-FC was the regimen of choice for cryptococcal meningitis (26).

Laboratory diagnosis. The high incidence of *Candida* infections led to the development of improved and efficient tools for the diagnosis of candidemia in the clinical laboratory. These new diagnostic tools were evaluated by medical mycologists working in service laboratories, thereby developing applied research data. Conventional blood culture techniques were compared with Sterifil lysis-filtration (224); commercially prepared vacuum blood-culture bottles (Albimi and Difco) were evaluated (138); biphasic and vacuum blood culture bottles were compared (347); the diagnostic efficiency of two commercially prepared biphasic media (BBL Microbiology Systems) were assessed (53); and the diagnostic value of a new blood culture centrifugation technique was examined (102). Data from the evaluation of these new systems indicated that vented biphasic blood culture bottles, venting of broth cultures, and the centrifugation technique yielded more rapid and optimal recovery (83%) than the conventional broth culture technique (67%). During the 1980s, the commercially available lysis centrifugation Isolator (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) was found to be the most sensitive and rapid method for isolating yeasts and filamentous fungi from blood (30). The detection of fungemia was increased 36.6% by the Isolator, which also was superior to the previously evaluated Bactec Radiometric System (Johnston Laboratories, Cockeysville, Md.) (184).

In addition to improved techniques for the direct demonstration of *Candida* spp. in blood cultures, indirect methods were developed for the diagnosis of candidiasis. Immunologically based tests, such as skin tests and the measurement of serum antibodies, had provided unsatisfactory results as diagnostic tools for candidiasis. Investigators began to develop assays to detect *Candida* antigens or metabolites (215, 291, 369, 441). Although these alternatives were promising, they lacked specificity and sensitivity. The efforts in the ensuing years were directed toward the detection in serum of *Candida* D-arabinitol, enolase, and cell wall mannoproteins by more advanced technological approaches, such as DNA-based methods.

Two approaches for the immunodiagnosis of aspergillosis were investigated. Schaefer and coworkers found that biweekly immunodiffusion tests (modified Ouchterlony's test) for *A. fumigatus* antibodies were helpful for the clinical diagnosis of invasive aspergillosis in a high-risk population (360). The second approach was the development of tests for the detection of antigens of *A. fumigatus* in serum (243, 442, 443). Although the first approach is used frequently today for the diagnosis of noninvasive aspergillosis, the second approach seems to have a greater potential for the immunodiagnosis of invasive disease. Negative precipitin tests do not rule out invasive disease. The nature of the antigen detected, a galactomannan, was reported by Reiss and Lehmann in 1979 (334), while Paul Lehmann was a postdoctoral fellow with Errol Reiss at the CDC.

Several commercial kits for the identification of yeasts in the clinical laboratory were developed in the 1970s. These kits became very helpful in the clinical laboratory, because conventional methods were tedious and time-consuming despite the modifications made in 1975 (238) to the methods of Wickerham and Burton (455). The first evaluation of the API 20C Yeast Identification System (API Laboratory Products, Ltd., Plainview, N.Y.) was performed by Roberts et al. in 1976 (348). They compared the API 20C system and conventional methods and found that the API 20C system was a useful method for yeast identification. A modified version of this system is the test used today in most clinical laboratories; the API computerized database was developed by Davis Pincus from API (117). This new version was evaluated in 1979 by Land and coworkers (240). Based on Staib's discovery in 1962 that *C. neoformans* produced melanin when grown on agar containing an extract of *Guizotia abyssinica* seeds (396), Hopfer and Groschel (183) and Salkin (355) developed a 6-h test that screened for *C. neoformans* colonies.

Standard and Kaufman developed the exoantigen test between 1976 and 1977 (399, 400). This important advance was accomplished while Paul Standard was performing research for his doctoral dissertation at the University of North Carolina at Chapel Hill under the guidance of Leo Kaufman from the CDC (117). By 1975, the identification of the fungal pathogens *B. dermatitidis*, *C. immitis*, and *H. capsulatum* involved the demonstration of their diagnostic morphology in the mycelial form and the subsequent conversion from the mycelial to the yeast form found in tissue. The exoantigen test eliminated these steps because it was based on the direct 3-day detection of specific soluble antigens from mature cultures of each fungus. The exoantigen method was adapted later for many other fungal pathogens, and commercial exoantigen kits have been available since the 1980s. However, a negative exoantigen test is not always absolute proof that the culture is not *H. capsulatum*.

The frequent use of coccidioidin (derived from mycelium) indicated in the 1970s that improvement in this skin test reagent was needed in certain clinical situations. In 1973, Levine et al. compared the reagent prepared by them in 1969 from cultured spherules (247) with the classic coccidioidin of Smith and his coworkers (385). Levine et al. found that spherulin was more sensitive than coccidioidin. Both reagents are used either together or alone today. Many approaches to purifying these heterogeneous extracts and other immunoreactive antigens have been attempted.

Taxonomy and classification. The perfect states of three fungal pathogens were discovered by Kwon-Chung (231–233). By 1969, Kwon-Chung had studied the cytology of *C. immitis* and discovered that it had three chromosomes (237). She then studied the ecology of *H. capsulatum* and discovered the sexual reproductive cycle of this fungus in 1972 (231). She identified the two mating types (+ and −) of *H. capsulatum* among natural as well as clinical isolates and named the teleomorph *Emmonsiella capsulata* in honor of Emmons. As a sequela of this discovery, Kwon-Chung reported that the sexual state of *Histoplasma duboisii* was identical to *E. capsulata* because the cleistothecia and ascospores produced by mating *H. capsulatum* and *H. duboisii* were identical to those of *E. capsulata* (232). Therefore, she treated *H. duboisii* as a variety of *H. capsulatum* rather than a separate species. In 1979, McGinnis and Katz transferred *E. capsulata* to the genus *Ajellomyces* (277), which was named in honor of Ajello.

J. Shadomy had reported from the NIH in 1964 that *C. neoformans* may produce true hyphae (372). In 1970, she demonstrated that these hyphae had clamp connections, which linked *C. neoformans* to the Basidiomycetes (371). As a result of this information, Kwon-Chung found a basidiomycetous state in four pairs of *C. neoformans* after a 3-week incubation period (233). In order to have a stable *C. neoformans* sexual state, she had developed a sucrose (40%)–agar medium that contained all the ingredients useful for fungal growth. Although the basidial structures found by Kwon-Chung showed a close relationship with *Filobasidium*, a genus described by Olive (233, 237), she knew that she had a new genus, which she named *Filobasidiella*. It is now known that *F. neoformans* has two varieties, neoformans (anamorph, *C. neoformans* var. neoformans) and bacillisporus (anamorph, *C. neoformans* var. gatti) (237).

Michael McGinnis obtained his doctoral degree under the guidance of L. Tiffany at Iowa State University in the Department of Botany. He was trained as a classical mycologist and became involved in medical mycology at the CDC, where he had his postdoctoral training with Ajello and Haley (117). McGinnis' *Laboratory Handbook of Medical Mycology*, published in 1980, became important for the development of the discipline. In 1977, McGinnis summarized and established the clinical nomenclature and clarified the taxonomy of nine human pathogenic species of *Exophiala*, *Phialophora*, and *Wangiella* (276). Saccardo's original classification was based on an artificial system that did not consider natural relationships and had created a great deal of confusion because of the pleomorphic nature of many of the black fungi. McGinnis provided excellent descriptions of the taxonomic features of each species, which were accompanied by photomicrographs helpful for the identification of these fungi in the clinical laboratory.

Etiology and morphology. Paul Szaniszlo and his students' main research interest at the University of Texas at Austin has been the morphological and ultrastructural changes associated with the growth of both the yeast and hyphal forms of phaeoid (as dematiaceous) fungi (117). Szaniszlo et al. described for the first time in 1976 the induction of *Wangiella dermatitidis* multicellular muriform (as sclerotic bodies) cells in vitro (417). These bodies appeared to be an intermediate form between muriform cells and hyphal growth.

Immunochemistry. The immunochemistry of fungal products was also studied in the 1970s by Judith Domer and Reiss, among others. In 1971, Domer investigated the content of the mycelial and yeast phases of *H. capsulatum* and *B. dermatitidis* and found two chemotypes of *H. capsulatum*, based on chitin and glucose content (98). She found no evidence for chemotypes of *B. dermatitidis*. Syverson et al., on the other hand, compared the cytoplasmic antigenic composition of the two morphological forms of *C. albicans* by two-dimensional crossed-line electrophoresis (416). They found six distinct, soluble antigenic constituents in each form that were not present in the other form.

Reiss et al. demonstrated in 1974 that the cell walls of *C. albicans* contained three fractions and mannan (333), while Reiss was conducting his postdoctoral research in the laboratory of H. Hasenclever at the NIH (117). The cell wall fractions, peptidoglucomannan, soluble mannoglucan, insoluble mannoglucan, and mannan, were antigenic and elicited delayed-type hypersensitivity, as measured by skin tests and specific inhibition of macrophage migration. The structure of the cell wall mannan separated *C. albicans* into two serotypes, A and B (174). Lupan and Cazin isolated and characterized in 1976 the two major active antigens from culture filtrates of *P. boydii* (antigen 1) and its anamorph, *Scedosporium apiospermum* (as *M. apiospermum*) (antigen 2) by ion-exchange chromatography (263). Both antigens were immunologically identical by immunodiffusion tests. D. Lupan and T. Kozel were J.

Cazin's students at the University of Iowa (117). The pathogenicity of this fungus was found to be related to both the sexual and asexual forms (262).

Host defenses against *C. neoformans*. Preliminary basic research advances in understanding pathogenesis and host-fungus interactions, such as phagocytosis and cell-mediated immunity, were made during the 1970s. These observations were made both in animal models and in vitro. By 1974, it had been established that the host's major defense mechanism against *C. neoformans* was cell mediated and could be measured by detecting delayed hypersensitivity. Murphy et al. in 1974 developed their first murine model to study this mechanism in cryptococcosis (300). By the late 1970s, T-lymphocyte-mediated mechanisms appeared to be the primary means of host defense in this disease in the murine model.

Diamond and Allison established in 1976 that human peripheral blood leukocytes, excluding T cells, could kill *C. neoformans* in the presence of anticryptococcal antibodies in vitro (84). The killing of *C. neoformans* cells appeared to occur by a nonphagocytic mechanism. Peripheral blood neutrophils, monocytes, and "activated" macrophages had a limited capacity to "ingest" and kill *C. neoformans*. However, ingestion was not essential for the anticryptococcal action of phagocytes. In 1977, Diamond developed a guinea pig model of intraperitoneal cryptococcosis, using intravenous infection to define the relative importance of these killing mechanisms in vivo and the effects of immunosuppression and immunostimulation (85). After the finding of Bulmer and Sans that cryptococcal polysaccharide inhibits the attachment, not the ingestion, stage of phagocytosis (45), Kozel and McGaw discovered in 1979 that immunoglobulin G (IgG) is the principal, if not the sole, opsonin in human serum for phagocytosis of *C. neoformans* by normal mouse macrophages (225). Opsonization involved a direct interaction between IgG and the macrophage via an Fc-mediated process.

Host defenses against *H. capsulatum*. By 1977, Howard had made the following important discoveries on cellular immunity in murine histoplasmosis: macrophages from immunized animals restricted intracellular growth of *H. capsulatum*; the lymphocyte acted as the mediator cell and the macrophage acted as the effector cell; and partially purified lymphocytes "armed" macrophages to suppress the intracellular growth of this fungus. However, supernatants from cultures of immune lymphocytes did not activate macrophages (190). Artz and Bullock studied in 1979 the suppression activity induced by disseminated histoplasmosis in mice in order to understand immune function in humans (12). They noted a shift of host cell activity or immunoregulatory disturbances from a suppressor (1 to 3 weeks) to a helper mode by week 8, when mice recovered from the infection. These parameters were similar to those observed in humans.

Host defenses against *C. immitis*. During the 1970s, Demosthenes Pappagianis (University of California, Davis) and David Stevens (Santa Clara Valley Medical Center) initiated research on the host immune response to *C. immitis* (117). Beaman et al. reviewed the literature and concluded in 1977 that although T cells were required for the cell-mediated immune response of bacterial infections, their role in *C. immitis* infections was not well defined (16). Cell-mediated immunity appeared to be associated with recovery from coccidioidomycosis (skin test positive), and severe infections were regulated by humoral response (high CF titer and skin test negative). They concluded that the effector cells that inactivate the spherules or endospores during infection are presumably macrophages and polymorphonuclear phagocytes and that the T-cell population was essential for effective immunity in mice (16).

Galgiani et al. studied, for the first time in fungi, the substances derived from *C. immitis* that cause chemotactic migration of PMNs to the site of infection in vitro (135). This cellular defense mechanism was mediated by the generation of complement-split (heat-labile) fragments, but direct leukotactic properties in the absence of serum could be displayed. These results suggested to them that alternative and classical complement pathways were activated by mycelial and spherulin filtrates, without antibodies being required. Therefore, the inhibition of migration of PMNs by high concentrations of spherule-derived substances might obstruct efforts to limit *C. immitis* infection (135).

Host defenses against opportunistic filamentous fungi. Diamond and coworkers demonstrated in 1978 that neutrophils were also important as host defense mechanisms in invasive aspergillosis and zygomycosis (as mucormycosis), even though hyphae in the lesions were too large to be phagocytized (87). Light electron microscopic observations showed that neutrophils attached to and spread over the surfaces of hyphae even in the absence of serum. This nonphagocytic mechanism was followed by severe damage and probably death of the hyphae. Damage to the hyphae was inhibited by compounds that affected neutrophil surface functions, motility, and metabolism. The attachment appeared to activate potential fungicidal mechanisms in neutrophils (87).

Host defenses against *C. albicans*. For phagocytosis of fungi to occur, phagocytes must come in contact with fungal cells. Diamond and Krzesicki showed in 1978 that human peripheral blood neutrophils could ingest and probably kill *C. albicans* in vitro (86). They also demonstrated that attachment to neutrophils was inhibited by *Candida* mannans. The neutrophils recognized a protein component on the *Candida* surface which was sensitive to chymotrypsin. On the other hand, the neutrophil receptors for *Candida* spp. were susceptible to chymotrypsin as well as trypsin. The damage to *Candida* spp. occurred by oxidative mechanisms, which confirmed Leher's and Cline's findings in 1969 (245). It is now known that both oxidative and nonoxidative mechanisms are involved in the killing of *C. albicans* by neutrophils and macrophages.

Fungal molecular genetics. The first report of the successful release of living protoplasts from albino and brown types of *H. capsulatum* was published by Berliner and Reca (28). The production of spheroplasts was important as a means to study the cell wall composition, physiology, cytology, and genetics of fungi. In 1979, Olaiya and Sogin microfluorometrically compared the flow of the DNA content of haploid, diploid, triploid, and tetraploid strains of *S. cerevisiae* with the DNA content of *C. albicans* (315). They found that *C. albicans* contained a diploid amount of DNA. This discovery ruled out the preconceived idea that *C. albicans* was a haploid yeast like the other species of this genus and led to important investigations on the genetics of fungi during the 1980s and 1990s.

Physiology and nutrition. Land et al. associated the morphogenesis of *C. albicans* with changes in respiration (239), while Geoffrey Land was conducting research for his doctoral dissertation under the guidance of Friedman (117). Hyphae produced more ethanol, evolved less CO_2, and consumed less oxygen than yeasts. This suggested to them that an abrupt change from an aerobic to a fermentative metabolism of glucose occurred. Nickerson's attempts (312, 313) to describe the morphogenesis of *C. albicans* at the cellular level had prompted these studies. Because differences between the predominant cytoplasmic proteins of the two phases were not

detected, Manning and Mitchell suggested in 1980 that proteins unique to each phase may serve a regulatory function (269).

In 1977, Maresca et al. examined further (270) the role of sulfhydryl groups in determining the forms of *H. capsulatum* growth (329, 357). Maresca et al. found that the elevated temperature of 37°C initiated a series of reactions leading to changes in the intracellular level of cyclic AMP. The level of cyclic AMP was fivefold higher in the mycelium form than in the yeast form, which was an important determinant of the morphological form and its disease-producing potential (270). They also showed that cysteine stimulated oxygen consumption in the yeast form but not in the mycelial form. This group proposed a model which outlined the unique biochemical events during the three stages of temperature-induced mycelium-to-yeast form transition of *H. capsulatum.*

Training and research centers. A postdoctoral training and research program in medical mycology was established in 1972 at Washington University under the joint direction of George Kobayashi and Medoff. This program was initiated with the aid of a Brown-Hazen Program grant (1973 to 1976), which served as the basis for subsequent support from NIH training grants (1976 to 1992) (222, 223). In the fall of 1974, Thomas Mitchell, who trained under Friedman at Tulane University, succeeded Conant as director of the Medical Mycology Laboratory at Duke University and assumed the responsibility for teaching medical mycology in the medical and graduate schools (292). Mitchell also reorganized and continued the renowned Duke Summer Mycology Course from 1975 to the present. In 1977, D. Durack came to Duke as chief of the Division of Infectious Diseases and recruited John Perfect, who focused his research on experimental cryptococcosis and has participated in several clinical trials conducted by the NIAID-MSG group. Reiss came to the CDC in 1974 and became chief of the molecular biology section. In the same year, William Merz came to Johns Hopkins from Columbia University, where he had trained under Silva-Hutner, to establish a medical mycology service laboratory. In the 1970s, Juneann Murphy and Domer, who had trained under George Cozad and Friedman, respectively, became active in the field at the University of Oklahoma and Tulane University, respectively.

From 1972 to 1977, Brown-Hazen funds were awarded to Furcolow and Ernest Chick and later to Goodman and Chick to offer short-term and long-term medical mycology courses for practicing physicians and laboratory personnel at the University of Kentucky. A similar training program was offered by Glenn Roberts at the Mayo Clinic; McGinnis at the University of North Carolina at Chapel Hill; and Carlyn Halde at the University of California at San Francisco. During the 1970s, medical mycology research laboratories were established by Richard Diamond at Boston University Medical Center; David Stevens at Santa Clara Medical Center; David Drutz, Milton Huppert, M. Weiner, and John Graybill at the University of Texas Health Science Center at San Antonio; W. Hoeprich and Pappagianis at the University of California, Davis; Eric Jacobson at MCV/VA Hospital, Richmond, Va.; and John Galgiani at the University of Arizona (117).

Beginning in 1978, the Brown-Hazen Program awarded the Dalldorf Postdoctoral Fellowship (1979 to 1991) to a total of nine physicians and one Ph.D. scientist (335). The purpose of the fellowship was to encourage individuals with appropriate education and training to undertake research careers in medical mycology by obtaining specialized training and experience.

THE ERA OF TRANSITION, 1980 TO 1994

Overview

The era of transition is characterized by major changes and conflicting events. In the early 1980s, a new population of high-risk individuals for severe mycoses was introduced with the onset of the AIDS pandemic (218, 296). Unfortunately, opportunistic and classical fungal pathogens were noted to cause severe and fatal disease in increasing numbers of patients with AIDS, cancer, and organ transplants (340). Although more funding support should have been available in response to this need, there was a substantial reduction in federal funds and a depletion of private sources. As a consequence, established and formal training centers and programs were either closed or substantially reduced in scope, and medical mycology courses as complete units also nearly ceased to exist. Formal education in the discipline currently is limited to sections in microbiology courses and lectures for medical students. As leaders of applied research and training programs began to retire, they were not replaced in many institutions because of the loss of financial support. These events led to an overall crisis in training and a decline in the number and quality of diagnostic laboratories. As researchers became more specialized and focused their studies on specific aspects of a fungal disease or its etiologic agent and individuals from other medical disciplines initiated research in medical mycology, a major transition in leadership was evident. Leadership shifted from traditionally trained medical mycologists to a more diversified number of scientists directing research and training resources at isolated laboratories. Their contributions facilitated the transition to molecular biology and genetics-oriented research in the field (117) (Table 1).

During the last 15 years, much research in molecular biology, cellular immunity, host-parasite interactions, genetics, taxonomy, and the immunochemistry of fungi was initiated and intensified. The development of more accurate and rapid laboratory diagnostic tests was accomplished by the application of DNA-based methods and numerous other technological advances. DNA-based procedures were also employed as reliable epidemiologic tools (Table 1). During the early 1980s, two new oral antifungal agents were developed, fluconazole by Roerig Pfizer in England and itraconazole by Janssen Pharmaceuticals in Belgium. Clinical trials and applied research were supported by pharmaceutical and corporate funds, while basic research was funded by intramural and extramural NIH grants. Two collaborative groups may be singled out as important contributors to the development of medical mycology in the United States during this period, the NIAID-MSG and the National Committee for Clinical Laboratory Standards (NCCLS) Antifungal Susceptibility Testing Subcommittee (117).

Scientific Contributions

Antifungal therapy for cryptococcosis. The medical records of 41 patients with cryptococcosis diagnosed at MCV from January 1966 through June 1980, were reviewed in 1981 by Kerkering et al. (211). It was observed that pulmonary cryptococcosis was rarely included in the differential diagnosis, which led to missed diagnoses and therapeutic errors. Since the natural history of untreated pulmonary disease was dissemination in immunocompromised patients, these patients should be treated with antifungal therapy.

Dismukes and the members of NIAID-MSG hypothesized in 1987 that the duration of the established combined flucytosine-amphotericin B therapy for *C. neoformans* meningitis (26) might be shortened further from 6 to 4 weeks, thus reducing

toxicity without compromising efficacy (93). This multicenter, prospective, randomized clinical trial enrolled 194 patients with cryptococcal meningitis. The results of this study demonstrated that a 4-week regimen should be reserved for patients who have meningitis without neurologic complications, underlying disease, or immunosuppressive therapy. Patients who did not meet these criteria should have 6 weeks of therapy. The several side effects of this combined regimen were also evaluated (398).

As cryptococcal infections were diagnosed in up to 10% of AIDS patients, it became evident that the established regimen for cryptococcal meningitis was unsuccessful for the treatment of this syndrome in these patients (75 to 85% versus 40 to 50% success rate). Most patients relapsed after treatment was ended. Preliminary reports demonstrated that the newly developed fluconazole and itraconazole could be alternatives to lifelong amphotericin B for the treatment of *C. neoformans* meningitis in AIDS patients (79, 403, 411). Thrush also could be prevented in AIDS patients with fluconazole therapy (405). However, the efficacy of these antifungal agents could be established only by controlled trials with large numbers of patients. Three collaborative trials were conducted to evaluate fluconazole (38, 331, 353). Bozzette et al. evaluated in 1991 the efficacy of maintenance therapy with fluconazole in 84 AIDS patients who had completed successful primary therapy for *C. neoformans* meningitis (38). They compared fluconazole with placebo and found a 37% relapse rate among patients assigned to placebo versus a 3% relapse rate among the patients receiving oral fluconazole. Their conclusion was that in patients with AIDS, silent persistent infection is common after clinically successful treatment for this disease (38).

In 1992, Powderly et al. compared maintenance therapy with fluconazole versus amphotericin B in 218 AIDS patients with *C. neoformans* meningitis (331). Oral fluconazole was superior (97% relapse free) to amphotericin B (78% relapse free). In the same year, Saag et al. compared intravenous amphotericin B with oral fluconazole as primary therapy for AIDS-associated acute cryptococcal meningitis (353). Treatment was successful in 40% of 63 patients receiving amphotericin B and in 34% of the 131 fluconazole recipients. There was no significant difference between the groups in overall mortality (18% on fluconazole versus 14% on amphotericin B). Saag et al. concluded in 1992 that although fluconazole was an effective alternative to amphotericin B as primary treatment for patients who were at low risk for treatment failure, the optimal therapy for patients at high risk (abnormal mental status) remained to be determined (353). Other approaches to primary therapy have been advocated, including the use of higher doses of fluconazole and itraconazole and combined flucytosine and fluconazole therapy. The NIAID-MSG is currently conducting a trial consisting of a 2-week induction phase with either amphotericin B alone or combined with flucytosine, followed by an 8-week treatment with either fluconazole (400 mg/day or itraconazole (200 mg twice a day) (117). Preliminary data show no significant difference between the two regimens (354a).

Antifungal therapy for blastomycosis and histoplasmosis. Ketoconazole was approved by the Food and Drug Administration in 1981. This oral antifungal agent was developed in the 1970s by Janssen Pharmaceuticals in Belgium. Preliminary studies of ketoconazole by the NIAID-MSG during its phase II evaluation in 1985 had shown encouraging results in patients with histoplasmosis, blastomycosis, nonmeningeal cryptococcosis, and coccidioidomycosis (307). A prospective, multicenter, randomized clinical trial was conducted by the NIAID-MSG members to compare the efficacy and toxicity of a low-dose regimen (400 mg/day) of ketoconazole with a high-dose regimen (800 mg/day) in 80 patients with blastomycosis and 54 with histoplasmosis (307). Ketoconazole proved to be effective (85%) for immunocompetent patients with non-life-threatening, nonmeningeal forms of these infections. However, high dosages were associated with side effects.

By 1992, amphotericin B and ketoconazole were considered highly effective therapies for blastomycosis and histoplasmosis (success rates from 70 to 95%). Dismukes and the NIAID-MSG members assessed in 1992 the efficacy and toxicity of orally administered itraconazole in the treatment of nonmeningeal, non-life-threatening forms of blastomycosis and histoplasmosis (94). This multicenter trial (14 university centers) evaluated 85 patients with culture or histopathologic evidence of blastomycosis and 37 patients with histoplasmosis. They found a 90 to 95% success rate among blastomycosis patients and an 81 to 86% success rate among histoplasmosis patients. Itraconazole became an alternative to either amphotericin B or ketoconazole for the treatment of these infections. Unfortunately, a review of the medical records of 185 patients with blastomycosis at the University of Alabama and affiliated hospitals between 1956 and 1991 revealed a marked increase (24% since 1981) in the number of immunocompromised patients with blastomycosis (324). This had been the mycosis least often associated with immune disorders. Because the disease appeared to be more aggressive in immunocompromised patients than in normal patients, early and intensive amphotericin B therapy was required instead of the alternative oral agents (324). The role of fluconazole in the treatment of histoplasmosis is under investigation. Preliminary data indicate that 400-mg doses or higher are necessary for a successful outcome (354).

Histoplasmosis was found among 2 to 5% of patients with AIDS who reside in areas endemic for *H. capsulatum* in the United States and Latin America (450). In the Kansas City and Indianapolis areas, the incidence was as high as 25%. Relapses occurred in as many as 50% of patients receiving ketoconazole and in 10 to 20% of patients receiving amphotericin B maintenance therapy. Wheat et al. developed in 1986 a radioimmunoassay for *H. capsulatum* polysaccharide antigen, which became a useful new method for the rapid diagnosis of disseminated histoplasmosis (449). In 1991, Wheat et al. established that antigen levels of 2 units or more strongly suggested histoplasmosis relapse (450). The next strategy was to prevent relapse among these patients. Wheat and the members of the NIAID-MSG assessed the efficacy and safety of 400-mg/day oral itraconazole in preventing histoplasmosis relapse after successful induction amphotericin B therapy in 42 AIDS patients in 1993. They concluded that itraconazole, 200 mg twice daily, is safe and effective in preventing relapse of disseminated histoplasmosis in AIDS patients (451). The clearance of antigen correlated with clinical efficacy. In 1990, Spitzer et al. found by using genetic markers that AIDS patients were infected with less virulent (for mice) strains of *H. capsulatum* in the St. Louis area (393).

Antifungal therapy for coccidioidomycosis. Graybill led an evaluation of escalating high doses of ketoconazole in 1988 for the treatment of 15 patients with coccidioidal meningitis (161). They found that high-dose ketoconazole alone and combined with intrathecal amphotericin B had similar therapeutic benefits. However, a dose of ketoconazole higher than 800 mg/day was associated with intolerable nausea and vomiting. In 1990, Graybill and the NIAID-MSG elected to evaluate itraconazole as an alternative to the more toxic amphotericin B and ketoconazole for coccidioidomycosis (162). Forty-nine patients with nonmeningeal coccidioidomycosis were treated with oral itraconazole doses of 100 to 400 mg/day for periods of up to 39

months. An important accomplishment of this study was that the clinical response to therapy was evaluated with a scoring system accounting for number and size of lesions, symptoms, culture, and serologic titer. The scoring system was needed to impose criteria for measuring clinical response; prior results had been based on variable criteria for defining complete remission and lesser degrees of improvement. The preliminary conclusion of this study was that itraconazole appeared to be superior to ketoconazole for the treatment of nonmeningeal coccidioidomycosis (162).

Prior to the development of ketoconazole and miconazole, the established therapy for coccidioidal meningitis, the most fatal type of infection with *C. immitis*, was frequent administration of intrathecal amphotericin B. Later, the second-line therapies became ketoconazole and miconazole. Galgiani and the NIAID-MSG members concluded by 1990 that safer and more effective therapies were needed; intrathecal administration of drug was technically difficult, produced irritation and discomfort, was associated with other infections, and was not always effective (136). With the advent of fluconazole and itraconazole, and before the latter two drugs were approved, Galgiani and the NIAID-MSG members evaluated in 1993 the safety and efficacy of fluconazole treatment (400 mg/day for up to 4 years) in 50 patients with active coccidioidal meningitis (136). Thirty-seven of 47 (78%) evaluable patients responded to treatment within 4 to 8 months. Their conclusion was that fluconazole therapy is often effective in suppressing coccidioidal meningitis.

Antifungal therapy for aspergillosis, candidiasis, and sporotrichosis. Denning et al. evaluated in 1989 an oral itraconazole regimen of 400 mg/day in 18 patients with invasive aspergillosis (78). They concluded that itraconazole may be an important advance in the therapy of aspergillosis. A multicenter study was conducted by Denning and the NIAID-MSG members in 1994 to further evaluate itraconazole as a therapeutic agent for invasive aspergillosis (80). Seventy-six evaluable patients received oral itraconazole for from 0.3 to 97 weeks. The data demonstrated that the response rate to oral itraconazole was comparable to that to amphotericin B therapy. However, although the results suggested that oral itraconazole was an alternative therapy for this fungal infection, relapse was noted among immunocompromised patients.

Patients with profound neutropenia and corticosteroid-induced phagocyte dysfunction are susceptible to candidiasis and aspergillosis. Animal models to evaluate the treatment of these infections in this patient population were developed at the NCI between 1990 and 1993. Walsh et al. evaluated oral fluconazole for the prevention and early treatment of disseminated candidiasis in granulocytopenic rabbits (437). They found that fluconazole was as effective as amphotericin B for prevention but not for treatment. Before 1993, the treatment of choice for pulmonary aspergillosis in granulocytopenic patients was desoxycholate amphotericin B, which is toxic. In 1994, Francis et al. developed a model of pulmonary aspergillosis in rabbits to reproduce the persistent levels of profound granulocytopenia in humans (128). With this model, they evaluated the efficacy of a new formulation of unilamellar liposomal amphotericin B (approved in western Europe) and the detection of *A. fumigatus* D-mannitol in bronchoalveolar lavage fluid and galactomannan in serum. Both diagnostic approaches were useful markers of pulmonary aspergillosis. The new therapeutic agent was more effective and safer than desoxycholate amphotericin B. The next step will be to evaluate these novel approaches in humans. Evaluation of itraconazole doses of 100 to 600 mg/day for the treatment of 27 patients with sporotrichosis showed that this drug was a successful therapy in the treatment of cutaneous and systemic sporotrichosis (375).

Antifungal susceptibility testing standard. The NCCLS approved the formation of a subcommittee for antifungal susceptibility testing in April 1982 (137). The chair of the subcommittee was John Galgiani from the University of Arizona, and he proposed to constitute the subcommittee with six voting members and five advisors in July 1982. Following several studies (116, 131, 327), the subcommittee proposed a reference method for the antifungal susceptibility testing of yeasts in December 1992. By 1995, the activities of the subcommittee had diversified, and four working groups were formed within the subcommittee. These groups are investigating the role of the proposed reference method in the needed evaluations of in vitro versus in vivo correlations of drug efficacy as well as further definition of the reference method parameters. The subcommittee efforts are also directed to adapting and tailoring the reference method for the testing of filamentous fungi and for the development of more convenient procedures for routine use in the clinical laboratory.

Laboratory diagnosis. The search for more sensitive, reliable, and rapid tests for the diagnosis of early invasive candidiasis and other fungal diseases continued during the 1980s and 1990s. Three different avenues were investigated for the diagnosis of early disseminated candidiasis: the detection of protein or cell wall mannoprotein marker antigens, the detection of the fungal metabolites D-arabinitol and enolase, and DNA probes. Different technological approaches were explored for this purpose: solid-phase sandwich radioimmunoassay for the detection of soluble cytoplasmic protein antigens (404), gas-liquid chromatography for the detection of mannose (293), latex-coated particles with heterogeneous antibodies (143, 309), combined microbiological and gas chromatographic methods to detect D-arabinitol (29), a sandwich enzyme immunoassay to detect mannan (82), a combined gas chromatographic and enzymatic assay to detect D-arabinitol (463), mouse monoclonal IgG to detect an immunodominant 48-kDa enolase antigen of *C. albicans* (273), and a double-sandwich liposomal immunoassay for *Candida* enolase (438).

Much progress has been accomplished since Kiehn et al. discovered arabinitol in the serum of patients with disseminated candidiasis in 1979 while they were testing for the presence of serum mannose (215). Wong and coworkers have studied arabinitol as a possible diagnostic marker since the late 1980s (463). In 1988, Wong and Brauer reported that infected patients had elevated serum levels of D-arabinitol and normal levels of L-arabinitol, thereby identifying the diagnostic marker (463). In 1993, Wong et al. elucidated the D-arabinitol biosynthetic pentose pathway and cloned the gene that encoded the regulation of intracellular NAD-dependent D-arabinitol dehydrogenase (464).

Commercial kits have been developed for the diagnosis of systemic candidiasis. The Cand-TEC latex agglutination system (Ramco Laboratories, Houston, Tex.) was based on the detection of antigen as described by Gentry et al. in 1983 (143). The ICON *Candida* assay (ICON Hybritech Inc., San Diego, Calif.) is an enzyme immunoassay for the detection of circulating mannan. Although the ICON appears to be more reliable than the Cand-TEC, both kits had been evaluated and found to be lacking in specificity and sensitivity (47, 309, 328). In the early 1990s, a commercial rapid test was developed in Japan for the enzymatic-fluorometric determination of serum D-arabinitol. Switchenko et al. described in 1994 an automated enzymatic method for this purpose (415). The reaction steps of the assay were adapted so that they could be performed automatically in the clinical laboratory on a chemistry analyzer. Many of the

problems associated with the current procedures for the detection of D-arabinitol, including the cross-reactivity associated with the enzymatic-fluorometric method, could be avoided. Preliminary results demonstrated that detection of D-arabinitol permitted detection of invasive candidiasis and early recognition of fungemia in serially collected sera from high-risk cancer patients as well as therapeutic monitoring of the infection (439). Weiner developed a promising radioimmunoassay for the detection *C. immitis* antigen in 1983 (444). It detected antigenemia in five of nine (56%) patients with active coccidioidomycosis. These tests need further evaluation and may became valuable diagnostic and prognostic tests. They may complement rather than replace blood cultures.

In 1984, Hageage and Harrington observed that calcofluor white, a nonspecific fluorochrome with affinity for chitin and cellulose, could be used to detect fungal elements in clinical specimens (166). This technique eliminated some of the inaccuracies associated with KOH preparations as well as being faster than the selective PAS and silver stains. Although calcofluor white stain requires a fluorescence microscope, this stain has replaced India ink and KOH preparations for the detection of fungi in many clinical microbiology laboratories.

DNA-based laboratory diagnosis. In 1988, Cutler et al. isolated for the first time a DNA probe that may become useful in the direct detection of *C. albicans* in clinical specimens (72). They isolated a DNA fragment that hybridized specifically with DNA from *C. albicans* but did not hybridize with DNA from other infectious agents or from the host. Another avenue that has been explored is the amplification of a DNA segment that lies within a fungus-specific gene by PCR. Buchman et al. reported in 1990 the unambiguous detection of *C. albicans* by PCR within 6 h from the time that a clinical specimen is obtained (43). Their chosen DNA sequence was the *Candida* gene coding for the cytochrome P450L1A1. In 1993, Hopfer et al. simplified sample preparation for the detection of 18 species of fungi, including *C. albicans*, from clinical specimens (185). However, further simplification is needed to make these tests more convenient for use in the clinical laboratory. In addition, their sensitivity in clinical specimens, including blood, also remains to be evaluated.

More rapid laboratory tools for the detection of fungi in tissue as well as their identification were developed in 1987, such as the DNA probes for the identification of *Candida* spp., *T. glabrata* (274), and *H. capsulatum* (209). Another novel approach was the adaptation of random amplified polymorphic DNA (RAPD) (458) for characterization of isolates of *Candida* spp. by Lehmann et al. (244). Clark at Gen-Probe Inc. (San Diego, Calif.) simplified the early probe assays for the identification of fungi by developing a nonisotopic chemiluminescent probe system (117). This system utilizes a single-stranded DNA probe that is complementary to the rRNA of the fungus being identified. Commercial probes for the identification of *B. dermatitidis*, *C. immitis*, *C. neoformans*, and *H. capsulatum* have been evaluated (170, 408).

DNA-based epidemiology. By 1987, various methods had been developed as epidemiologic tools to identify a specific strain as the source of infection. This early methodology relied on nonreproducible, cumbersome, and insensitive tests which measured phenotypic characteristics. DNA-based typing procedures, such as molecular probes and the determination of electrophoretic karyotypes, replaced earlier methods. The development of pulsed-field gel electrophoresis procedures demonstrated that the chromosome mobility of *C. albicans* and *S. cerevisiae* was extremely variable under apparently identical testing conditions. Lott et al. defined in 1987 an electrophoretic karyotype for *C. albicans* by field inversion gel electrophoresis (255), while T. Lott was conducting his postdoctoral studies with Reiss (117). This karyotype, the result of the migration of intact chromosomes, was distinct from that of other species of *Candida* and thus species specific. B. Magee and P. T. Magee went a step further in 1987 and determined that isolates of *C. albicans* and other species of *Candida* had different electrophoretic karyotypes by using orthogonal-field alternating gel electrophoresis (266). In 1989, C. S. Kaufmann and Merz determined different electrophoretic karyotypes among isolates of *T. glabrata* (207), and Perfect and coworkers identified different electrophoretic karyotypes of *C. neoformans* (325). These studies suggested that electrophoretic karyotypes could be used as stable genetic markers to delineate strains for use in epidemiologic studies.

Recombinant DNA technology made possible the replacement of phenotyping methods by the direct examination of the genomes of fungi (422). Molecular probes provided a high resolution for this examination. Scherer and Stevens used this approach to study *C. albicans* and described a DNA extraction and digestion method for the whole *Candida* genome in 1987 (363). By using restriction endonuclease digestion and electrophoresis, chromosomal DNA restriction fragments of various lengths produced unique patterns in the gel, analogous to fingerprints (363). These gel patterns could be used to type *Candida* isolates in a more precise manner. By 1988, Scherer and Stevens reported that only a limited number of sites in the genome of a given species could be scored for differences with their 1987 procedure (364). Therefore, they isolated a repeated DNA segment that provided a species-specific DNA probe for *C. albicans*. E. D. Spitzer and S. G. Spitzer demonstrated in 1992 that the CNRE-1 hybridization probe, based on repetitive DNA sequences from *C. neoformans*, could discriminate among 10 strains of this species isolated from eight patients (394). Thus, DNA polymorphism could be measured in the laboratory by using DNA probes as another epidemiologic tool. Controversy began regarding which method was more sensitive, and the contour-clamped homogeneous electric field (CHEF) method appeared to provide greater sensitivity than the enzymatic tests for recognizing strain variations among certain fungi (430).

In addition, DNA fingerprinting and RAPD were adapted as epidemiologic tools. The RAPD assay was described in 1990 by Williams et al. (458). Since DNA purification for the RAPD assay was time-consuming, Woods and colleagues shortened their earlier method for *H. capsulatum* DNA purification from a 2-day to a 2-h procedure (465). In 1993, Meyer et al. combined RAPD and conventional DNA fingerprinting to detect *C. neoformans* strain variation (289) during Meyer's postdoctoral training with Mitchell (117). They found that this combination was more reliable than classical DNA procedures alone for detecting polymorphic DNA in *C. neoformans*. The same year, Lott et al. demonstrated that pulsed-field gel electrophoresis and RAPD can be used to show *C. parapsilosis* intraspecies variation (257). In 1994, fragment length polymorphism analysis with two probes was used to characterize and compare *C. neoformans* isolates from pigeon excreta with those isolated from human cryptococcal meningitis (71).

Taxonomy and classification. In 1983, McGinnis stated that the nomenclature for the mycoses caused by the black fungi is in a state of confusion (278). He considered that clarification of the conceptual basis for the infections known as chromoblastomycosis and phaeohyphomycosis was warranted. McGinnis defined chromoblastomycosis as a chronic, localized cutaneous or subcutaneous infection (278). The infected tissues contain muriform cells and histologically show pseudoepitheliomatous hyperplasia, dermal granulomas, abscess formation, and fibro-

sis. Phaeohyphomycosis had been described by Ajello et al. in 1974 (7) to include mycotic diseases in which the etiologic agent (heterogeneous group) was present in tissue as phaeoid pseudohyphae or hyphae (with no muriform cells).

Several taxonomic problems were clarified during this era. In 1995, de Hoog et al. proposed that the hyphomycete *X. bantiana* should be transferred to the genus *Cladophialophora* (76a). McGinnis et al. studied isolates of the genera *Bipolaris*, *Drechslera*, *Exserohilum*, and *Helminthosporium* and clarified the taxonomy of human pathogenic species belonging to these genera (279). Weitzman et al. concluded that the genera *Arthroderma* and *Nannizzia* were congeneric (447). Because of priority, *Nannizia*, which was established by Stockdale in 1963 (407), was considered a synonym of *Arthroderma* (proposed by Currey in 1854) (447).

Vaccines. Primitive vaccines had stimulated an immune response against *T. mentagrophytes* and *C. immitis* during the 1950s (192, 321). A study conducted from 1980 to 1985 of 2,867 healthy human volunteers randomized to either a vaccine (*C. immitis*) group (1,436 subjects) or a placebo control group (1,431 subjects) showed a slight but not statistically significant difference between the two groups (322). Pappagianis et al. believed that the fungal fractions (e.g., proteinases) isolated from *C. immitis* in the 1980s and 1990s may be the approach of the future for the production of a more effective vaccine (322). Gordon and Lapa had found that a globulin fraction of serum from rabbits hyperimmunized against *C. neoformans* significantly enhanced the therapeutic effect of amphotericin B in experimental cryptococcosis in mice (159). A. Casadevall is attempting to adapt this procedure to enhance the treatment of fungal diseases in AIDS patients. This concept aroused a great deal of interest among the members of the NIAID-MSG during their annual meeting in April 1994.

Host defenses against *C. neoformans*. Important findings were reported regarding the host-parasite interaction variables in cryptococcosis by Murphy and her graduate and postdoctoral students at the University of Oklahoma at Norman and Oklahoma City. Lim et al. demonstrated in 1980 a direct correlation between high *C. neoformans* antigen levels and depressed delayed-type hypersensitivity (DTH) response (251). This group reported for the first time the developmental profiles of the DTH response, antigen levels, and antibody titers induced after intranasal inoculation of mice with *C. neoformans*. In 1986, Mosley et al. observed that intravenous injection of *C. neoformans* antigen triggered the induction of a cascade of *C. neoformans* suppressor cells (T cells) and factors which downregulated the protective anticryptococcal cell-mediated immune response (299). Of this cascade of suppressor T cells (Ts1, Ts2, and Ts3), Ts3 worked in conjunction with Ts2 to inhibit the anticryptococcal DTH response in the murine model. The Ts1 cells induced the Ts2 cells and so on (214). Murphy believed that most likely a similar induction of suppressor cells occurred in humans with high serum levels of *C. neoformans* antigen. In 1988, Murphy, in collaboration with investigators at the CDC and the University of Nevada, concluded that mannoprotein was the primary component recognized by the anticryptococcal cell-mediated immune response in mice (302). In 1989, Fidel and Murphy found that cyclosporin A, a potent immunosuppressive drug, affected DTH cells (123).

Because Murphy had demonstrated that cell-mediated immunity is a key host cell defense in cryptococcosis, she directed her research toward defining the lymphokines associated with induction and regulation of the anticryptococcal cell-mediated immune response (304). Her studies have shown that in mice immunized with cryptococcal antigen, two other populations of $CD4^+$ T lymphocytes were induced in addition to suppressor cells: T_{DTH} cells, responsible for the anticryptococcal DTH response, and Tamp cells, responsible for amplification of this response. Murphy's data showed in 1993 that spleen cell populations which contain T_{DTH} and Tamp cells produced more lymphokines (gamma interferon and interleukin-2 [IL-2]) than spleen cell populations that contained only T_{DTH} cells (304). These lymphokines were influential in the development of the anti-cryptococcal response amplified by the Tamp cells. She stated in 1993 that these results may serve as a basis for future studies in understanding the mechanisms by which lymphokines affect clearance of *C. neoformans* from infected human tissues (304).

The activity of the cytotoxic effector cells, or natural killer (NK) cells, was described in 1975 (216). NK cells were found in lymphoid tissues but were absent at birth and in the thymus of normal, unimmunized individuals. The role of NK cells as primary mammalian host defense mechanisms against viral and tumor targets had been studied by the early 1980s, but their role as nonphagocytic killer cells, a natural defense mechanism in infectious diseases, had not been established (301). Murphy and McDaniel provided for the first time in 1982 substantial indirect evidence that unstimulated murine splenic cells with the characteristics of NK cells could inhibit the in vitro growth of *C. neoformans* (301). Therefore, NK cells were potentially the third means of natural cellular immunity, the other two being macrophages and neutrophils. In 1985, Navabi and Murphy "elucidated" some mechanisms by which NK cells affected *C. neoformans* (305). In vitro, NK cells bound and formed conjugates with *C. neoformans*, and the number of these conjugates was directly proportional to the degree of growth inhibition. In 1991, Hidore and coworkers demonstrated that NK cells participated in the clearance of *C. neoformans* in vivo by exocytosis of cytolytic material, which resulted in the death of *C. neoformans* cells (180). This was the same sequence of events observed in the interactions of NK cells with tumor targets. By 1993, Murphy et al. showed that human NK cells, monocytes, and T lymphocytes bound and inhibited the growth of *C. neoformans* cells in the absence of cryptococcal antibody or complement (a nonphagocytosis process) (303).

Murphy's group also became interested in the potential surface components of *C. neoformans* involved in chemotaxis of neutrophils. Dong and Murphy had postulated in 1993 that two chemotactic factors were involved; one was *C. neoformans* derived, and the second was serum derived (101). Both factors were activated by cryptococcal components. They concluded that capsular glucuronoxylomannan contributed to the direct chemotactic activity of human neutrophils and that capsular mannoprotein only stimulated indirect chemotactic activity. In 1989, Kozel and associates demonstrated that the classical complement pathway had little or no role in the opsonization of *C. neoformans* (226).

Host defenses against *C. immitis*. It had been shown by Beaman and coinvestigators that T lymphocytes were essential to protect mice against *C. immitis* (16). These cells did not kill the fungus but had a role in initiating its killing by macrophages (phagosome-lysosome fusion). In 1983, Beaman et al. demonstrated that exposure of macrophages to antigen-stimulated splenic lymphocytes induced the release of lymphokines, which activated macrophages, resulting in their enhanced phagocytosis and killing (17). This killing mechanism had not been described before.

In 1991, Kirkland et al. reported that murine T cells were stimulated in vitro with a soluble conidial wall fraction (SCWF) of *C. immitis* (217). Their generation of an antigen-

specific murine T-cell line for SCWF demonstrated that the most antigenic subfractions of SCWF had molecular masses of 43 to 66 kDa. A portion of the gene which encoded one T-cell antigen was cloned and expressed by this group as a λgt11 fusion protein (217). On the other hand, Cox and Britt isolated and identified three clinically relevant antigens (diagnostic serology) from coccidioidin: the immunodiffusion and precipitin antigen (IDTP), the immunodiffusion and complement-fixing antigen (IDCF), and the heat-stable antigen in extracts of mycelium (IDHS) (69). By using two-dimensional immunoelectrophoresis, they demonstrated that these three antigens were contained in both spherulin and coccidioidin preparations.

Host defenses against *H. capsulatum*. Howard and his graduate and postdoctoral students continued investigating the host defense mechanisms in histoplasmosis. In 1984, Wu-Hsieh and Howard demonstrated that lymphokines armed macrophages against *H. capsulatum* in vitro and that lymphokines had a high interferon activity (heat stable and acid labile) (468). Georg Deepe also investigated the immune disturbances associated with *H. capsulatum* while he was doing his postdoctoral training under Ward Bullock at the University of Cincinnati (117). Deepe had isolated and propagated *H. capsulatum*-reactive murine T-cell lines (TCL; $CD3^+$ and $CD4^+$) and clones (76). These cells released IL-2 and a factor that stimulated macrophages to limit *H. capsulatum* growth in vitro. In 1988, Deepe demonstrated that neither TCL nor cloned T cells could transfer resistance to this fungus or confer a DTH response in vivo. However, $CD4^+$ cells from immunized mice could enhance an immune response (76). By 1994, Howard's group demonstrated that recombinant murine gamma interferon was the key element within the lymphokine-containing supernatants which was responsible for activating macrophages to the growth-inhibitory state (306). Arginine metabolism was required for this activation.

Host defenses against *B. dermatitidis*. Brummer, at Santa Clara Valley Medical Center, focused his research on the phagocytic host defense mechanisms of resistance in blastomycosis. It had been shown by Drutz and Frey in 1985 that human phagocytes were an important nonspecific defense against *B. dermatitidis* conidia and hyphae but that the parasitic form (yeast) was resistant to killing by phagocytes (104). Brummer and Stevens investigated in 1984 the hypothesis that neutrophil fungicidal activity was specifically enhanced by T-cell products, such as chemotactic factors (lymphokines), and by leukocyte and monocyte migration-inhibitory factors (39). They demonstrated for the first time a link between the soluble factors produced by stimulation of sensitized lymphoid cells with *B. dermatitidis* antigen and the induction of enhanced antimicrobial activity of neutrophils against *B. dermatitidis* in vitro. In 1992, Brummer et al. investigated several mechanisms of resistance of *B. dermatitidis* to killing by neutrophils (40). They concluded that resistance to killing was dependent on the inefficient generation of products from the peroxidase-dependent neutrophil microbicidal system. That lymphokines could modulate host phagocyte fungicidal activity had been demonstrated in vitro by Brummer in the mid-1980s. Purified interferon gamma stimulated the fungicidal activity of macrophages and neutrophils.

In 1992, Brummer and coworkers examined the in vitro interaction between macrophages and azole antifungal agents against *B. dermatitidis* (41). They found that fungistatic concentrations of itraconazole acted synergistically with murine peritoneal macrophages to kill *B. dermatitidis*. Brummer et al. also demonstrated in 1993 that progression of blastomycosis and elevated serum levels of IgE had a direct relationship with the production of the IL-4 lymphokine by antigen-stimulated murine spleen cells (42). On the other hand, they observed an inverse relationship between serum IgE and gamma interferon levels, which was associated with resistance to infection in immunized mice and healing in the mice treated with an azole. Elevated serum IgE levels had been reported in association with chronic parasitic disease progression in mice. Brummer et al. were the first to extend this association to murine blastomycosis (42). The induced chronic infection in immunized mice was postulated as a new animal model for the study of immunoregulation in fungal diseases. In 1991, Sugar and Picard used the animal model developed by Brummer in the early 1980s to demonstrate that both macrophages and H_2O_2 could selectively block the transition of germinating conidia to the yeast form of *B. dermatitidis* at 37°C (412).

Host defenses against opportunistic filamentous fungi. In human and experimental animal infections, neutropenia and neutrophil or macrophage dysfunction are risk factors for invasive aspergillosis. In the mid-1980s, Stuart Levitz focused his research on the role of the phagocytic host defense (neutrophils and macrophages) in aspergillosis and zygomycosis. Levitz and Diamond found in 1985 that resting conidia of *A. fumigatus* were highly resistant to oxidative and nonoxidative fungicidal products of neutrophils, because in addition to their natural resistance, resting conidia stimulated suboptimal release of neutrophil products (249). In 1986, Levitz and coworkers (250) demonstrated that rabbit neutrophil and macrophage antimicrobial cationic peptides (defensins) (370) did not kill dormant, resting conidia of *A. fumigatus* and *R. arrhizus* (as *A. oryzae*). However, once the invasive form of these fungi began to grow, phagocytic cells and their cationic peptides could exert their fungicidal activity. These results were consistent with prior data reported by Diamond's group in the late 1970s (86, 87). In 1988, Lehrer et al. reported that human neutrophils contained four defensins (six in rabbits), but only two of them, HNP-1 and HNP-2, killed *C. albicans* effectively in vitro (246).

Waldorf et al. found in 1984 that bronchoalveolar macrophages from normal mice participated in the defense against *R. arrhizus* (as *R. oryzae*) by inhibiting the germination of conidia or conversion to the tissue-invasive stage (435). In contrast, defense against *A. fumigatus* was not dependent on inhibition of conidial germination but on early killing of conidia. In diabetic and cortisone-treated mice, bronchoalveolar macrophages allowed conidial germination or infection by *R. arrhizus*. In the cortisone-treated mice, macrophages did not kill fungal conidia, and aspergillosis occurred. Levitz et al. concluded in 1986 that only when phagocytic defenses are profoundly disturbed are invasive infections seen (250).

Because patients infected by the human immunodeficiency virus (HIV) are susceptible to invasive aspergillosis (without neutropenia or corticosteroid therapy risk factors) and neutrophils are an important component of the host defense in aspergillosis, Roilides and coworkers studied in 1993 the antifungal activity of neutrophils in 31 HIV-infected children (351). They also examined whether serum circulating factors and active HIV or viral proteins from these patients might suppress neutrophil function, as well as the possibility of improving the impaired patient's neutrophil antifungal function with granulocyte colony-stimulating factor (G-CSF). They concluded that impairment of serum-mediated antifungal activity was present in patients with low CD4 cell counts (<25% of normal median value) and that G-CSF partially corrected the defective function of neutrophils in five patients. In the same year, Roilides et al. evaluated the hypothesis that corticosteroids had a deleterious effect on neutrophil antifungal function (350). The potential preventive utility of G-CSF and gamma

interferon was assessed to investigate this hypothesis. Their conclusion was that corticosteroids impair neutrophil function in response to *A. fumigatus* and that G-CSF and gamma interferon prevent this impairment in vitro. This mechanism has potential clinical significance, because it may serve as an adjunct to antifungal chemotherapy for the prevention and treatment of invasive aspergillosis in corticosteroid-treated patients.

Host defenses against *C. albicans*. Djeu at the University of South Florida became interested in the antifungal activity of neutrophils against *C. albicans*. She investigated the factors released by T lymphocytes and NK cells (as large granular lymphocytes) that stimulated killing by neutrophils (96). The growth inhibition of *C. albicans* by lymphokine regulation of neutrophils was measured by Djeu in a rapid radiolabel microassay. Djeu and coworkers showed in 1986 that neutrophils could be activated by two lymphokines, tumor necrosis factor and gamma interferon, and that neutrophils were better than peripheral blood lymphocytes in inhibiting growth of *C. albicans* in vitro (96). NK cells also produced those two lymphokines and granulocyte-macrophage colony-stimulating factor (GM-CSF), among others, which had the ability to directly activate and mobilize neutrophils. Since the neutrophil-activating activity in NK cells was not neutralized by antibodies to tumor necrosis factor and gamma interferon, another factor was also involved. In 1991, Blanchard et al. identified NK cells as the source of neutrophil-activating factors, of which GM-CSF played a central role as a mediator between NK cells and neutrophils (32). This factor was not produced by monocytes or small mature T cells. Djeu stated that it is possible that other cytokines are produced by these cells following activation by *C. albicans* and that future research is needed to understand the role of neutrophils in host resistance to infection (96).

At Georgetown University, Calderone's group focused on the identification of the receptors on *C. albicans* that bind complement as well as the potential pathogenic role of these receptors. Several membrane glycoproteins (CR1, CR2, CR3, and CR4) are found on human peripheral blood cells; these glycoproteins serve as receptors for the binding of cleavage products of the third (C3) component of complement (e.g., CR2 binds C3d). Similar specific receptors also are found on fungal cells (49). Study of the functional significance of the receptors for C3 complement fragments on *C. albicans* was initiated for the first time by Calderone et al. in 1988 (49). They identified two protein receptors, ic3b (7 kDa) and C3d (60 kDa), which bound the corresponding C3 fragments of complement in extracts of *C. albicans* hyphae. In 1991, by immunofluorescence and immunoelectron microscopy, it was shown that the C3d receptors (CR2) were produced by both forms of *C. albicans* under in vitro and in vivo (murine model) conditions (202). The receptors of blastoconidia were buried at the level of the plasma membrane. By 1993, Calderone's group had purified the mannoprotein receptors from blastoconidia (50 kDa) and hyphae (60 kDa) of *C. albicans* (434). Both mannoproteins inhibited the binding of antibody-sensitized sheep erythrocytes conjugated with iC3b or C3d by hyphae of *C. albicans*. Therefore, although the two mannoproteins had dissimilar properties, they had a common antigenic determinant (434).

Domer and her students concentrated their efforts on studying the regulation of the cellular immune responses mediated by candidal cell wall mannan and glycoproteins in a murine model. In 1989, Domer et al. extracted mannan from *C. albicans* and found that the cell-mediated response to mannan was greater than the response to cell wall glycoprotein in vivo (99). The effect was reversed in vitro. However, it was unclear which component of the mannan extract, e.g., mannan, protein, or the intact mannoprotein, was responsible for the detection as well as the suppression of cell-mediated immunity. They concluded that complete separation of the mannan component from the protein component was needed to clarify this issue (99).

Since oropharyngeal and gastric infections with *Candida* spp. occur frequently in AIDS patients but systemic candidiasis is rare in the early stages of AIDS, an animal model of a retrovirus-induced immunodeficiency syndrome was needed to examine the exacerbation of gastric candidiasis. In 1992, Cole et al. reported the first murine model of inavasive gastrointestinal candidiasis associated with an AIDS-related murine immunodeficiency syndrome (62). Infant mice were infected by oral-gastric inoculation with the retrovirus complex. This model will allow investigations of *Candida* infections in the immunosuppressed host during progressive stages of AIDS-associated infection in mice.

Fungal enzymes and pathogenicity. Following Rippon's association of enzymes with virulence in the late 1960s (343), various investigators studied the role of different fungal enzymes in pathogenicity. In 1986, Lupan and Nziramasanga reported on the collagenolytic and elastinolytic activity of *C. immitis* in vitro (264). In 1987, Yuan and Cole characterized a single proteinase (enzyme) from *C. immitis* with those two activities (470). This enzyme was capable of degrading human immunoglublins and could represent an important virulence factor in the development of coccidioidomycosis. The suggested molecular size of the purified proteinase was 34 kDa. The enzyme was localized in the walls of parasitic cells of *C. immitis* (immunoelectron microscopic studies) (63). In 1992, Cole et al. also reported isolation and expression of the gene that encoded the enzyme (63). In the same year, Kruse and Cole isolated and characterized another enzyme, α-glucosidase, which was identical to the 120-kDa tube precipitin (TP) antibody-reactive mycelial fraction of *C. immitis* (227). This enzyme was expressed by both forms of the fungus. Kruse and Cole suggested in 1992 that this enzyme could be responsible for plasticization of the cell wall, which leads to spherule differentiation during the rapid diametric growth of the parasitic form (227).

Pappagianis and his graduate students also investigated the enzymology of *C. immitis*. Resnick et al. reported in 1987 the isolation and characterization of two major proteolytic components of *C. immitis*, a potent serine elastase and a metalloproteinase (337), and hypothesized their association with virulence. Johnson et al. in 1993 isolated a chitinase from *C. immitis* spherule-endospores which was identical to the CF antigen, based on its biochemical properties and serologic activity (201). Amino-terminal protein sequence analysis of the chitinase linked the IDCF antigen with chitinase activity.

The discovery of *Candida* proteinase was reported from Germany by Staib in 1965 (397). *Candida* proteinase isoenzymes were also characterized in Germany in 1981 by Ruchel, who found three strain-dependent enzymes (352). Kwon-Chung and colleagues in 1985 examined the virulence of a proteinase-deficient mutant, its parent, and one proteinase-producing revertant in a mouse model (234). They concluded that the extracellular proteinase produced by *C. albicans* is one of the virulence factors associated with this organism (234). The degree of virulence correlated with the level of proteinase produced. In 1989, Lott et al. reported the sequence of the *C. albicans* aspartyl proteinase (AP) gene, which included a total of 1,705 bp (256). Based on evidence reported from England (413) that there was more than one gene encoding the extracellular AP of *C. albicans*, Morrison et al. considered the

possibility that there was more than one protein present in AP preparations in 1993 (297). These investigators detected three dominant proteins (41, 48, and 49 kDa) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in purified AP preparations.

Indications that the extracellular acid proteinase from *C. albicans* was a probable virulence factor led to the cloning of genes for this enzyme at four laboratories in Europe, New Zealand, and the United States. Morrow et al. reported the identification of the first gene regulated by switching between white and opaque colony formation in *C. albicans* in 1992 (298). The nucleotide sequence revealed that this gene codes for an acid proteinase in *C. albicans*. This gene was identical to the one isolated by Hube and his collaborators in Europe in 1991 (191). This discovery suggested that two homologous genes could have different functions. B. Magee, in collaboration with her colleagues from Europe and New Zealand, assigned the clones of the extracellular proteinase genes to the electrophoretic karyotypes of *C. albicans* in 1993 (268). They reported that the two genes map to different chromosomes and that every strain tested so far contains both genes, and the nonidentity of the two genes was confirmed (268). They also found evidence for additional genes, which suggested that there was a gene family in *C. albicans*, and named this gene family the secreted acid proteinase (SAP) family.

Fungal molecular genetics. The genetics of *C. albicans* was the subject of considerable study in the 1980s. Auxotrophic mutants were isolated and characterized in the 1970s, but mapping and recombination studies began in the 1980s. The leading discovery was the recognition by Whelan et al. in 1980 that *C. albicans* was a diploid organism when studied by UV-induced mitotic recombination, which yielded auxotrophs (452). This discovery confirmed Olaiya and Sogin's earlier conclusion, which was based on DNA content (315). Another important contribution in this area of research was the evidence that protoplasts of complementing auxotrophs of *C. albicans* could fuse in the presence of polyethylene glycol and generate prototrophic cells (359). This became another tool for the study of fungi.

Studies of DNA transformation and gene replacement in fungi with *S. cerevisiae* as a model began to appear in the literature during the late 1970s. An important property of the yeast transformation system was that cloned DNA sequences could be introduced into chromosomes by homologous recombination. This allowed the development of procedures for precise replacement of chromosomal regions with in vitro-altered DNA sequences (e.g., gene replacement). With this information, Miller et al. demonstrated in 1985 that *A. nidulans* genes can be replaced with mutant alleles made in vitro (290) by either the one- or two-step procedure developed in 1979 for *S. cerevisiae* (362). Gene replacement permitted rapid proof of the identity of new genes that are isolated by complementation of mutations (290). It also made possible investigation of the biochemical and biological consequences of introducing a specific, preselected mutation into the genome of otherwise unaltered cells.

Kurtz et al. developed a DNA-mediated transformation system in 1986, further improving the genetic analysis of *C. albicans* and other fungi (229). Since transformation was a rare event, this system required a gene that functions in the host and a system for selecting individuals that have taken up and expressed this gene (229). The gene chosen for transformation was the cloned *C. albicans ADE2* gene. This DNA plasmid became integrated into host DNA at the site of the *ADE2* gene and was the first DNA-mediated transformation in *C. albicans*. It facilitated the creation of selective markers, was a valuable tool for the genetic manipulation of this yeast, and led to the transformation of other fungi. One of these markers was the *URA5* gene from *C. neoformans* that Edman and Kwon-Chung isolated in 1990 (105).

The transformation system of *C. albicans* stimulated efforts to clone many of its genes. Libraries of *Candida* DNA for complementation of auxotrophic mutations led to the identification of a significant number of DNA sequences. At the same time, electrophoretic procedures were developed to visualize fungal chromosomes. Schwartz and Cantor (366) and Carle and coworkers (54, 55) developed a new agarose gel electrophoresis system, called pulsed-field gradient electrophoresis, that was capable of fractionating up to 2-Mb chromosome molecules. This system separated intact *S. cerevisiae* chromosomes for the first time. By using Southern blotting (392) and hybridization with chromosomally assigned probes, gel bands could be assigned to their corresponding chromosomes (366). In 1986, Chu et al. manipulated electric fields by arranging multiple electrodes along a polygonal contour clamped to predetermined electric potentials (60). The CHEF technique overcame the problem of a nonuniform electric field. The following year, Vollrath and Davis addressed the problem of resolving DNA molecules larger than 2 Mb by using the CHEF procedure (433). This was an important breakthrough because pathogenic yeasts contain chromosomes that are larger than 2 Mb.

Pulsed-field electrophoresis procedures allowed B. B. Magee and coworkers to suggest in 1988 that *C. albicans* had seven chromosomes and to assign genes to them by hybridization (267). In the ensuing year, other identified karyotypes gave an estimate of six to nine chromosomes. On the basis of more advanced methods of pulsed-field electrophoresis and studies of genetic linkages, Wickes et al. reported in 1991 that the basic number of chromosomes for *C. albicans* was eight (457). Failure to separate the two largest chromosomes (including the rDNA gene) had provided a previous count of seven. During the same year, Wickes et al. reported that phenotypic change occurred in conjunction with chromosomal rearrangement in *Candida stellatoidea* type I (a subspecies of *C. albicans*) (456). Pulsed-field electrophoresis also enabled Kwon-Chung et al. in 1992 to separate the chromosomes of the type culture of *F. neoformans* var. neoformans and the tester strains (type a and type b) (236). They found that the two mating types had different karyotypes. Studies in mice of the virulence of mated congenic strains demonstrated that type b was more virulent than type a (236). Wickes obtained her doctoral degree at Catholic University, Washington, D.C., under the guidance of Kwon-Chung and pursued her postdoctoral studies at the laboratory of J. C. Edman, University of California at San Francisco (117).

Jacobson studied the genetics of two virulence factors of *C. neoformans*, the capsule and melanin, at the VA Hospital and MCV in Richmond. Jacobson et al. in 1982 examined the inheritance of the cryptococcal capsule (197), a virulence factor that had been described in the 1970s (44, 45). Jacobson and coworkers described the production of the phenotypic and genetic characteristics of capsule mutants. They concluded that chromosomal genes are responsible for synthesis of the cryptococcal capsule and that these genes were not linked to auxotrophic markers (197). In 1991, Jacobson and Emery provided genetic support for a model that linked melanization with resistance to oxygen toxicity and virulence of *C. neoformans* (198). In 1993, Jacobson and Tinnell reported that the antioxidant capacity conferred by melanin had a value that approximated antimicrobial oxidant production by stimulated macrophages (199). They concluded that melanin appeared to

protect *C. neoformans* against leukocyte oxidants. Geis et al. confirmed for the first time in 1984 that melanin, a possible virulence factor in *W. dermatitidis*, was produced by the pentaketide pathway (141). Mutants with a decrease or loss in melanin synthesis were induced and isolated.

Slutsky et al. demonstrated in 1985 that *C. albicans* switched heritably and reversibly at high frequency between several general phenotypes identified by colony morphology on agar (383). This discovery suggested to them that switching may provide *C. albicans* and other fungi with the capacity to invade tissue and diverse body locations, evade the immune system, or change antibiotic resistance (383). In 1987, Soll and coworkers reported for the first time that switching was occurring at the site of infection and provided a minimum estimate of the number of types of switching systems and strains that were involved in vaginal candidiasis (391). In 1992, Morrow et al. identified the gene (*PEP1*) regulated by switching in *C. albicans*, which encoded an acid protease of the pepsinogen family (298). By 1993, two other genes had been identified by this group: *Op4*, which is on a different chromosome than *PEP1*, and the white-specific gene *cWh11*, which is not transcribed in opaque cells (395).

Physiology and nutrition. In 1981, Maresca and coworkers discovered that cysteine stimulation of oxygen consumption is due to a cytosolic cysteine oxidase activity, which is present only in yeast-phase cells (271). By 1983, Kumar et al. were able to isolate and purify the cysteine oxidase from yeast cells of *H. capsulatum*. This enzyme appeared to play an important role in the phase conversion of *H. capsulatum* (228). In 1993, Szaniszlo's group reported that an extremely low pH medium containing 0.1 mmol of 1^{-1} Ca^{2+} induced large numbers of muriform cells in three etiologic agents of chromoblastomycosis (286). Mendoza et al. indicated that high Ca^{2+} concentrations reversed this tendency and promoted maintenance of hyphal growth, which may play a role in infections in human tissue (286). In the same year, Cooper and Szaniszlo developed an artificial parasexual cycle in *W. dermatitidis* and identified two of its cell division cycle genes, *CDC1* and *CDC2* (68).

Sokol-Anderson et al. in 1986 examined the role of oxidative injury induced by amphotericin B (390). It had been known that amphotericin B and other polyene macrolide compounds bind to the cell membrane ergosterol, a major sterol in fungal cells (cholesterol in mammalian cells). The mechanism of cytotoxicity had been poorly defined. Sokol-Anderson et al. described two distinctive actions of amphotericin B on fungal cells, a prelethal leakage of potassium (low amphotericin B doses) and a lytic effect caused by doses used clinically (390). The latter was the only effect closely tied to oxidative damage.

Training and research centers. As a result of the substantial reduction in federal funds and depletion of private support, the training programs at Washington University, Tulane University, the University of California at Los Angeles, the University of Kentucky, and the University of Oklahoma at Norman were terminated. When Silva-Hutner retired in 1981 from Columbia University, this training and research center ceased to exist. The Naval Biological Laboratory in Oakland, Calif., was also closed in the early 1990s following Levine's retirement. Kaplan retired from the CDC in the late 1980s, Ajello retired in September 1990, and this team was disbanded. At CDC, the focus has changed as new areas for training and research emerge and emphasis is placed on basic research. Gordon and Salkin retired from the New York State Laboratory, and Dennis Dixon and Chester Cooper left this institution in the early 1990s and were not replaced. S. Shadomy died in 1991, and J. Shadomy retired in 1992 from MCV in Richmond, Va. Since neither scientist was replaced, formal training at this center was also terminated.

CONCLUSIONS

This historical study has traced the development of medical mycology as a discipline in the United States from 1894 to 1994. The discipline began within the context of the sweeping technological and social changes occurring at the end of the nineteenth century. The establishment of major medical centers with state-of-the-art equipment and the best trained personnel available initiated advances in patient care during those early years. Although a great deal of progress has been made since the late 1890s, the crisis in formal and informal training that began in the 1980s presents significant problems for medical mycologists today. On the other hand, in the last 15 years, important research contributions have come from a more diversified group of scientists as well as from the research and training resources that replaced formal programs established since the 1930s. These advances have made possible the shift to fungal molecular biology, cellular immunology, and genetics which appears to be necessary to combat the increased virulence and resistance of fungi to chemotherapies. While these new technologies are being applied, the cost of equipment and the dearth of properly trained personnel may limit their use. These trends suggest that the current status of medical mycology may be compared with that during its early years of uncertainty.

Increased public pressure for downsizing, cost containment, and accountability for expenditures has narrowed the focus of research activities, and the creativity of researchers is often channeled toward goal-oriented studies. This trend has been strengthened by the dependence of investigators on corporate support. Therefore, scientists have become more specialized and must, of necessity, form partnerships and collaborative groups. Educational methods have also been significantly altered. Rapid developments in electronic media are replacing laboratory training under the guidance of skilled investigators. Since it is costly to train, staff, and equip the needed laboratories, there are fewer places with the necessary resources for complete medical mycology diagnostic work. Furthermore, the rapidity of change raises uncertainties about what would constitute proper education in medical mycology even if more resources were available.

What should leaders in the field do in response? Suggested areas of study include a formal evaluation of baseline competency in the field as well as appropriate educational models to prepare individuals for their various roles. There is a critical need to combine effective training with the new technologies. Also, it is important to take into account emerging trends in the incidence and prevalence of fungal infections, for further educational and scientific developments that will lead to improvements in patient care. For this strategy to be successful, medical mycologists must work to increase the range and availability of reliable resources. Furthermore, organizations in control of resources must recognize the importance of basic research advances without neglecting the practical aspects of the field, including training and education. Ongoing funding is indispensable for both applied and basic medical mycology education and research to thrive.

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