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Session 1

Tuesday, 24 February 1970, 9:15 a.m.

THE MYCOSES AS A MAJOR PUBLIC HEALTH PROBLEM

Chairman

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THE MEDICAL MYCOLOGICAL ICEBERG

Libero Ajello

Any attempt to quantitate the impact of the mycoses on public health is doomed to failure. Since they are not universally classified among the notifiable diseases, hard data on their incidence and prevalence, as well as information on their morbidity and mortality, are either fragmentary or simply not available. Numerical data on the mycoses are not compiled by any nation or organization. The size of the medical mycological problem is further obscured by trade secrecy, which makes it difficult to obtain or to publish figures on the dollar and cents value of the antifungal pharmaceutical preparations marketed.

The situation that confronts us can well be likened to an iceberg. The only visible portions of the vast bulk of the mycoses problem are a few peaks and crags. Even these are only dimly revealed at best by the few scattered reports that are available on the incidence and prevalence of fungus infections.

The bulk of the problem lies submerged in a murky sea of ignorance. The true dimensions of the medical mycological burden that weighs on the people of the world remain unknown. As a consequence, the public is apathetic, and public health organizations have not given any truly significant or sustained support to programs in this area.

The medical mycological problem is large indeed. Data indicating the size of it have been culled for this presentation from numerous case reports, reviews, and surveys published by investigators throughout the world. They have been organized under three broad headings: cutaneous mycoses, subcutaneous mycoses, and systemic mycoses.

Cutaneous mycoses

Among this group of diseases are some that approach dental caries and the common cold in both incidence and prevalence. Untold numbers of people throughout the world are afflicted by the fungi that invade and destroy our skin, hair, and nails.

In tropical regions of the world, tinea versicolor is extremely widespread. Millions of individuals are infected in Africa, Asia, and Latin America. For example, in the Democratic Republic of the Congo, Vanbreuseghem (*67*) found that this disease was the most prevalent of all the mycoses. The coastal areas of Mexico, the so-called *tierras calientes*, are particularly rife with this disease, and González Ochoa (*20*) has observed a 50 per cent rate of infection in the general population. An equally high prevalence of this disease was encountered by Marples (*44*) in Western Samoa. She noted that the disease "did not appear to be regarded by the Samoans as worthy of attention and in most cases was untreated." A similar situation must exist throughout Melanesia and Polynesia. Indifference to tinea versicolor is universal; many victims are either unaware of their infection or are resigned to live with it because of limited financial resources and lack of medical facilities. Usually only individuals concerned about the cosmetic effects of the disease are motivated to seek medical care.

Although it is especially prevalent in the tropics, tinea versicolor occurs elsewhere as well. Stein's data (*62*) show that it is responsible for approximately 5 per cent of the fungus infections in temperate regions. Certainly this disease is not rare in the United States. Dermatologists are well acquainted with it and are consulted by many patients.

Tinea pedis is another cosmopolitan disease; myriads of cases occur in all countries of the world. In contrast to tinea versicolor, this disease is more widespread in temperate than in tropical areas. As it happens, "athlete's foot" is virtually unknown in those regions where large numbers of inhabitants go without shoes because of the combined factors of warm climate and low levels of income. In other areas, however, it may affect from 50 to 90 per cent of the people in the course of their lives (*37*). English (*14*) estimates that up to 70 per cent of the general population may have clinical signs of tinea pedis, although only a small proportion of such individuals can be proven to have a mycotic infection. In certain population groups, however, the rate of confirmed cases may be quite high. Hulsey and Jordan (*29*) demonstrated fungus elements in 63 per cent of the university students they examined. During World War II, Hopkins and co-workers (*28*) found foot lesions in more than 80 per cent of the men on an infantry post. Microscopic studies of the skin revealed fungus elements in 70 per cent of those who had intertrigo of the toes and in over 90 per cent of those with dyshidrotic lesions on the soles.

Blank, Taplin, and Zaias (*7*) report that skin diseases among the American troops in Vietnam are the commonest cause of disability. In the Mekong Delta, for example, 77 per cent of 209 men required hospitalization for "foot infections." The etiologic agent involved most frequently in the dermatomycoses was *Trichophyton mentagrophytes.*

Despite such optimistic statements as "Ringworm of the scalp, a scourge of childhood for more than 2,000 years, has finally yielded to treatment with griseofulvin" (*27*), this disease still flourishes in many parts of the world (*36, 49, 61*). It is especially prevalent in the underdeveloped areas of Africa, Asia, and Latin America, where funds for specific medication with griseofulvin are not readily available. The prevalence of tinea capitis is directly related to the economic status of the families and of the country in which they live. For example, a survey by Vanbreuseghem (*68*) in Somalia showed a 36 per cent prevalence of tinea capitis among boys 5 to 10 years of age. In the Sudan, Mahgoub (*42*) noted that the rate of infection in a boys' boarding school was 17 per cent.

The incidence of scalp infections is also high in the Middle East and parts of Asia. Rates reached 23 per cent in a home for boys in Poona, India (*48*), and 10 per cent in a school in Kashmir (*33*).

In general, scalp infections in Europe and the United States are relatively infrequent. As in other parts of the world, however, their prevalence is greatest among the socially deprived groups. Beginning in 1960, one of the most extensive tinea capitis surveys in history was conducted in Yugoslavia under the direction of Dr. E. I. Grin (*24*). A total of 1,782,000 people were screened. Among them, 94,296 cases were diagnosed, corresponding to an infection rate of 5.3 per cent. In some villages, morbidity was as high as 8.6 per cent. In Greece, a recent survey (*63*) revealed a 1.4 per cent level of infection among 4,701 children examined. However, in one village, the incidence was 17 per cent. A 1959 Washington, D.C., survey (*32*) showed that 0.8 per cent of the elementary school population was infected, and an Atlanta, Georgia, study revealed that 2.6 per cent of 1,753 schoolchildren had tinea capitis (*5*).

Although the tineas are not usually disabling, they do constitute an important public health problem. In many countries, children with ringworm of the scalp are barred from school until they are cured. Thus, at a critical age in their lives, they are deprived of their educational rights. In addition, they may be subjected to psychological traumas by being forced to wear

distinctive headwear and by being shunned by their peers and by neighborhood families.

The social consequences of *Trichophyton concentricum* infections in Melanesia and Polynesia merit special attention. Tinea imbricata is well established in many islands in the southern part of the Pacific Ocean. Infection rates as high as 18 per cent have been found in some villages of Papua and New Guinea (*39*). In a carefully conducted epidemiological study in New Guinea (*58*), the social consequences stemming from tinea imbricata were discovered to be profound. The shunning of infected males as prospective husbands contributes to bachelorhood among men. Infected women are married at a later age than uninfected ones, and then most often they become the second wife of a polygamous husband. In addition, infected children and adults are discriminated against in respect to educational and employment opportunities. Lack of funds for mass treatment and control programs prevents reduction or elimination of the disease and its attendant social problems.

Tinea corporis and nail infections are quite prevalent throughout the world. Data on their frequency are not available, but the general opinion is that these conditions are not rare, and some, such as nail infections, are increasing in prevalence (*27*).

An indirect estimate of the size of the cutaneous mycoses problem can be obtained through data on expenditures for antifungal preparations. Information obtained in 1960 (*3*) revealed that $25,000,000 had been spent for ringworm medications during the previous year. More recently, the *Wall Street Journal* of 6 March 1968 quoted the 1966 sales of griseofulvin at $6,700,000. If we assume, conservatively, that $25,000,000 has been spent in the United States for ringworm every year since 1959, their dollar value to date in this country alone comes to $275,000,000 for the past eleven years.

It should be obvious to all that the cutaneous mycoses do, indeed, constitute a serious public health problem. Their toll in terms of suffering, disability, man-hour losses, psychological trauma, and monetary expenditure is much greater than is generally realized.

Subcutaneous mycoses

Under the heading of subcutaneous mycoses the following three diseases will be discussed: chromoblastomycosis, mycetomas, and sporotrichosis. Here the data on prevalence and incidence are even more fragmentary and incomplete than those on the cutaneous mycoses. Nevertheless, occasional surveys give fleeting glimpses of the dimly sensed bulk of their numbers.

Cases of chromoblastomycosis are especially prevalent in Africa and Latin America. The disease also occurs with less frequency in Asia, Australia, Europe, the United States, and Canada.

Every public health worker in Latin America and anyone who has visited hospitals there cannot fail to be impressed by the number of patients with chromoblastomycosis in the wards and outpatient clinics. Data compiled by Romero and Trejos (*53*) reveal how common this crippling and disfiguring disease may be. In Costa Rica, they estimated that the case rate was approximately 1 per 24,000 inhabitants. The prevalence rate in the Republic of Malagasy is also high. During the four-year period 1955-1959, Brygoo and Segretain (*8*) recorded 129 cases, signifying a case rate of 1 per 32,500 population. In one district, the incidence reached an astounding 1 per 7,000 inhabitants.

Such estimates, few and crude as they may be, provide an insight into the size of the problem that must exist in these and many other countries. Due to the therapeutic intractability of this infection and its high incidence, chromoblastomycosis looms as a disease of considerable public health importance.

Mycetomas occur with striking frequency and devastating effect in the tropical regions of the world. A survey by Abbott (*1*) in the Sudan revealed that over a 30-month period 1,231 cases had been admitted to hospitals and "a great many more were seen in outpatient departments."

Studies carried out in other parts of Africa reveal that mycetomas are prevalent in Algeria, Cameroun, Chad, Malagasy, Niger, Somalia, Tanzania, and Uganda (*43*). Rey (*52*) presents data to support the thesis that mycetoma prevalence rates comparable to those of the Sudan exist across Africa in a belt characterized by an annual rainfall of 250 to 500 mm of rain.

In Latin America, a survey conducted by Mariat (*43*) documented a high number of cases in Argentina, Mexico, and Venezuela. By far the greatest number was registered in Mexico. Over a 20-year period, a list of 206 cases was compiled by Dr. Latapi (*43*). Venezuela, with 68 cases, and Argentina, with 23, were the other countries with a relatively high frequency of mycetomas.

The disease is less common in temperate regions. Green and Adams (*23*) supported the validity of reports of only 63 cases for the United States for the years 1896 to 1964. Approximately 100 cases have been reported in Europe (*47*).

Since Asian publications on mycetomas are few in number, we have only a vague idea of their prevalence in that vast part of the world. A spot survey of material filed in the pathology departments of five medical colleges in southern India brought to light 187 cases (*34*). This report gives an inkling of the true size of the problem as it must exist not only in this area of India but throughout Asia as well.

Mycetomas are not as rare as currently available data would indicate. They occur with high frequency in a broad zone around the world. The numerous victims lead lives of resigned desperation, since in the absence of medical services and effective chemotherapy they face the inevitable and irreparable loss of limbs and a desolate future. These infections are a challenge to public health workers everywhere to develop preventive programs and to establish centers for early diagnosis and prompt surgical intervention.

In recent years, sporotrichosis has been shown to crop up with surprising frequency in both temperate and tropical regions throughout the world. The greatest recorded outbreak of this or any other subcutaneous mycosis occurred in the deep subterranean gold mines of South Africa. Over a period of 28 months, 2,825 miners became infected after contact with timber overgrown with *Sporothrix schenckii* (*25*). Sporotrichosis is well known as an occupational hazard for florists, pottery packers, and others who come in contact with sphagnum moss (*12, 16*), straw (*19*), and wood products (*6*). But the majority of infections occur sporadically, usually following some traumatic incident in which soil-engendered spores of *Sporothrix schenckii* enter the wound. In parts of Brazil, sporotrichosis is estimated to account for 0.5 per cent of all the dermatoses (*56*). The disease is especially common in Mexico (*35*); in the city of Guadalajara, it is considered to be the most prevalent of the noncutaneous mycoses (*2*). So many cases go unreported, however, that its true incidence remains unknown.

The development and use of skin test antigens for sporotrichosis have begun to reveal the occurrence of widespread subclinical infections by *S. schenckii* in the general population. Small-scale surveys carried out in Louisiana showed a sensitivity level of 11 per cent among prison and hospital inmates. In contrast, high-risk plant nursery workers had a 33 per cent sensitivity rate, and the levels rose to 58 per cent among those who had been employed ten years or longer (*57*). In Arizona, the same antigen elicited positive reactions in 10 per cent of a group of 203 hospital patients (*30*). Sporotrichin prepared in Brazil elicited a 24 per cent level of reactions in a small group of Brazilians and no reactions among 55 individuals in Germany (*69*).

Systemic mycoses

Five diseases—blastomycosis, coccidioidomycosis, cryptococcosis, histoplasmosis, and paracoccidioidomycosis—will be discussed under the heading of systemic mycoses.

Although blastomycosis first came to medical attention in 1894 (*17*), much is still unknown

about its geographic distribution, prevalence, and the natural habitat of its etiologic agent, *Blastomyces dermatitidis*.

At present, blastomycosis is known with certainty to be endemic only in the United States, Canada, and eight African countries: Democratic Republic of the Congo (*4*), Morocco (*62*), Mozambique (*40, 41*), Republic of South Africa (*4*), Rhodesia (*56*), Tanzania (*4*), Tunisia (*4*), and Uganda (*4*).

By far the greatest occurrence has been recorded in the United States. Dr. John F. Busey (personal communication) has tabulated 1,470 cases dating from 1894 to 1968. A survey of the records of 170 Veterans Administration hospitals for the 12-year period 1946–1957 disclosed reports on 198 proven cases, or an average of close to 17 a year (*9*). Another survey by Schwarz and Goldman (*59*) revealed that 99 patients were hospitalized in the United States during the first six months of 1953. A study of mortality from selected nonnotifiable diseases published by the National Communicable Disease Center (*64*) showed 188 deaths attributed to blastomycosis—an average of 19 a year over the 10-year period 1958–1967. Thus, the disease is a matter of considerable public health importance within the United States.

The prevalence of blastomycosis in Canada is relatively low compared to that in the United States. In the latest available compilation, 114 cases had been registered from 1906 to 1962, for a yearly average of 1.8 (*22*).

More time is needed before we can assess the nature and size of the blastomycosis problem in Africa. So far, only 11 cases have been diagnosed, or a least published, from there.

Coccidioidomycosis is a disease of limited distribution. It is only known with certainty to occur in North, Central, and South America, where its etiologic agent, *Coccidioides immitis*, flourishes in semiarid regions.

In the endemic areas of the United States, coccidioidomycosis is a major disease. Some 35,000 new infections are said to occur yearly in California alone (*15*). For the entire endemic area in Arizona, California, New Mexico, Nevada, Texas, and Utah, the annual total is believed to be in the neighborhood of 100,000. An estimated one third of these cases develop overt signs of infection. The latest compilation of deaths attributed to coccidioidomycosis in the United States reveals a yearly average of 53.3, for a total of 533 over the 10-year period 1958–1967. As Fiese (*15*) has pointed out, however, it is morbidity rather than mortality that makes coccidioidomycosis a serious disease. "In the most highly endemic areas—Bakersfield, California; Phoenix, Arizona; and El Paso, Texas—nearly 100 per cent of the population will have been infected in a few years, and about a fifth of them will have had an illness severe enough to cause temporary incapacity and to warrant medical care."

Unfortunately, data from Latin America on coccidioidomycosis are much less complete than those from the United States. In Mexico, skin test surveys have hinted at prevalence rates ranging from 5 to over 50 per cent in many states: Baja California, Chihuahua, Coahuila, Durango, Guanajuato, Jalisco, Nayarit, Nuevo León, San Luis Potosí, Sinaloa, Sonora, and Tamaulipas (*21*). The states of Colima, Guerrero, and Michoacán, despite their tropical climate, also have significant coccidioidin sensitivity levels among their native populations—10 to 30 per cent in Colima and Michoacán, and 5 to 10 per cent in Guerrero.

Endemic areas are small in Central America, existing only in Guatemala and Honduras. Coccidioidin sensitivity levels of 26 per cent were found by Mayorga (*45*) in two villages located in the Motagua Valley of Guatemala. In Honduras, Trejos (*45*) found a reactivity level of 16 per cent in the Comayagua Valley. A 1969 survey showed that 9 per cent of 448 residents in the city of Comayagua had positive reactions (*31*).

In South America, Venezuela and Argentina have the most extensive endemic areas. The status of coccidioidomycosis in Venezuela was recently studied by Campins (*10*). The disease

is endemic only in the states of Falcón, Lara, and Zulia. Coccidioidin sensitivity levels of 46 per cent have been found in Lara, and of 24 per cent in Falcón. Data on Zulia are not available.

Few skin test surveys have been carried out in the remaining coccidioidomycosis areas in South America. In Santiago del Estero, Argentina, a sensitivity level of 19 per cent was recorded among 2,213 children between the ages of 6 and 16 (*46*). Only two coccidioidin surveys have been carried out in Paraguay, and none have been made in Bolivia. The Paraguayan studies revealed a 44 per cent level of reactivity among a group of 82 Indians (*4*) and less than 3 per cent reactivity in the city of Asunción (*18*).

Much remains to be done before the full extent of the coccidioidomycosis problem in Latin America becomes known.

Cryptococcosis is one of the most serious and dreaded of the systemic mycoses. Its etiologic agent, *Cryptococcus neoformans,* has a marked tendency to invade the central nervous system and cause meningitis. Cases of this disease have been recorded in virtually all parts of the world. They present a diagnostic challenge, since the symptoms induce clinical and pathological changes that resemble tuberculosis, neoplasms, brain tumors, and insanity. Failure to recognize this mimicry leads to delays in accurate diagnosis and prompt administration of specific therapy, and has even resulted in commitment to mental institutions.

An accurate estimate of the prevalence of cryptococcosis and the morbidity that it causes is impossible to make at this time. Although cases are not required to be registered, other types of data indicate that this disease causes great suffering and that mortality is high. In the United States, 734 deaths have been attributed to cryptococcosis over the 10-year span 1958–1967, for a yearly average of 73 (*64*). No statistics of this kind are available for other countries.

A few years ago, Utz (*66*) estimated that 200 to 300 cases of cryptococcal meningitis occurred annually in the United States. This figure, although based on an educated guess, may not be too far from reality. If the annual average of deaths attributed to *C. neoformans* is 73, and if we assume in this era of amphotericin B therapy that fewer than one fourth of the cryptococcosis patients die, then about 290 clinical cases of this disease probably occur annually.

Last year, the Fungus Immunology Unit of the U.S. National Communicable Disease Center received 666 sera and spinal fluids from 478 patients with suspected cryptococcosis, and 85 of these specimens gave positive reactions. If other diagnostic centers released or recorded similar information, we could begin to get an idea of the prevalence of cryptococcosis not only in the United States but in the rest of the world as well.

It is the writer's belief that cryptococcosis is the sleeping giant among the deep mycoses. When reporting and surveillance programs are established, the number of cases will prove to be astonishingly high. The tip of the iceberg in this case is deceptively small.

Information on the prevalence and incidence of histoplasmosis is extensive when compared to that available for the other mycoses. Much remains to be learned, however, before we have the full picture of its impact on human welfare. Histoplasmosis cases have been diagnosed in virtually all parts of the world, but the frequency of infection varies considerably from region to region. Histoplasmin skin test surveys have revealed many areas where levels of infection are high among certain groups of individuals. Reaction levels of 10 per cent or higher were found in one or more regions of 25 countries: Algeria, Argentina, Brazil, Burma, Canada, Colombia, Cuba, Democratic Republic of the Congo, Ecuador, French Guiana, Honduras, Italy, Liberia, Malaya, Mexico, New Guinea, Nicaragua, Pakistan, Panama, Paraguay, Puerto Rico, Ruanda-Urundi, Surinam, the United States, and Venezuela (*26*).

Absence of reporting makes it impossible to cite morbidity and mortality data for histoplas-

mosis. In the United States, estimated infections number in the millions. On the basis of one of the best planned and most extensive histoplasmin surveys ever carried out, it has been determined that the sensitivity level in the 48 contiguous states averages 20 per cent (*13*). Using the latest U.S. Census Bureau estimate of 200,485,000 people for the 48 states and assuming that the yearly sensitization rate is constant and the histoplasmin reaction is specific, we can calculate that approximately 40,000,000 people have been infected. On the basis of earlier data, Furcolow estimated that approximately 200,000 cases of acute pulmonary histoplasmosis occur yearly in the United States (*65*). From 1958 to 1967, 736 deaths were attributed to this disease, for an annual average of 74 (*64*).

Information of this kind suggests the magnitude of the histoplasmosis problem. Additional attention is needed to ensure that facilities are made generally available for the prompt and accurate diagnosis of the infection so that specific therapy can be initiated in the early and more responsive stages of the disease.

Of all the systemic mycoses, paracoccidioidomycosis has the most restricted geographic distribution. As far as is currently known, this disease occurs only in Latin America. Its domain extends from Mexico to Argentina. The only places in this region with no reported cases so far are Chile, Guyana, and Surinam, in South America; British Honduras and Panama, in Central America; and the islands of the West Indies.

Case reports from Ghana (*38*) and Malagasy (*55*) are believed to be erroneous.

In the endemic areas, the incidence and prevalence varies greatly from country to country and from region to region within the countries. The greatest number of cases have been encountered in Brazil, Colombia, and Venezuela. Chirife and del Río (*11*) found that 1,724 cases had been recorded in Brazil, for a morbidity rate of 2.5 per 100,000 inhabitants. Venezuelan cases totaled 300, giving a rate of 5 per 100,000. Restrepo and Sigifredo Espinol (*50*) cited 373 cases for Colombia—337 more than were listed by Chirife and del Río (*11*) three years earlier. For all of Latin America, 3,037 cases have been recorded. Such figures should be regarded as only an approximation of the true prevalence of paracoccidioidomycosis. The actual number of clinically manifest cases is probably much higher.

Until recently, lack of potent and specific skin test antigens prevented epidemiological surveys from being used in determining the prevalence of infections and in locating endemic areas. Dr. Angela Restrepo, however, has now developed such an antigen. With it, she and her collaborators (*51*) have begun population surveys. Among 3,938 individuals tested, 10 per cent were positive to a mycelial antigen, and 6 per cent to a yeast-form reagent. Variation among the countries ranged from 6 to 13 per cent.

Despite some evidence of cross-reactivity with histoplasmosis, the paracoccidioidin survey indicated that an asymptomatic benign form of paracoccidioidomycosis may occur in the endemic areas. There is an obvious need for more extensive field studies with standardized antigens. When these studies and surveillance programs are under way, we will begin to obtain a more objective picture of the paracoccidioidomycosis problem.

Discussion

An attempt has been made to reveal the dimensions of the medical mycological iceberg. The information we have been able to gather, based on the meager data available and on educated guesses, only refers to the visible peak of an enormous submerged body. But this information, faulted as it may be, indirectly permits us to visualize and quantitate the dimensions of the entire mass. The writer is convinced that the mycoses represent a greater health burden and challenge than is realized by the public or by their health officials. Morbidity and mortality associated with the

pathogenic fungi have been continuously underreported.

It is a well-known observation that whenever properly trained and motivated individuals begin to study mycological problems a host of cases are uncovered where none had been thought previously to occur. As a result of this phenonemon, geographic distribution maps and prevalence and incidence data are misleading. The records generally reflect the location and activities of an investigator rather than true distribution patterns of the diseases. Many regions considered to be relatively free of mycotic infections can properly be said to lack medical mycologists rather than mycoses.

The medical mycological picture is not all bleak, however. The present meeting reflects growing interest in the mycoses on the part of the Pan American Health Organization.

In the United States, at the recent Second National Conference on Histoplasmosis (Atlanta, Georgia, 6–8 October 1969), a resolution was passed recommending that steps be taken by the U.S. National Communicable Disease Center to have the mycoses classified as notifiable diseases. The lengthy process for implementing this resolution has already been initiated. In addition, the NCDC, through its Ecological Investigations Program, has begun a publication entitled *Mycoses Surveillance* (65), which promises to provide much-needed data.

At Buenos Aires in 1966 the XV Pan American Congress on Tuberculosis and Pulmonary Diseases passed a resolution sponsored by the Union of Latin American Tuberculosis Societies (ULAST), under the guidance of Dr. José I. Baldó, recommending that all member countries establish coordinating commissions for study of the mycoses at the national level. Several countries have already done this. All others should be urged to follow their example. Once the commissions start to function, reporting mechanisms will be developed and implemented. Conceivably, the work of these groups could be coordinated under the auspices of WHO and the Pan American Health Organization. Global morbidity and mortality data would then be systematically collected, evaluated, and distributed to all persons interested in public health.

Until we can show that the apparent size of the mycoses problem is deceptively small, that in reality the mycoses are common diseases, and that the toll they take in misery and mortality is high, we cannot expect to obtain the support we need for the development and implementation of control programs, research projects, and training courses.

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PREVALENCE OF CUTANEOUS MYCOSES IN LATIN AMERICA

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Generally under the term "cutaneous mycoses" are classified both dermatophyte and *Candida sp.* infections (*2, 20, 28, 30*). This report, however, will deal only with the prevalence of dermatophytoses, or tinea infections, which are the most important of the cutaneous mycoses.

The dermatophytoses are infections of the skin, hair, and nails caused by several species of dermatophytes: fungi belonging to the genera Epidermophyton, Microsporum, and Trichophyton. Since the same clinical picture in various parts of the body can be caused by different genera and species of dermatophytes, the classification of the dermatophytoses is based on the part of the body infected: tinea capitis, tinea corporis, etc. Only the *Trichophyton schoenleinii* and *T. concentricum* infections have characteristic clinical pictures. These are named, respectively, favus and tinea imbricata.

The prevalence of the dermatophytoses will be considered first in terms of the skin diseases seen in dermatologic practice and second in terms of the mycotic skin diseases diagnosed in mycological laboratories. Consideration will also be given to the prevalence of the different clinical forms of the dermatophytoses and to the incidence of tinea infections according to their etiologic agents.

Studies on the prevalence of the dermatophytoses in terms of the skin diseases seen in dermatologic practice have been carried out in Mexico (*10, 21, 38*), Central America (*10*), and Brazil (*39, 44*), and data on mycotic skin diseases diagnosed in mycological laboratories have been compiled in Venezuela (*34*) and Brazil (*11, 26*). Other investigations have been carried out on the prevalence of the clinical types of tinea infections and of ringworm according to their agents. In the last decade (1960–1969), dermatophytoses have been investigated in Mexico (*28, 38*), El Salvador (*23*), Colombia (*40, 47*), Venezuela (*6, 7, 34*), and Brazil (*11, 26*). Before 1960, research on this subject had been done in Puerto Rico (*12*), Uruguay (*31*), Argentina (*36*), and Chile (*46*). Information is also available from Ecuador (*41*), Cuba (*17*), Peru (*33*), Paraguay (*9*), and Central America (*10*).

The following summary is presented on the basis of the incomplete and fragmentary data available.

Prevalence of dermatophytoses in dermatologic practice

Superficial mycoses and cutaneous mycoses, which together are generally designated as dermatomycoses, constitute one of the most important dermatologic problems in the Latin American countries. In Mexico (*38*), they account for 17 per cent of the skin diseases seen in dermatologic practice and, in Brazil (*3, 44*), for 15 to 22 per cent. The prevalence of tinea infections, as observed in outpatient dermatologic clinics in selected countries of Latin America, is summarized in Table 1. The incidence of these infections varies with the economic status of the population. This accounts

Table 1

Prevalence of tinea infections in outpatient dermatologic clinics in selected Latin American countries

Country	Period	No. of patients examined	Types of tinea infection	Occurrence	
				Abs.	Rel.
Mexico (*10*)	1952	2,490	Of the scalp and skin	295	11.7
Mexico (*10*)	1958	2,960	Of the scalp and skin	485	16.0
Mexico (*10*)	1958	1,318	Of the scalp and foot	119	9.0
Mexico (*10*)	1959	3,433	Of the scalp and foot	441	12.7
Mexico (*10*)	1955–59	2,768	Of the scalp and foot	284	10.2
Mexico (*38*)	1949–56	10,000	Of the scalp	1,200	12.0
Mexico (*38*)	1954–58	11,360	Of the scalp	954	8.4
Mexico (*38*)	—	10,000	Of the scalp	331	3.3
Mexico (*21*)	1966	7,020	Of the foot	392	5.5
Guatemala (*10*)	1959	1,189	Of the scalp	130	10.9
Costa Rica (*10*)	1959	2,660	All types	193	7.3
El Salvador (*10*)	1959	1,672	All types	93	5.6
Brazil (*44*)	1954–66	10,841	All types	1,561	14.4
Brazil (*39*)	1964	2,773	All types	205	7.6

for the variable percentage of infected people seen, for example, in Mexican hospitals. In Brazil, climatic conditions explain to a certain extent the differences in prevalence between the northern and central parts of the country.

Prevalence of dermatophytoses in mycological laboratories

Tinea infections, compared to the other mycotic skin diseases seen in mycological laboratories, are the major diagnostic problem. The prevalence of dermatophytoses in relation to superficial mycoses and cutaneous mycoses diagnosed in several Latin American mycologic laboratories is presented in Table 2.

Prevalence of clinical types of dermatophytoses

Tinea capitis has been the most common clinical form of the tinea infections throughout Latin America, except in Puerto Rico and the southern part of Brazil. Its prevalence ranges from 39 per cent in Brazil (*25*) to 77 per cent in El Salvador (*23*). The incidence of the clinical types of tinea infections varies from country to country and within the same country from one time to another. Such variations are shown in Table 3.

Table 2

Prevalence of dermatophytoses in relation to superficial and cutaneous mycoses diagnosed in selected mycological laboratories in Latin America

Country	Period	Patients with dermatomycoses	Patients with tinea infections	
Venezuela (*34*)	1952–59	394 [a]	264	(67.1%)
Brazil, north (*44*)	1954–66	2,096 [b]	1,561	(74.4%)
Brazil, central (*11*)	—	298 [a]	194	(65.1%)
Brazil, south (*26*)	1956–66	1,463	842	(57.5%)

[a] Excluding candidiasis.

[b] Excluding candidiasis and onychomycosis.

Table 3

Prevalence of tinea infections according to clinical types in selected Latin American countries

Country	Period	Part of the body infected (%)					
		Scalp	Body	Feet	Groin	Beard	Nails
Uruguay (*31*)	1949	59.0	25.0	9.2	2.7	2.4	0.9
Puerto Rico (*12*)	1930–49	11.6	21.1	39.4	—	0.6	24.5
Brazil, south (*25*)	1957–63	27.9	33.2	12.2	23.4	1.4	1.7
Brazil, south (*30*)	1964–69	11.9	32.1	20.2	28.7	1.1	5.8

Prevalence of dermatophytoses according to etiologic agents

Autochthonous cases of human ringworm are widespread throughout Latin America. The species involved are *Epidermophyton floccosum, Microsporum canis, M. gypseum, Trichophyton rubrum, T. mentagrophytes,* and *T. tonsurans.* Four species are agents of autochthonous tinea infection distributed within restricted areas: *T. schoenleinii, T. violaceum, T. verrucosum,* and *T. concentricum.* Four species are isolated from sporadic autochthonous cases of tinea infections: *M. nanum, T. equinum, T. gallinae,* and *T. soudanense.* Infections caused by *M. audouinii* and *T. megninii* have been noted in patients coming from other countries.

T. tonsurans has been the predominant agent of tinea capitis in Mexico (*18, 38*), Puerto Rico (*12*), El Salvador (*23*), and the northern and central regions of Brazil (*8, 11, 35*). *M. canis* is the organism most frequently responsible for this infection in Colombia (*47*), Venezuela (*6, 7*), Uruguay (*31*), Chile (*46*), Argentina (*36*), southern Brazil (*25, 26*), and elsewhere generally in Latin America. It is also the agent most commonly isolated from other dermatophytoses throughout the region except in Puerto Rico and the central and southern regions of Brazil. With regard to ringworm, *T. mentagrophytes* has been the predominant species isolated in Puerto Rico (*12*), and *T. rubrum* is now the most frequent etiologic agent in certain parts of Brazil, replacing *T. tonsurans* in the central area (*11*) and *M. canis* in the southern area (*30*).

The order of prevalence of the etiologic agents of tinea infection varies from country to country and sometimes from region to region within the same country (*8, 11, 25*). It also varies in the same region from one time to another (Table 4). *M. gypseum* infections occur sporad-

Table 4

The five most frequently isolated dermatophyte species in Brazil, 1963–1969

Frequency of isolation	1963	1964	1965	1966	1967	1968	1969
Major	*M.c.*	*M.c.*	*M.c.*	*T.r.*	*T.r.*	*T.r.*	*T.r.*
↓	*T.r.*	*T.r.*	*T.r.*	*T.m.*	*M.c.*	*T.m.*	*T.m.*
to	*E.f.*	*E.f.*	*T.m.*	*M.c.*	*T.m.*	*M.c.*	*T.v.*
↓ minor	*T.m.*	*T.m.*	*E.f.*	*E.f.*	*E.f.*	*E.f.*	*M.c.*

M.c. = *M. canis*
E.f. = *E. floccosum*
T.r. = *T. rubrum*
T.m. = *T. mentagrophytes*
T.v. = *T. verrucosum*

ically in most Latin American countries, but they are frequent in central Brazil (*11*), Costa Rica (*32*), and Guatemala (*32*). Favus by *T. schoenleinii* is highly prevalent in some big cities or small rural areas where there are large numbers of people of Italian or Dutch descent (*5, 13, 15, 29*). Tinea infections by *T. violaceum* are highly prevalent in São Paulo, Brazil, where epidemic outbreaks occur (*13, 14*). *T. violaceum* was introduced by Italian immigrants and has become established in this part of Brazil. Tinea imbricata, caused by *T. concentricum,* is endemic among natives in restricted areas of Mexico (*42*), Guatemala (*37*), and Brazil (*22*). *T. verrucosum* occurs in Colombia (*40*), Uruguay (*31*), and Brazil (*27*). Sporadic cases of tinea infections have been caused by *T. gallinae* in Brazil (*42*) and Puerto Rico (*45*); by *T. equinum* in Uruguay (*31*) and Mexico (*19*); by *M. nanum* in Cuba (*16*) and Mexico (*4*); and by *T. soudanense* in Brazil (*1*).

Comment

In some of the Latin American countries, the incidence of cutaneous mycoses has not yet been investigated; in others, the studies are not recent, or they have been incomplete or limited to a small sample. In most countries, investigations have not been carried out uniformly over a long period of time. Consequently, it is impossible to draw a complete picture on the prevalence of the dermatophytoses. This situation also makes it difficult to document the dynamic changes in species frequency among the dermatophytes that take place under the influence of man's social activities, economic status, habits, and developments in hygiene and therapy.

There is no doubt that the dermatophytoses are of broad occurrence in Latin America. They are among the most common diseases seen in dermatologic practice. The dermatophytes are the pathogenic fungi most frequently isolated in diagnostic laboratories.

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PREVALENCE OF SUBCUTANEOUS MYCOSES IN LATIN AMERICA

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Introduction

This survey refers specifically to the prevalence and characteristics of subcutaneous mycoses in Latin America. It will be concerned primarily with sporotrichosis, mycetoma, chromoblastomycosis, and subcutaneous zygomycosis. Rhinosporidiosis, generally considered of fungal etiology, will also be included.

In reviewing the literature, several situations became evident. First, there are countries from which virtually no mycological reports were available (Nicaragua and Bolivia), so that there is no adequate knowledge of the existence and prevalence of subcutaneous mycoses in those areas. Second, many reports were deficient or faulty in terms of description of species and could not be considered for that reason. Third, most reports referred to the areas accessible to laboratories, and consequently no adequate panorama of the problem at the level of any single country could be constructed.

With these considerations in mind, a summary survey will be presented by disease and by country, with emphasis on the peculiarities or differentiating features of each mycosis in the various regions.

Sporotrichosis

Many cases of sporotrichosis have been described in Mexico in the last 15 years (*2, 33, 41, 49, 52, 108*). In 1954, González Ochoa (*41*) reported that more than 300 cases had been recognized in patients from different parts of the country, mainly Guerrero, Guanajuato, and Nuevo León. In a series of 257 cases, of which 80 per cent were males, lesions were more frequent in the lower extremities (73 per cent). In Morelos, however, the lesions appeared on the trunk in 40 per cent of the cases seen (*75*). In another series of 180 cases diagnosed in Mexico City, Latapí (*49*) reported that 82 per cent were of the lymphangitic type, while Aceves (*2*) found that the fixed form was more prevalent in a study of 118 patients from Jalisco. One of the Mexican reports refers to a child infected on the second day of life (*108*). Lavalle (*52*) described four family outbreaks all related to inoculation through contaminated straw.

Sporotrichosis has been described in all the countries of Central America except Nicaragua. In Guatemala, Solórzano (*104*), García (*38*), and Mayorga (unpublished) have diagnosed 80 cases. In El Salvador, Llerena (*55*) recognized 36 cases, while Corrales *et al.* (*27*) described 14 in Honduras. Lesions were more frequent in the extremities, and the lymphangitic type was predominant. In Costa Rica, Mata and Trejos (personal communications) diagnosed 125 cases in a period of three years (1956–1959), and they mentioned many children with facial lesions. Only five cases appear reported from Panama (*18, 74*), and only three have been recorded in Cuba (*107*). Recent publications from the Dominican Republic (*79*) and Guadeloupe (*9*) confirmed the presence of sporotrichosis on those islands.

Mention of the disease in Ecuador was made

by Rodríguez (*89, 90*). In Colombia, among 123 cases (*83, 84*), the fixed type was present in 77 per cent of the patients and the lymphangitic in 23 per cent—an opposite situation from that prevailing in the other Latin American countries. Fifteen additional cases were reported by Peña (*77*). In Venezuela, 76 cases were reviewed by Lizardo *et al.* (*54*). One report is available from French Guiana (*101*).

Numerous cases have been described in Brazil. In a series of 104 patients from São Paulo studied by Sampaio and co-workers (*96*), the lymphangitic type occurred most frequently. Similar findings were reported by Bopp and Bernardi (*11*) and Londero *et al.* (*57*) in Rio Grande do Sul. The studies of Rotberg *et al.* (*93*) in São Paulo and of Londero *et al.* (*59*) in Rio Grande do Sul showed a higher prevalence among the urban population. In the dermatology outpatient clinic of a hospital in Pará, Silva and Nazaré (*98*) described 56 cases among a total of 10,841 subjects. In Rio de Janeiro, Gonçalves and Peryassú (*40*) diagnosed 68 cases, and Ramos e Silva (*82*), 195. Family outbreaks have been registered in laborers handling straw (*71, 99*).

Sporotrichosis has seldom been reported from Peru. Miranda and Troncoso (*70*) and Miranda *et al.* (*69*) described five cases. A national survey made by Canese and Silva in Paraguay (*20*) resulted in the finding of 37 cases, with prevalence of the lymphangitic type.

In Uruguay, the disease has been studied by Mackinnon *et al.* (*63*), who found 157 cases, 50 per cent of which appeared to be related to armadillo hunting. In Argentina the disease has been reported by Pessano and Negroni (*78*) and by Grinspan and Madeo (*45*).

With regard to animal cases, infected dogs and cats (*37*) and mules (*95*) were observed in Brazil. One case was described in a horse in Colombia (*3*).

Mycetoma

The prevalence of mycetoma in Mexico was described by Aceves (*1*), Latapí and Ortiz (*50*), Saúl *et al.* (*97*), González Ochoa (*43*), and Lavalle (*51*). A review of the literature (*97*) shows that approximately 300 cases have been diagnosed in that country. About 20 per cent of those encountered by Lavalle (*51*) came from the State of Morelos. Aceves (*1*) studied 75 cases from Guadalajara. Most Mexican authors recognized *Nocardia brasiliensis* as the etiologic agent in 90 per cent of the cases. *N. madurae* is responsible for approximately 6 per cent, while *Streptomyces somaliensis* and *N. pelletieri* account for 2 per cent. Four cases of mycetoma caused by *Allescheria boydii* and *Cephalosporium sp.* appear in the literature (*51*). A review by González Ochoa (*43*) points out that lower extremities are involved in 63 per cent of the patients, while 20 per cent present thoracic wall lesions and 10 per cent are localized in the upper extremities. Abdominal wall lesions account for 3 per cent. Lavalle (*51*) demonstrated a higher frequency in males (82 per cent) than in females (18 per cent), and a higher prevalence in adults over 16 years of age (75 per cent).

In Central America, mycetomas are caused mainly by *N. brasiliensis*. In Guatemala, García (*38*) diagnosed seven cases, and Mayorga, 20 (unpublished). Most had lesions in the extremities. Cases due to *Madurella grisea* and *Aspergillus oryzae* were reported by Mayorga *et al.* (*66, 67*). Fifty patients with mycetoma caused by *N. brasiliensis* were found in El Salvador. *M. grisea, M. mycetomi, A. boydii, Cephalosporium falciforme,* and *Botryodiplodia sp.* also have been isolated (Llerena, personal communication). In Honduras, Fernández (*35*) reported 15 cases, 14 of which were due to *N. brasiliensis* and one to *N. asteroides*. Lesions were not observed in the feet, while localization in the back was common. Only one case has been registered in Costa Rica and one in Panama (*10, 16*).

N. brasiliensis was isolated from the few cases found in Cuba (*76*). *A. boydii* was the etiologic agent in one case from Puerto Rico (*62*). In the rest of the Caribbean Islands,

mycetomas caused by *Monosporium sp.* in Guadeloupe (*34*), *M. grisea* in Grenada (*25*), *A. boydii* in St. Croix (*24*), and *M. mycetomi* in Curaçao (*32*) have been reported.

In Ecuador, Rodríguez (*86, 88*) diagnosed three cases caused by *Nocardia sp.* and one by *A. boydii.* In Colombia, Cárdenas *et al.* (*22*) reported that out of 36,000 patients only five had mycetomas, two of which were traced to *N. asteroides* and *N. brasiliensis.* Of a series of 68 cases in Venezuela summarized by Mariat (*65*), 35 per cent were due to *N. brasiliensis* and 28 per cent to *M. grisea. Pyrenochaeta romeroi, C. recifei,* and *A. boydii* have been isolated from mycetomas in Venezuela (*4, 14, 62*).

Almeida and Simões-Barbosa (*5*) isolated *M. mycetomi* from 13 Brazilian mycetomas. Lobo (*56*) diagnosed nine cases in Pernambuco, isolating *N. pelletieri, N. madurae, N. brasiliensis, A. boydii,* and *C. recifei.* According to Lacaz (*48*), *N. brasiliensis* is the most common etiologic agent in Brazil. He reported 20 cases, isolating *A. boydii, C. falciforme,* and *M. grisea.* Silva and Nazaré (*98*) described five mycetomas caused by fungi and 14 by actinomycetes. Mackinnon (*62*) refers to one case of mycetoma from Peru in which *M. mycetomi* was the responsible agent, and Miranda and Troncoso (*70*) reported one caused by an actinomycete. Six cases from Paraguay were reviewed by Canese *et al.* (*20*), with *A. boydii* cultured from two of them and *M. mycetomi* from one. Mycetomas have been observed in Chile—eight caused by actinomycetes and one by *M. grisea* (*80*).

Two cases with *N. brasiliensis* from Uruguay were reported by Mariat (*65*). In Argentina, one case caused by *N. madurae* was described by Girardi and Khoury (*44*). Madeo and Cordero (*64*) isolated *N. brasiliensis* from another patient. Zapater (*111*) demonstrated 13 mycetomas caused by *M. mycetomi,* four by *M. grisea,* one by *Madurella sp.,* and five by *A. boydii.* Niño and Freire (*73*) reported five by *M. grisea,* one by *M. mycetomi,* one by *A. boydii,* and another by *Monosporium sp.*

Tables 1 and 2 summarize the etiology of actinomycotic and eumycotic mycetomas found in Latin America.

Chromoblastomycosis

Approximately 50 cases, all caused by *Fonsecaea pedrosoi,* have been reported in Mexico (*29, 75*). *Cladosporium carrionii* was recently isolated in four patients from a village in Oaxaca (*7*). There is evidence that the disease is most prevalent in the states of Veracruz and Tabasco, but sporadic cases have also been reported from Sinaloa, Jalisco, and San Luis Potosí (*75*).

Table 1

Etiologic agents isolated from actinomycotic mycetomas in Latin America

N. brasiliensis	*N. madurae*	*N. pelletieri*	*N. asteroides*	*S. somaliensis*
Mexico	Mexico	Mexico		Mexico
Guatemala				
El Salvador				
Honduras			Honduras	
Costa Rica				
Cuba				
Colombia			Colombia	
Venezuela				
Brazil	Brazil	Brazil		
Uruguay				
Argentina	Argentina			

Table 2

Etiologic agents isolated from eumycotic mycetomas in Latin America

M. grisea	*M. mycetomi*	*A. boydii*	*Cephalosporium sp.*	*A. oryzae*	*P. romeroi*	*Botryodiplodia sp.*
		Mexico	Mexico			
Guatemala				Guatemala		
El Salvador	El Salvador	El Salvador	El Salvador			El Salvador
		Puerto Rico				
		Guadeloupe				
		St. Croix				
Grenada						
	Curaçao					
		Ecuador				
Venezuela		Venezuela	Venezuela		Venezuela	
Brazil	Brazil	Brazil	Brazil			
	Peru					
	Paraguay	Paraguay				
Chile						
Argentina	Argentina	Argentina				

Numerous cases are found throughout Central America, except in Nicaragua and El Salvador, where only one and two cases, respectively, have been diagnosed (Montero-Gei, personal communication) (*21, 81*). In Guatemala, García (*38*) reported 15 cases, and Mayorga (unpublished) has diagnosed 15 more. In Honduras, Cueva (*28*) reported six patients, and according to Corrales-Padilla (personal communication), 20 cases were diagnosed in Tegucigalpa. Of all the countries, Costa Rica appears to have the highest prevalence of chromoblastomycosis, as judged by 53 cases reported by Romero and Trejos (*92*) among a population of 1.5 million people. Studies carried out in that country showed that 97 per cent of the patients were peasants between 20 and 60 years old. Lower extremities were involved in 80 per cent of them. The etiologic agent in all the Central American cases was *F. pedrosoi*. A report by Solano (*103*) that *P. verrucosa* and *F. compacta* were present in cases from Costa Rica requires confirmation. In Panama, 10 cases have been described. The first report was by Snow *et al.* (*102*), followed by others by Calero (*15, 17*), López (*61*), and Ospino (*74*).

In Cuba, 42 cases due to *F. pedrosoi* were reported by Léon (*53*) and Argüelles (*8*), and 24 additional patients were seen by Fernández and Reaud (*36*). Recent observation by Goiscou and Kourie (*39*) place the Dominican Republic among the countries with a high prevalence of chromoblastomycosis, 36 cases having been described in a two-year period.

In Puerto Rico, Carrión (*23*) found six cases caused by *F. pedrosoi* and one by the new species *F. compacta* (*Hormodendrum compactum*). Audebaud *et al.* (*9*) reported one case in Guadeloupe in which *F. pedrosoi* was the etiologic agent. Chromoblastomycosis was reported from Ecuador by Rodríguez (*87, 90*). In Colombia, 28 cases were described by Restrepo (*84*), three fourths of them caused by *F. pedrosoi* and the rest by *F. compacta* and *P. verrucosa*. Peña (77) found 17 additional cases.

Venezuela presents striking differences from other Latin American countries in regard to chromoblastomycosis. Lesions appeared localized mainly in the upper half of the body, and the most frequent agent was *C. carrionii*, as reviewed among 34 cases by Campins and Scharyj (*19*). Other authors referred to additional cases (*26, 46*) with the same characteristics. The states of Lara and Falcón (dry and arid) account for 65 per cent of the *C. carrionii* cases, whereas 35 per cent caused by *F. pedrosoi* come from

Table 3.

Etiologic agents isolated from chromoblastomycosis in Latin America

F. pedrosoi	*F. compacta*	*P. verrucosa*	*C. carrionii*
Mexico			Mexico
Guatemala			
El Salvador			
Honduras			
Nicaragua			
Costa Rica	Costa Rica (?)	Costa Rica (?)	
Panama	Panama		
Cuba			
Puerto Rico	Puerto Rico		
Guadeloupe			
French Guiana			
Venezuela	Venezuela	Venezuela	Venezuela
Brazil	Brazil	Brazil	
Colombia	Colombia	Colombia	
Peru			
Paraguay		Paraguay	
Argentina			

humid, forested places. Two cases in French Guiana were traced to *F. pedrosoi* (*100*).

The status of chromoblastomycosis in Brazil was reviewed by Lacaz (*48*) in 168 cases recognized up to 1955. Most were due to *F. pedrosoi*. Lesions in the lower extremities were most frequent among the Brazilian patients (*60*). In Peru, Miranda and Troncoso (*70*) isolated *F. pedrosoi* from three patients. In Paraguay, Canese *et al.* (*20*) found 29 cases. In Argentina, only six cases have been reported (Negroni, personal communication), and all of them were caused by *F. pedrosoi*.

Table 3 shows the distribution of the etiologic agents isolated from chromoblastomycosis in Latin America.

Rhinosporidiosis

The causal agent of rhinosporidiosis, *Rhinosporidium seeberi*, has not been cultured from lesions or from nature, but its role is generally recognized. The disease has been described in Mexico and South America, but not in Central America. Of 153 reported cases, 108 were human and 45 were animal. Paraguay had one third of all the cases and Argentina had one fourth. It should be pointed out that most of the Paraguayan and Argentine cases came from the Chaco region. The distribution of human and animal rhinosporidiosis in Latin America is shown in Table 4.

Subcutaneous zygomycosis

A few cases of this rare mycosis have been observed in Latin America. Andrade *et al.* (*6*) diagnosed a case in a Brazilian girl presenting paranasal nodules. Another case was found in

Table 4

Rhinosporidiosis in Latin America

Country	Human	Animal	Total	Reference
Argentina	6	35	41	(*72*)
Brazil	13	5	18	(*31*)
Chile	7	—	7	(*30*)
Colombia	4	—	4	(*77*)
Cuba	1	—	1	(*31*)
Ecuador	2	—	2	(*87*)
Mexico	4	—	4	(*68,109,112*)
Paraguay	56	—	56	(*20*)
Uruguay	2	5	7	(*31*)
Venezuela	13	—	13	(*105*)
Totals	108	45	153	

Jamaica by Bras *et al.* (*13*), and Resptrepo *et al.* (*85*) reported one case from Colombia. All patients described were Negroes. *Entomophtora coronata,* a parasitic fungus of insects, was isolated from all of them. Vignale *et al.* (*110*) recorded one case of destructive mucocutaneous zygomycosis in a white woman from Brazil. The etiologic agent was *Mucor ramosissimus.*

Johnston *et al.* (*47*) described a case in a mule, with lesions in the nasal mucosa, also due to *E. coronata.*

Discussion

As evidenced by the number of reported cases, the subcutaneous mycoses do not rank as major public health problems in Latin America. Nevertheless, if one considers the damage that any of these mycoses inflict on the individual in terms of discomfort, disability, days of hospitalization, and consequences of sequelae, their significance is very great.

The survey of the literature has revealed an abundance of cases of sporotrichosis, mycetoma, and chromoblastomycosis throughout Latin America. Considering the difficulty of diagnosis, the lack of comprehensive surveys, and the fact that these are not reportable diseases, an even greater number of cases can reasonably be expected. Their true prevalence, as yet unknown, is a matter of great interest to those engaged in the practice of public health.

This paper dealt with the distribution of subcutaneous mycoses in Latin America. Several problems were encountered; these related mainly to lack of uniformity in description and terminology and to the fact that in some instances the same cases were reported by more than one author.

Sporotrichosis has been reported from all countries except Nicaragua, Bolivia, and Chile. In adults lesions were more frequent in the extremities, and in children they were mainly on the face. It is of epidemiologic interest that *Sporothrix schenckii* was isolated from soil and from cactus thorns in Brazil (*58, 91*), and from natural substrates in Uruguay (*63*).

Mycetomas have been described in all the countries except Nicaragua and Bolivia. In Costa Rica and in Panama, only one case was reported. The etiologic agents, described in order of frequency, were *N. brasiliensis* and *N. madurae* for the actinomycotic mycetoma, and *M. grisea, M. mycetomi,* and *A. boydii* for the eumycotic mycetoma. *N. asteroides, N. pelletieri, S. somaliensis, P. romeroi,* and *Cephalosporium sp.* were rare. *N. brasiliensis* was isolated from soil in Mexico (*42*), and *N. asteroides* was reported in Brazil (*94*). *A. boydii* was found in soil in different parts of Brazil (*91*), and *M. grisea* was isolated in Venezuela (*12*).

With regard to chromoblastomycosis, cases have been reported from all the countries except Bolivia, Chile, and Uruguay. It appears to be rare in Nicaragua and El Salvador, with only one case described from each of these countries. Six cases have been registered in Argentina. *F. pedrosoi* was almost exclusively the etiologic agent in all the countries but Venezuela, where *C. carrionii* was the most frequent agent and *F. pedrosoi* was second in importance. It should be noted that this latter fungus has been isolated from soil in Venezuela (*94*).

Several reports on rhinosporidiosis and subcutaneous zygomycosis were available from various countries of Latin America.

Interest should be stimulated in official public health departments, universities, hospitals, and other centers to gather information on subcutaneous and other mycoses in a consistent manner, following standardized procedures so as to provide uniform and comparable data. Short-term mycology courses should be promoted by international organizations in order to provide local personnel with the knowledge necessary for the isolation and preliminary identification of the etiologic agents. Recognized mycology laboratories, strategically distributed throughout Latin America, should serve as reference centers for the proper identification of species that are difficult to characterize and for the investigation of other problems. Such centers

should have support—moral and perhaps economic—from international organizations. These and other measures could lead eventually to a more accurate estimate of the prevalence of subcutaneous mycoses and to a better knowledge of agent, host, and environmental factors and their interaction. With more complete information of this kind in hand, additional steps could be initiated for the prevention and control of these mycoses.

Summary

There is a greater abundance of mycoses in Latin America than in any other part of the world. Sporotrichosis, mycetomas, and chromoblastomycosis are, in that order, the most prevalent subcutaneous mycoses, and they are found in practically all the countries of the area. Oddly, sporotrichosis does not seem to be present in Chile, and mycetomas do not appear to be found in Costa Rica and Panama. There are endemic foci of rhinosporidiosis in Argentina, Paraguay, and Brazil, and occasional cases of zygomycosis have been reported in South America. As more complete mycological knowledge in the region becomes available, this panorama could change.

Sporotrichosis does not constitute a serious health hazard because of its usually nonsevere clinical course and its easy diagnosis and treatment. On the other hand, mycetomas and chromoblastomycosis are difficult to treat, long in duration, and often crippling or incapacitating.

Rhinosporidiosis and zygomycosis are rare in humans and are not considered public health problems, although rhinosporidiosis is of relative importance in endemic areas.

Existing data point to the need for specific studies to answer the following questions: What are the agent, host, and environmental factors accounting for the different distribution of subcutaneous mycoses throughout Latin America? Why do some countries seem to be apparently free of certain mycotic diseases? Why do the mycoses present varying degrees of severity in different regions?

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PREVALENCE OF SYSTEMIC MYCOSES IN LATIN AMERICA

Dante Borelli

General background

It would appear appropriate to characterize as "systemic" [1] only those mycotic diseases that ordinarily present the tendency to affect the body as a whole. These fall in the category of deep mycoses. The most important are systemic candidiasis, histoplasmosis, blastomycosis, paracoccidioidomycosis, coccidioidomycosis, cryptococcosis, and nocardiasis. The present summary will focus on histoplasmosis, coccidioidomycosis, cryptococcosis, and paracoccidioidomycosis.

A considerable amount of material on the prevalence of mycotic endemics in Latin America is available in the literature. In particular, there is the global review by Ajello, which devotes special attention to the situation in Latin America (*2*); a review by Lacaz on South America (*9*); one by Londero, Fischman, and Ramos on Brazil (*5*); one by González Ochoa on Mexico and Central America (*4*); one by Campins on Venezuela (*3*); one by Pardo and Trespalacios (*7*) on Cuba; one by Mackinnon on Uruguay (*6*); and one by Rodríguez on Ecuador (*8*).

These publications are the result of careful scientific work, but they only touch on superficial details in an immense, largely unexplored field. Moreover, they are not concurrent; they cover different time periods over a total span of more than 20 years.

A point of paramount significance is that the published findings from medical mycology carried on in Latin America seldom reach secondary dissemination outside the region. Ainsworth (*1*) found that in 1964 only 4.7 per cent of the titles contained in the *Review of Medical and Veterinary Mycology* had originated in South America. *Dermatología Iberolatinoamericana,* the journal of the Colegio Iberolatinoamericano de Dermatología, which appears also in English with selected articles, represents perhaps the most outstanding effort to establish a secondary review of the Latin American papers on subjects in the field of medical mycology.

Histoplasmosis

The following summary is based on 94 scientific reports that have been collected on the prevalence of histoplasmosis in various parts of Latin America.

More or less numerous series of cases of progressive disease have been reported from Mexico (*37, 38, 39, 40, 41*), Honduras, Costa Rica (*42, 90*), Panama (*27, 79*), Colombia (*73, 74*), Venezuela (*9, 11, 12, 18, 26, 44, 54, 65, 66, 67, 71*), French Guiana (*30, 31*), Ecuador (*45, 75, 76*), Peru (*10, 34*), Chile (*62*), Argentina (*21, 53, 60, 61, 64*), Brazil (*1, 7, 8, 66*), and Cuba. Two facts are to be noted: the great frequency of infantile cases and the apparent rarity of the disease in Brazil.

Accidental infections of two mycologists were

[1] "Systemic" is defined in *Dorland's American Illustrated Medical Dictionary* as "pertaining to or affecting the body as a whole."

reported in Venezuela; permanent cure resulted in one case, but death followed in the other (*44*).

Mass histoplasmin surveys, some of them covering dozens of localities and several thousands of persons, have been conducted in almost all the Latin American countries: Mexico (*41*), Honduras (*46, 47, 77*), Costa Rica (*42*), Panama (*85*), Colombia (*35, 36, 63, 69, 72, 92*), Venezuela (*17, 56, 58, 66, 68*), French Guiana (*30*), Surinam (*22, 93*), Peru (*13, 15*), Uruguay (*25, 70*), Brazil (*81, 82, 83*), and Cuba (*32, 33*). There was also a global survey (*28*) that included data on Latin America. The prevalence of reactions has been shown to vary from one place to another and also according to the age structure of the population tested, but on the whole it has become quite clear that practically all the human population in Latin America is living either within or near areas in which histoplasmic infection can be contracted. Of course, there are areas that have not yet been explored, and repeat surveys will be needed in order to detect and interpret future changes and/or incongruities in the prevalence of reactors. There is little doubt that permanent facilities are needed throughout Latin America for the mycological, histological, and serological diagnosis of histoplasmosis, since cases of progressive disease or epidemic outbreaks are capable of occurring anywhere in the region.

Histoplasmosis has been diagnosed in lower animals as well: in bats in Colombia (*52, 88*), Panama (*48*), El Salvador (*49*), and Trinidad (*29*); in several small mammals in Panama (*78, 86*) and Surinam (*23*); in cattle in Panama (*91*); in a dog in Venezuela (*55*); in Proechimys in French Guiana (*32*); and in rats in Brazil (*80*).

Histoplasma capsulatum has been isolated from soil on five occasions in Venezuela (*3, 14, 19, 20, 57*); twice each in Panama (*43, 87*), Peru (*5, 50*), and Trinidad (*4, 6*); and once each in Puerto Rico (*89*), Uruguay (*24*), Brazil (*79*), and French Guiana (*31*).

Coccidioidomycosis

Some 50 papers at hand bear data on the prevalence of coccidioidomycosis in Latin America. Among others, there are the excellent reports presented at the Phoenix Symposium in 1965 by Drs. González Ochoa, Mayorga, Campins, and Negroni, each of which covers an important endemic area.

Of Latin America's total surface of 20 million square kilometers, 7.5 per cent, or 1.5 million km^2, corresponds to the *reserváreas* of *Coccidioides immitis*. This area, according to estimates, is principally distributed as follows: over 1 million km^2 in Mexico; 200,000 km^2 in Argentina; and between 30,000 and 40,000 km^2 in Venezuela.

Almost all the territory in Mexico has been covered by coccidoidin surveys. The prevalence of reactions was as high as 75 per cent in one place. As of 1955, 64 cases were known to have occurred. Of these, 20 were published in the literature (*1, 9, 17, 20, 21, 22, 38, 46*), 36 were known through personal communications to authors, and 8 were studied by the authors but not published.

In Central America there was knowledge of four cases in Guatemala, two in Honduras, and three in El Salvador, and the existence of small *reserváreas* was demonstrated (*3, 12, 13, 27, 30, 42*).

From Venezuela, Campins (*5*) has reported on 35 known cases as of 1965. The proportion of males to females was 2:1. Several coccidioidin surveys (*4, 5, 10, 28, 31, 46*) helped in recognizing endemic areas. One place attained a peak reaction level of 46.4 per cent. The *reservárea* has not yet been delimited; additional areas are still to be explored, and new, broader surveys need to be performed in the "old" areas (*2, 6, 7, 11, 14, 15, 19, 36, 49, 50*).

In Colombia, two cases of coccidoidomycosis occurred in a dry region near the Atlantic coast (*39, 41*). In one locality within that region, 18 per cent of the adult subjects reacted to intradermal injection of coccidioidin (*40*).

Reports from Ecuador (*25, 26*) suggest that the disease is endemic in certain arid areas of the

Andean cordillera. It would appear desirable to collect new and more specific evidence from the same areas and to search for more appropriate sites at lower levels of elevation.

No data were found on the incidence of coccidioidomycosis in Peru, although small endemic areas may exist between the Andes and the Pacific Ocean where the climate is favorable.

The reports from Chile and Brazil are negative.

Cases of coccidioidomycosis have been recorded in Paraguay (*29*), Bolivia (*28*), and Argentina—in the last instance originating probably in the Dry Chaco region and continuing southward (from 27° to 40° S) into the pampean region. As of 1966, 27 cases had been diagnosed in Argentina (*8, 29, 32, 33, 34, 35, 37, 48*). Here again the male-female ratio among the patients was approximately 2:1. Among the children tested, the reaction levels ranged from 10 to 20 per cent. The adult population has not been studied to any extent.

In addition to the many surveys—some of them quite broad—carried out in Mexico, Venezuela, and Argentina, several others have been performed in Brazil (*43*), French Guiana (*16*), Paraguay (*18*), Honduras (*42*), Ecuador (*25, 26*), and Guatemala (*30*).

An accidental infection during a survey in the pampean endemic area was observed in Dr. Briz de Negroni (*35*). Dr. Alfonso Trejos (personal communication) acquired the infection in a laboratory in the United States. A Venezuelan physician contracted coccidioidomycosis while visiting Southern California as a tourist and then traveled to Paris, where his case was diagnosed at the Pasteur Institute (*47*).

Infections among Mexican laborers migrating to the United States are a well-known occurrence (*24*).

Cases of infection in dogs and cattle have been reported from Guatemala. A dog from Nicaragua was found to have been infected with *Coccidioides immitis* in the examination following its death just after arrival in Norway.

Cryptococcosis

Cryptococcosis is an infectious disease of ecumenical distribution. Its prevalence in Latin America seems to be as low or lower than it is in the other inhabited regions of the world. The extrahuman life of the agent has been associated with the dung of pigeons.

In the literature at hand there are only 28 published studies on the prevalence of cryptococcosis in the region. Brazil is the country best represented, with 11 papers, (*1, 2, 4, 5, 10, 11, 15, 17, 20, 24, 26*); Venezuela follows with five (*3, 16, 18, 19, 22*); and then comes Argentina (*14*), Mexico (*28*), Colombia (*12*), Cuba (*9*), and Paraguay (*6*), with one each. A report from Venezuela cites 24 cases, but usually in other places the recorded figure is much lower—for example, six cases in Minas Gerais.

While it is claimed that the endemic is increasing in the city of São Paulo, only 14 cases have been reported over a period of five years, thus giving an annual incidence of 1 case per 1.5 million inhabitants.

Evidence indicates that cryptococcosis infection is likely to be much more frequent than cryptococcosis disease. However, practical methods for demonstrating its true extent are lacking.

It would be reasonable to say that cryptococcosis is sporadically present in Latin America. Owing to its preference for the adult human male, its incidence is likely to increase as life expectancy is prolonged.

There are a few examples on record that demonstrate the presence of cryptococcosis in lower animals in Latin America. Marmosets of the species *Leontocebus geoffroyi* have been found infected with *Cryptococcus neoformans* in Panama (*27*), as have been goats (*Capra hircus*) in Brazil (*8*) and Aruba (*25*). The goats studied in Aruba had been bred in Venezuela (Paraguaná peninsula), Colombia (Goajira peninsula), and on the island of Aruba itself. Approximately 1 per cent of the slaughtered animals were found to present small lung torulomata. It would be of interest to study the life of Cryptococcus in the arid habitat of goats.

The presence of *Cryptococcus neoformans* in soil has been demonstrated by isolations from Brazil (*23*), Colombia (*13*), and Venezuela (*21*).

Paracoccidioidomycosis

This disease is without doubt the most widespread of the systemic mycoses in Latin America.

It would be foolhardy to venture any statistical estimate of its prevalence. Indeed, the records published to date are only partial contributions as against the large numbers of cases known to have been diagnosed, studied, or treated in different places. While on the one hand it is evident sometimes that several papers are reporting on the same group of patients, on the other hand it is well known that there are groups of thoroughly studied cases which have never been published.

Although the prevalence of this endemic is basically unknown, the high proportion of male patients in relation to females—ranging from 7:1 to 70:1—is strikingly evident.

Paracoccidioidomycosis is most prevalent among adult human males. It can be expected that its frequency will keep pace with the increase in the over-all susceptible human population and the extension of life expectancy.

It is widely believed that living and working in the field is a factor in the etiology of paracoccidioidomycosis. Accordingly, it is thought that the increasing flow of migration to the cities should help to reduce its prevalence in another 20 or 30 years. This hypothesis, however, is not on entirely solid ground. For one thing, it is rather difficult to find people in Latin America who have not had some direct contact with soil and vegetation. Also, there are well documented cases of European immigrants who came and established themselves in the very center of a Latin American capital (within the endemic area) and initiated a clinical course of paracoccidioidomycosis while working and living in a carpentry or shoe-repair shop. Perhaps a comprehensive study in the city of São Paulo would give a better idea of the role of urban life in the future prevalence of the disease.

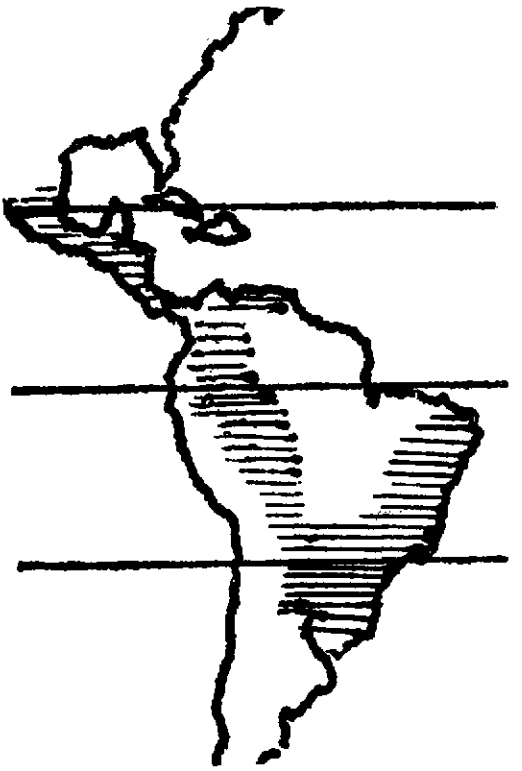

Fig. 1. The endemic areas of paracoccidioidomycosis, according to an hypothesis of the author.

Despite earlier warnings against any attempt to estimate the prevalence of paracoccidioidomycosis, it is still tempting to hazard a guess. On this basis, it might be said, after much speculation, that 5,000 cases have been diagnosed as of 1970. Of these, perhaps 2,500 may be from Brazil, 600 from Venezuela, 600 from Colombia, 100 from Argentina, 100 from Peru, 50 from Ecuador, 50 from Uruguay, 30 from Paraguay, 20 from the Central American countries, and 20 from Mexico. It might be estimated that within the endemic area of paracoccidioidomycosis, as defined by the author, there are currently 45 million Brazilians, 5 million Uruguayans, 10 million Argentines, 1 million Paraguayans, 1 million Bolivians, 3 million Peruvians, 1.5 million Ecuadoreans, 10 million

Colombians, 6 million Venezuelans, 4 million Central Americans, and 6 million Mexicans—for an over-all total of almost 90 million Latin Americans. Further, it might be guessed that the annual incidence in the areas of greatest endemicity is as high as 5 cases per million, whereas in the areas of lower endemicity it is around 1 per million—for a mean of 2.5 cases per million each year. This would translate to a total of 225 new cases during 1970 for the whole of Latin America.

Comment

The foregoing summary is based on a personal collection of papers on the prevalence of some of the systemic mycoses in Latin America. They correspond to studies carried out over periods of from 30 to 70 years.

What informative value, if any, do these publications have? Do they really represent the present status of knowledge, or are they only a fraction of the information published on the subject? Finally, do they truly reflect the prevalence of these mycoses in Latin America? These questions are difficult to answer.

An example of the gap between what is known and what has actually been published is offered by González Ochoa in regard to the prevalence of coccidioidomycosis in Mexico. He reported in 1965 that in Mexico City during the 10-year period from 1952 to 1961 a total of 14 cases had been published. In the next four years, however, an additional 44 unpublished cases were known of in two hospitals alone.

This agrees with the author's personal experience. Of four cases of histoplasmosis, only one has been published; of four cases of coccidioidomycosis, only two; of 70 cases of paracoccidioidomycosis, none has been published; a sole case of cryptococcosis remains unreported in the literature. Similarly, the author has published only one of 140 cases of sporotrichosis and only two of 80 cases of chromomycosis.

The comparison of this situation with an iceberg, as proposed by Dr. Ajello, is quite reasonable—particularly if it is kept in mind that some icebergs are almost entirely submerged.

What is of most interest is the *future* prevalence. The dynamics of the problem is the main concern. Thus, attention should be given to the foreseeable changes in the susceptible population—vegetative growth and shifts in age structure—and the trends that affect it: migration; urban development; penetration and colonization of the forest; and irrigation, exploitation, and population of arid areas.

Speculation about the future prevalence of paracoccidioidomycosis, for example, reveals several conditions pointing to the possibility of an explosive process: (1) a numerical increase in the human population, particularly within the endemic area, (2) a relative aging of this same population owing to prolonged life expectancy, (3) the occupation of extensive areas in the warm-temperate floor following the construction of roads and the consequent colonization of the cis-Andean region, and (4) a migratory trend from cold, eroded areas to less cold and more fertile ones that are free of malaria.

It may be asked whether the prevalence of mycoses is truly a public health problem. The fragmentary evidence at hand would seem to deny that the mycoses, either individually or taken together, currently constitute a serious health problem. This, to some extent, is because the superficial mycoses, very frequent and partly communicable, are usually mild, whereas the deep mycoses produce only rare cases of potentially lethal disease. The only aspects of considerable public health importance are the epidemics of coccidioidomycosis and histoplasmosis that break out when large groups of susceptible people enter the respective *reserváreas*.

Still, the mycoses do constitute a serious medical problem in Latin America. Those who practice mycology as a specialty of medicine know that, from the medical point of view, the possible mycoses that are overlooked are just as important as the infections that are diagnosed. The usefulness of a mycologist in a community

is to be measured not only by the number of mycological cases he may discover, but also by the specialized diagnostic and therapeutic function he is carrying out on a continuing basis. For example, mycology is definitely useful in the control of diseases of recognized public health importance such as tuberculosis, leprosy, and cancer. In other words, the importance of the control of mycoses is not based only on their prevalence; the mere proved or probable existence of certain mycoses in a region is already a clue to the presence of diagnostic problems. Continuously increasing migrations contribute to the seriousness of this matter. The need for medical mycologists in Latin America is acute, and the absence of such workers is, of itself, creating a public health problem.

Although one of the ways to begin learning the real prevalence of mycoses is to introduce and enforce the compulsory reporting of new cases, thought should be given to the possibility of exercising a technical control on diagnostic procedures, since not even in the United States is there the necessary number of medical mycologists, properly distributed throughout the country.

In Latin America, the problem is twofold: first, there is a lack of trained mycologists, and, second, there is no real possibility of enforcing compulsory notification. The result would be that most cases would go unreported, while some cases would be reported on the basis of a wrong diagnosis. In any event, it would be impossible to test the value of the reports. It is suggested, therefore, that the Pan American Health Organization counsel its Member States to establish compulsory reporting for mycotic diseases only when they are prepared to take such a step.

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OPPORTUNISTIC MYCOSES

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Opportunistic mycoses are those infections produced by saprophytic fungi. Human beings are constantly exposed to these microorganisms, and under normal conditions they are not pathogenic for man.

Among all the fungi, macro- and micromycetes, fortunately only a few species are capable of producing disease in man. The pathogenicity and virulence of these primarily pathogenic fungi is apparently explained by the ability of some of them to survive and proliferate in situations that are not suitable for the growth of most other fungi. In general, this pathogenic capacity is manifested by the dimorphic expression of the microorganism. For instance, it is well known that in deep mycoses most of the fungi adopt the yeast form. It represents their adaptation to a set of special conditions, such as temperatures (37.5° C) above the normal range preferred for their growth, low redox potentials, and carbon dioxide tensions greater than those in the external environment (*32*). Most of the microscopic saprophytic fungi whose spores continuously contaminate the air are unable to adapt to such conditions. They survive only in an environment having the temperature, oxygen, and CO_2 tensions that are optimal for the growth of most of the fungi (*9*). However, Rippon *et al.* (*32*) have recently shown that some species of Aspergillus and Penicillium, typical saprophytic fungi, are dimorphic and pathogenic for laboratory animals when they are cultured at 37° C with a low redox potential, using increasing concentrations of cysteine on gradient plates. Experiments of this kind may explain at least partially the ability of these low virulent opportunistic fungi to become pathogenic.

There are, in addition, some fundamental factors involving the host or the physician, that can facilitate these fungal infections or make them more serious. They may be categorized basically as follows:

1. Chronic diseases and other debilitating situations that afford suitable environmental conditions for the metabolism of the fungi, thus facilitating the infection—diabetes mellitus, malignant tumors, tuberculosis, amebic abscess of the liver, surgical procedures, transplantations, etc. (*1, 5, 36, 41, 44*).

2. Pathological conditions that decrease or inhibit the inflammatory reaction and the immunological response—leukemias, lymphomas, agranulocytosis (*4, 6, 7, 11, 13, 18, 24, 36*).

3. Prolonged medical treatments with substances that produce effects similar to those described in paragraph 2 above—corticosteroids, antimetabolites, etc. (*11, 12, 27, 36, 37, 40*).

4. Prolonged therapy with certain antibiotics, either through their direct irritating action on the tissues or through suppression of the normal bacterial flora (*14, 33, 34, 35, 36, 37, 40, 43*).

Numerous etiologic agents have been involved in the opportunistic mycoses. Occasional isolations of innocuous saprophytic fungi such as Alternaria (*29*), Fusarium (*31*), Cryptostroma (*15, 16*), and Curvularia (*19, 28*) from pathologic processes have been reported in the litera-

ture. However, the microorganisms that have been most frequently isolated are those of the genera Candida and Aspergillus and also some of the Zygomycetes, among which Absidia, Rhizopus, and Mucor are the most commonly found.

It is very important to note that when some of the predisposing factors for the opportunistic mycoses are present along with primary pathogenic fungal infections, the infections are more intense, and apparently the virulence of these pathogenic fungi is highly increased. This situation has been observed in cases of histoplasmosis (*17*) and cryptococcosis associated with lymphomas (*10, 18, 45, 46*), coccidioidomycosis associated with several different conditions (*42*), blastomycosis associated with tuberculosis (*41*), and even resistant fungal infections of the skin, produced by *Trichophyton rubrum*, associated with leukemia or systemic lymphomas (*8*).

The first publications dealing with the possibility that antibiotic or corticosteroid therapy could play an important role in the pathogenesis of the opportunistic mycoses appeared approximately 15 to 20 years ago in the American literature (*3, 18, 40, 43, 44*). Since then, most of the situations that facilitate infections produced by such fungi have been analyzed thoroughly (*1, 2, 4, 6, 14, 33, 34, 35, 36, 38*). It has also been shown that opportunistic fungal infections are a major problem the world over. Although their frequency varies from one country to another, it would appear from the literature that their incidence is highest in the United States.

We have reviewed the problem in Mexico using postmortem and surgical material from the Department of Pathology of the General Hospital, National Medical Center (Mexican Social Security Institute). In a previous communication (*21*) we reported that infections of this kind occurred at a rate of 6.6 per cent in 1,000 autopsies. With more than 2,000 autopsies now studied, we have observed that the incidence remains at about the same level.

In our material, the opportunistic mycoses correspond mainly to candidiasis, aspergillosis, and zygomycosis, in that order. Some of the peculiarities of these infections as they occur in Mexico are discussed below.

Candidiasis

This is probably the most frequently observed opportunistic mycosis. In our material, which consists of nonselected autopsies from a general hospital, its incidence is 5.4 per cent. The infection is most common in the gastrointestinal tract, particularly the esophagus, the stomach, and the duodenum, and less frequently in the small bowel. We have also observed involvement of the lungs with certain frequency, and we have occasionally found the infection in the brain, bladder, pharynx, and heart valves, especially in patients who have undergone surgery. In rare instances we have seen a generalized candidiasis. Our criterion for calling a case "generalized" is localization of the microorganism in more than four different organs.

In none of our cases, even the generalized infections, have there been any data suggesting hematogenous dissemination of the fungi. The predisposing factors have been those classically mentioned in the literature: diabetes mellitus, infectious diseases (peritonitis, tuberculosis, etc.), hematologic diseases (leukemias, lymphomas, bone marrow aplasia), amebiasis, malignant tumors, and postoperative conditions, in that order of frequency. In addition to the classical predisposing factors for the implantation and dissemination of the fungi, the administration of broad-spectrum antibiotics and/or steroids for long periods of time was involved in most of our cases.

Candidiasis is seldom suspected at the time of dissection with our routine postmortem and surgical material. Thus it is most likely to become known later from microscopic findings in the histologic studies. For this reason, in more than 50 per cent of our cases we have been unable to make a precise mycologic identification of the fungus. However, in those cases in which the lesions are suspected and cultures are made (approximately 25 per cent), the

responsible species has always been *Candida albicans*. Even when we cannot tell the exact species under the microscope, we assume that we can identify the genus with certainty.

Aspergillosis

In our material, this mycosis is second to candidiasis in frequency. Its proportion—namely, 1.2 per cent—is considerably lower than that of candidiasis, however. In contrast to candidiasis, which has a practically ubiquitous distribution, all cases of aspergillosis had pulmonary localization, either with single or multiple lesions. In one case there was a generalized aspergillosis with extensive lymphohematogenous dissemination to the lungs, liver, spleen, pericardium, myocardium, endocardium, pancreas, thyroid gland, and lymph nodes.

In all our cases of aspergillosis there was a basic predisposing disease and concomitant administration of antibiotics and/or cortical steroids that favored installation of the fungus. For the most part, the primary diseases in our series are similar to those reported elsewhere in the literature—leukemia, lymphomas, collagen diseases, etc. (*25, 26, 30*). However, in a third of our cases the initial predisposing illness was an amebic abscess of the liver. The coexistence was first reported in 1962 (*20*). Even though this was some time ago, and amebiasis is a rather frequent disease in Latin America and Asia, we have not seen the association described anywhere outside of Mexico. The etiopathogenic factors determining superinfection by Aspergillus in the cases of amebiasis are probably the same as for association with other debilitating chronic infectious diseases, such as tuberculosis. These factors are mainly malnutrition and related hypoproteinemia, which probably lead to depression of the specific and nonspecific immunological mechanisms.

It is also difficult to guess the nature of the lesions on gross examination of the organs in postmortem studies. For this reason, most of our cases have been diagnosed on the basis of the microscopic morphology of the microorganism in the lesions. In our postmortem studies we have been able to culture the fungus from pulmonary lesions in only two cases. In one, the responsible agent was *Aspergillus fumigatus;* in the other, the organism was only identified at the level of genus as *Aspergillus sp.*

Zygomycosis

The finding of zygomycosis in our material is a rarity. We have seen only three cases in more than 38,000 histopathological studies of both postmortem and surgical material—a frequency of 0.007 per cent. Our cases correspond to what Straatsma *et al.* (*39*) have described as "syndrome of nasal, paranasal sinus, orbital, and central nervous system disease."

We have found zygomycosis in patients with long-term poorly controlled diabetes with acidosis. In two of the cases, the diagnosis was made in a live patient from material obtained by antrostomy from the maxillary sinus. The other case was an autopsy finding with all the characteristics of the disease as described in detail in the classic report by Gregory *et al.* (*23*). In the first two cases, the disease could be partially kept in check by cleaning the affected maxillary sinus, controlling the diabetes, and administering amphotericin B. Mycological cultures were made in these two cases from material obtained during surgery. *Mucor sp.* was isolated in one of them and *Rhizopus nigricans* in the other. In the latter, the infectious process initially located in the paranasal sinus had spread to the orbit, producing orbital cellulitis and later on opthalmoplegia, which made it necessary to enucleate the affected eyeball. Although the infection was initially controlled, it was never possible to seat a prosthesis because mold was continuously developing in the orbit, despite all precautions, including cleansing of the area several times a day with amphotericin B.

In addition to the three types of mycosis discussed above, we have observed the association of candidiasis and aspergillosis in several patients, and the presence of unidentified mold at the histologic level in another one.

Comment

The mycoses produced by opportunistic fungi are found more often now than they were in the past. Their frequency keeps pace with advancements in medical and hospital attention. Even when the predisposing conditions of the host play a major role in the pathogenesis of the opportunistic mycoses, there is no doubt that the most important factor of all is the physician practicing modern medicine today. Even when leukemia or diabetes, for instance, constitute important predisposing factors for the opportunistic mycoses, the former have always existed, whereas the opportunistic mycoses were considered unusual until approximately 20 years ago. The use of antibiotics, corticosteroids, antimetabolites, immunosuppressive drugs, organ transplantation, and other means of prolonging survival has caused the opportunistic infections, especially those of iatrogenic origin, to become a real problem in medical mycology.

In a review of the literature on medical mycology published in Mexico between 1946 and 1958 (*22*), no reports of this type of opportunistic mycosis were found. At present there are hundreds of cases of candidiasis and aspergillosis and also some of zygomycosis in which one or more of the recognized predisposing factors can be found in the clinical history. It is also interesting to point out that in some of the primary deep mycoses, such as cryptococcosis, these factors are causing the fungal infection to become more intense and the lesions more severe than they were in the past. In these cases, the clinical history also shows the use of antimetabolites, steroids, antibiotics, or other drugs that facilitate the dissemination of the infections and aggravate the clinicopathological picture in general.

In Mexico City we have had the opportunity to corroborate the important role played by the quality and economic aspects of medicine in the incidence of opportunistic mycoses. As mentioned before, the frequency of aspergillosis in the present material, coming from a well-supported institution, is 1.2 per cent. Compared to this, some years ago (*20*) studies were made of similar material from a charity hospital with limited economic resources, where prolonged treatments, the use of drugs such as antibiotics and antimetabolites, and other expensive forms of therapy were only rarely seen. The frequency of aspergillosis was only 0.2 per cent. In the richest countries, where hospitals can afford the most advanced and complete treatments, including the use of expensive drugs, the frequency of this type of infection is very high. This fact is clearly reflected in the medical literature if reports of opportunistic fungal infections in the United States of America are compared with those from other parts of the world.

It can be seen, too, that the high frequency of diseases such as amebiasis, leprosy, malaria, cysticercosis, and the mycoses in tropical countries is simultaneously associated with low levels of economic development, which is the case throughout most of Latin America. This situation has been characterized picturesquely as the "pathology of poverty." In the light of the facts reviewed here, it could well be expected that when the underdeveloped countries outgrow the "pathology of poverty" as a result of economic progress they will then fall victim to a new kind of pathology—the "pathology of wealth." This will indeed happen unless adequate and efficient measures to prevent and treat the infections produced by opportunistic fungi are developed.

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DISCUSSION

Chairman Ajello: The morning session is now open for discussion. We will entertain questions.

Dr. Muchmore: In line with the "pathology of wealth" discussed by Dr. González-Mendoza, I should like to propose the term "iatromycoses" to refer to those fungal infections that occur as a result of the treatment a patient receives for other diseases. This term would be consistent with the word "iatrogenic," which is already in wide use.

Also, I should like to make a point in connection with Dr. Borelli's allusion to the sex incidence of fungal infections in speaking of cryptococcosis. Sexual differences in the incidence of mycoses have often been said to be due to exposure and to the work habits of the people involved. I wonder, however, if he has considered that this difference might be due to factors other than exposure. We have some evidence that perhaps females are considerably more resistant to these infections than males. Indeed, *in vitro,* the estrogen that they circulate so delightfully *in vivo* is highly antimycotic.

Dr. Borelli: In experimental infections with animals susceptible to these mycotic agents, we have found much higher resistance among females than among males. Sometimes the infected females have two or three pregnancies, bearing their offspring and nurturing them, while the males die. This is a general pattern present not only in the mycoses but also in other chronic infections.

Dr. Drouhet: First I would like to comment on histoplasmosis. We have diagnosed several cases on the islands of Guadeloupe and Martinique. The first case described by Darling was in a Negro male from Martinique who contracted the disease either before or during a stay in the Canal Zone.

Next I would like to ask Dr. González-Mendoza if he has found Cephalosporium infections as opportunistic mycoses. We have seen a case of Cephalosporium meningitis after corticosteroid therapy, and only last week we diagnosed a Cephalosporium endocarditis after surgical heart intervention.

Dr. González-Mendoza: We have not found opportunistic pulmonary mycoses produced by Cephalosporium, but we did find several cases that were diagnosed as aspergillosis on postmortem histological examination. Cultures were not performed, but we presume these cases were aspergillosis because the morphology of the hyphae looked like Aspergillus. Some of them could be Cephalosporium, however, since just hyaline mycelium, without sporulation, has been seen. In some instances, we have observed pigmented mycelia, but these were of the Cladosporium type.

Dr. Borelli: I should like to call attention to the need to delimit the endemic area of *Cladosporium carrionii.* It should be noted that whenever this organism was isolated the patient had been living in arid regions within the tropics, which are the *reservâreas* that I have assigned to this species.

Fonsecaea pedrosoi is found in all inhabited areas except the arid tropics. With regard to *Nocardia brasiliensis,* I have noted—and I would like to know whether this is true in other countries as well—that the organism is isolated from persons who have lived in hilly or mountainous areas but never from persons who have spent their entire lives in warm, tropical flatlands.

Dr. González Ochoa: I would not say that this is true in Mexico. The first cases that were described in this country came from the states of Sinaloa and Sonora, where there are large stretches of flatlands, and even deserts. We have had experience with a large number of cases of mycetoma due to *Nocardia brasiliensis,* and we have seen patients from both mountainous and lowland areas.

Chairman Ajello: I was wondering if Dr.

Kaplan would care to comment on the etiology of rhinocerebral zygomycosis, in view of the studies carried out with Dr. Donald Reinhardt in response to the recovery of *Rhizopus nigricans* in Mexico.

Dr. Kaplán: Dr. Reinhardt, a postdoctoral fellow, and I carried out studies on potential etiological agents of rhinocerebral zygomycosis. We were interested in determining the relationship between the ability to grow at 37°C *in vitro* and the potential capacity to produce rhinocerebral zygomycosis.

We tested a number of different zygomycetes for their ability to grow at 37° and for their potential pathogenicity, using rabbits that had been rendered acutely diabetic by the use of alloxan. We found that none of the fungi in this class that could not grow at 37° possessed pathogenicity for the diabetic rabbit. *Rhizopus nigricans* cannot grow at 37°, and it was not pathogenic.

We also found that not all the various fungi that could grow at 37° were potential pathogens. However, it is of interest to note that we did demonstrate potential pathogenicity in a number of fungi that to date have not been incriminated as causes of rhinocerebral zygomycosis in man. In other words, we feel we can alert the medical mycologist to the distinct possibility that there are fungi in addition to *Rhizopus oryzae, R. arrhizus,* and the like that are potentially capable of producing rhinocerebral zygomycosis in an animal (including man) with uncontrolled diabetes.

Dr. Mackinnon: In Uruguay we have only seen two cases of infection with *Nocardia brasiliensis*. These cases had some pecularities. They produced a lymphatic form of the disease. They were localized in exposed parts of the limbs, especially the hands and forearms. Although they resembled cases of sporotrichosis, they were more acute. No granules were found; only filaments. However, granules were observed in the inoculated guinea pigs.

These cases were diagnosed early—15 to 20 days after the infection was contracted. Thus, we suspect that the mycetoma due to *Nocardia brasiliensis* has a previous stage that does not look like a mycetoma.

I should like to ask my colleagues from Mexico, Venezuela, and Brazil if they have observed cases like this—which, incidentally, respond very readily to treatment with sulfamides.

Dr. Borelli: We saw a case of nodular suppurative lymphangitis produced by *Nocardia brasiliensis* in 1956, and we know of two or three more cases reported in the literature since that date. The symptomatology and course of the disease were the same as described by Professor Mackinnon: acute suppuration, rapid evolution, and cure within three to four weeks. I do not believe that cases of mycetoma usually display such a syndrome. This form evolves rapidly, with cure, and when we inoculate patients with the same strain we can observe suppurative lesions that heal spontaneously and rapidly. I doubt that this is a comparable situation, but it may be indicative.

In regard to the lowland areas in Mexico mentioned by Professor González Ochoa, these places, even though they have very hot temperatures during certain seasons, are located in the Temperate Zone.

Dr. Mariat: The species of Nocardia that we found from mycetomas and soil in Africa included *Nocardia brasiliensis* and *Nocardia asteroides,* and it is possible to find both these species in the same place. They do not depend on the nature of the terrain. Where we studied these cases, the terrain is almost flat.

Dr. Seabury: We have had three cases of *Nocardia brasiliensis* infection in New Orleans. The first appeared as a submental nodular lesion involving the lymph glands and was thought initially to be tuberculosis. The other two were both superficial lesions. One on the hand was believed at first to be sporotrichosis and the other on the arm was thought to be due to a Mycobacterium. In both, the correct diagnosis was only recognized after biopsy and culture.

Dr. Furcolow: The title of this morning's

session is "The Mycoses as a Major Public Health Problem." I think our difficulty is that we are dealing with too many scientists who are trying to count a few cases. You must remember that the estimate of 30 or 40 million Americans infected with histoplasmosis came about because a skin test survey was performed and it was decided that there was a major endemic area in one river valley of the United States.

What is quite clear from studies that have been presented here of scattered areas of South America is that there are three major river valleys infected with histoplasmosis. Perhaps there are not as many people in those three river valleys as there are in that valley in the United States, but I suspect there are.

In these same three central river valleys there is also the problem of South American blastomycosis, which is probably widely prevalent. So we are not talking about just one case of one thing. We are talking about millions of cases, probably, in South America, and it should be quite possible to set a more exact figure by looking at the river valley boundaries and estimating the populations. We are talking about millions of people infected with these two diseases alone, which are just the beginning of the major public health problem that concerns us.

Dr. Negroni: I believe that we will have a more complete idea of the incidence of this endemia—and of the endemic area of South American blastomycosis—once we have an adequate skin test antigen. Then we will know the extent of positive reactors. We hope that in the near future Dr. Restrepo, who has developed such a useful antigen for immunodiffusion tests, will achieve similar success with the skin test.

I should like to call attention to the need for improving techniques for the isolation of *Paracoccidioides brasiliensis* from soil. This will also help to give us an idea of the ecology and geographic distribution of the disease.

Right now we can do at least the following: each year we can record all the new cases and their incidence as diagnosed mycologically in various regions. This will give us a more accurate idea than what we get from reviewing the records of cases published in the literature since 1908.

Dr. Restrepo: I would like to ask Dr. Borelli whether he considers the term *reservárea* equivalent to "endemic area," or whether he means something else, because I believe we are talking about two different things.

Dr. Borelli: Epidemiological doctrine has been based on the study of contagious diseases. Accordingly, the concept of endemic area was originally intended to refer to the area in which normally diagnosed cases are reported. The concept of endemic foci was developed later. Sometimes we receive reports of cases from areas where it would have been impossible to contract an infection. The deep mycoses are not contagious. They have a long period of latency, and during this time a patient may have moved to another area. Thus, there are areas where the infection can be contracted because they are the source of the etiologic agents. I propose that we call these areas *reserváreas*. The areas in which you diagnose the cases and report them, following the traditional concept, should be called "endemic" areas.

The *reservárea* may coincide with the endemic area, but for some diseases there is no correspondence between the two. For example, in New York you have 150,000 bilharziasis patients; it is an endemic area, not a *reservárea* of schistosomiasis.

Dr. Restrepo: Thank you. I just wanted a clarification for the group here.

Now, I would like to tell Dr. Negroni that we do have the antigen for skin testing. The only thing we don't have is the money to go ahead with the ecological work. We used the antigen recently, and we think it works. We found considerable variations among the different areas in which the skin tests were carried out; in those where we found normal, healthy persons reacting to the antigen, the ecological conditions that favor paracoccidioidomycosis seemed to prevail. Thus, I think we can learn a lot from it.

Dr. Furcolow: I would like to introduce a word of caution against jumping into any big skin testing program. We must put priorities on the problems of disease as we know them. For example, I would suspect that in South America the problem of disease due to histoplasmosis is ten or a hundred times as great as that due to South American blastomycosis. Since you know you have a good skin testing antigen, if your money is limited, I think you should give thought to this sort of priority.

Dr. Larsh: I would like to ask Dr. González-Mendoza if there have been any surveys in chest hospitals or tuberculosis sanatoria in Mexico on the incidence of aspergillosis.

In the last few years we have diagnosed a number of aspergillosis cases at the Missouri State Sanatorium. Histoplasmosis and other systemic mycotic infections are common in this institution. We have not lost interest in histoplasmosis; however, opportunistic fungi, especially aspergilli, are being isolated and evaluated. Many of the cases have been of the large aspergilloma, but many have also been overt infections of the lung and other tissue. Are you seeing this in Mexico?

Dr. González-Mendoza: We have no exact figures on the incidence of aspergillosis and other opportunistic mycoses in tuberculosis hospitals or pneumonology clinics—or at least I do not know of any. But I do know, basically because of conversations held with colleagues, that it is not any greater than the frequency in general hospitals, where a variety of treatments—antibiotics, steroids, x-ray, antimetabolites, and the like—predispose to this infection. In other words, we do not find that the association of aspergillosis with tuberculosis is any more frequent than association with treatments that are classically considered to be predisposing to the disease.

Dr. Hasenclever: The observation of opportunistic fungus infections might depend on the kind of hospital in question. Certainly in a hospital specializing in the treatment of leukemics and organ transplantation one will find that candidiasis and aspergillosis, while maybe not overwhelming, are very serious problems.

I think we are all aware of the fact that the cultural diagnosis of candidiasis is quite difficult, particularly where one will be isolating Candidas from sputa and most other types of specimens submitted to a diagnostic laboratory. No one questions the significance of the isolation of a Candida from the blood, but we do not isolate the fungus very frequently from this type of specimen.

Aspergillosis presents the same kind of problem. In fact, in this case the isolation of the fungus seems to be even more infrequent, although at autopsy patients can be shown histologically to have been invaded with aspergilli.

Our approach has been to use serology to determine whether these patients may be infected. The patients we see frequently have leukemia in a state of relapse, and they are therefore being treated with immunosuppressive drugs, antimetabolites, steroids, and a whole host of things that may reduce the immunological response. In some cases, we see that these patients will respond immunologically. Particularly so far as candidiasis and aspergillosis are concerned, we hope to see a rise in antibody titer. Of course, to do this, one must have the patients available for some time and try to get a baseline antibody level. Therefore, if the patient shows a febrile disease that is not responding to the usual bacterial antibiotic therapy, one can therefore consider the use of amphotericin B, particularly if there is a serologic response to Candida or to Aspergillus. We have had some success. There are still many problems to be resolved, but the results seem useful enough to warrant continuing the work.

Dr. Muchmore: *Candida spp.* occur regularly in and on the human body, and we may presume that the patients who develop the infections Dr. Hasenclever mentioned serve as their own source for these organisms. But what is the source for the Aspergillus infections? And what, if anything, can we do to prevent them? It is very difficult to eradicate Candida from the

human body, but what can be done about the source of the Aspergillus? When this organism makes its appearance, was it previously present in the patient's body, or is the patient infected from an outside source *after* his underlying disease, or contributing medications (steroids, immunosuppressants, etc.) were begun?

Dr. Hasenclever: As to the source of *Aspergillus fumigatus* and other important species of Aspergillus, I think these are exogenous infections. If one tries to control the environment—and I think control of the environment is about the only possible way to control acquisition of the infection—this gets to be an overwhelming problem. Laminar airflow schemes are being employed, and hopefully they will be of some use. I do not know whether this approach has really been studied long enough to determine whether it is effective or not, but I feel quite certain that these infections with *A. fumigatus* are exogenous, and, as ubiquitous as this microorganism is, the control of its source is going to be a very difficult if not impossible problem.

Dr. Ahearn: I should like to comment briefly on the source of some of the opportunistic yeasts that we are discussing. The Candidas, such as *C. parapsilosis* and *C. tropicalis,* and some species of Torulopsis are capable of utilizing a broad variety of substrates. Their original source may have been the patient, but they can contaminate detergents, antibiotic solutions, cortisone creams, and the like. Many species can multiply in these kinds of environment. They may then be reapplied to the patient in concentrated form. We find this situation with certain types of cosmetics.

Dr. Drouhet: Concerning the opportunistic infections, I should like to say that out of 81 cases of septicemia with *Candida sp.,* we saw candidiasis of digestive endogenous origin in only 10; in the others, particularly those due to *Candida parapsilosis,* the origin was exogenous. We saw 24 cases of *Candida parapsilosis* septicemia, including six with endocarditis and three with Starr valves. Almost all the cases were catheter infections, and *C. parapsilosis,* as well as *C. tropicalis* and other species besides *C. albicans* were very frequent on the cutaneous surfaces. Thus, for septicemic infections due to yeasts other than *C. albicans,* the most important source of infection is the cutaneous surface.

It was very interesting for me to hear that aspergillosis now constitutes a problem in the Americas, since several years ago it was considered only a European problem. In Paris, each year for the last five years we have found more than 200 cases of pulmonary aspergillosis diagnosed by mycological and serological methods at the Pasteur Institute. Aspergillosis is frequently a complication of pulmonary tuberculosis after chemotherapy.

Dr. Furcolow: In the United States we estimate that there are 5,000 cases of cavitary histoplasmosis admitted to the tuberculosis sanatoria every year, and I am sure that there are many more cases of tuberculosis in South America than in the United States. Many patients reside in histoplasmosis endemic areas. So let's not talk in terms of five cases of aspergillosis. It seems to me that there is a real public health problem. We have the tools to work with. We have the treatment. What we need to consider is how to determine the extent of the problem and how to go about treating it.

Dr. Seabury: I would just like to point out that public health problems do vary from one place to another. For instance, Dr. Pepys was visiting us last week and reminded us that in the city of London there are at least 5,000 cases of aspergillosis a year. This is a somewhat different form of the disease. Most of what he is speaking about would now be called either alveolar-bronchiolar aspergillosis or, in some cases, fibrosing alveolitis of exogenous type. This has become a very major problem for them, and, as a matter of fact, in doing isolations by plate exposure during the winter months the fallout of *Aspergillus fumigatus* spores is tremendous.

Yet in the area of New Orleans, I isolated very, very few colonies from open-air exposure plates over a period of a year, using the same

sort of plate exposure. So apparently aspergillosis is not just an opportunistic disease.

Dr. Muchmore: We here in the United States of America have still a lot to learn from people in Europe. The problem of aspergillosis in patients with chronic pulmonary diseases is certainly not nearly so well known in the United States as in the European literature, just as we were slow to learn about the English disease, chronic bronchitis.

We seem to be reluctantly slow to learn that most of these patients have continuing difficulties after their tuberculosis has been treated, and that many of these problems are due to aspergillosis. In fact, the incidence of aspergillosis may be more frequent than that of histoplasmosis in the tuberculosis sanatoria.

Dr. Furcolow: I wonder about that. Dr. Larsh has a total incidence of 20 or 30 cases of aspergillosis in the State Sanatorium of Missouri, whereas some 500 cases of cavitary histoplasmosis were diagnosed in that same institution.

As to *Aspergillus fumigatus,* Dr. Cozad did air samples when he was with us, and it was found that aspergilli are the most common airborne fungi in Kansas City. And yet patients are not dying from it in large numbers.

Dr. Zaias: I recently finished studying abnormal toenails, and I find that the most common yeast is *Candida parapsilosis.* It occurs in a frequency of four to one over *Candida albicans.*

In Miami we have another problem: corneal mycotic infections produced by *Fusarium solani.* We have had about 26 cases in the past three years. Although this is not a very widespread disease, blindness is a problem no matter how many cases there are. I would like to know how many other persons here have encountered *Fusarium solani* keratomycosis.

Dr. Greer: We frequently see these infections in Cali, Colombia. During the past year alone we have had five cases of keratitis. *Fusarium solani* was the agent of one case, *Fusarium sp.* of three other cases, and *Cephalosporium sp.* of the fifth case. Mycotic infections of the eye apparently are common.

Dr. Louria: I should like to address myself to four points. First, it is interesting that in this morning's discussions of opportunistic fungi no mention was made of *Nocardia asteroides.* In certain hospitals treating patients with neoplasia, *N. asteroides* is every bit as great a problem as opportunistic infections with other fungi, including Cryptococcus.

Second, I think we are going to have to be very cautious in assessing the mechanisms of superinfections so as not to imply that we have a simplistic and adequate explanation for the way antibiotics predispose to them. We have all assumed, I think, that administration of antibiotics suppresses the existing flora and that this in itself predisposes the individual to superinfection. I am not sure that this is true. There are studies, for example, indicating that administration of certain antibiotics to newborns prior to the time enteric flora is established is associated with enhanced susceptibility to mycotic infection. There are other studies showing that if you eliminate the existing flora and then replace that flora with antibiotic-resistant organisms, the host is still susceptible. Clearly, we do not know the mechanism for susceptibility after administration of antibiotics.

Third, one of the things we are very interested in is the diagnosis of certain superinfections, and it may well be that we could increase the number of positive results, especially with Candida, if we were to use hypertonic media at times when cultures are negative and we think there might be fungus superinfection. There are a small number of cases where superinfections have been related to transitional forms of fungi, including Candida and Cryptococcus.

Finally, I think it is important to point out, as Dr. Hasenclever has, that we have no adequate serologic tests to help us predictably with Candida superinfection. As he said, if the titer rises, this is helpful, but in his laboratory, and in our laboratory, there are patients who have profound increases in agglutinating antibody

against Candida with no evidence at autopsy of any Candida infection. Serologically, we have both false positives and false negatives, and this applies to any test used: precipitating antibody, hemagglutinating antibody, or clear toxin sonicated antigen.

Now I would like to ask a general question. Is there evidence that malnutrition predisposes to any specific mycologic infection?

Dr. González-Mendoza: I should like first of all to refer to the fact of not having mentioned *Nocardia asteroides* as an opportunistic microorganism. The cases seen in Mexico by Professor González Ochoa and myself correspond to primary disease: mycetoma, or pulmonary or cerebral abscess. Nevertheless, we know from Professor Mariat, who very kindly provided us with information on several cases, that *N. asteroides* can occur as an opportunistic fungus, especially in cases of heart surgery.

With regard to malnutrition as a predisposing factor to infection by fungi, there are no conclusive data, as far as I know, at least for the human being. In studies of animals, the results have been varied. One thing is certain: if malnutrition exists, there is a reduction in the synthesis of protein, which might convey a reduction in the synthesis of antibodies.

Dr. Pollak: Dr. Furcolow is interested in hearing something about the major problems of the systemic mycoses. I recall his serologic work on histoplasmosis, and I should like to introduce some data from our serologic studies of paracoccidioidomycosis later in the Symposium.

Session II

Tuesday, 24 February 1970, 1:30 p.m.

RECENT ADVANCES IN DIAGNOSTIC PROCEDURES

Chairman

Angela Restrepo M.

Rapporteur

Lucille K. Georg

EXPERIENCE WITH A NEW INDICATOR MEDIUM (DTM) FOR THE ISOLATION OF DERMATOPHYTE FUNGI[1]

David Taplin, Alfred M. Allen, and Patricia Mann Mertz

In 1969, Taplin and co-workers (*3*) introduced Dermatophyte Test Medium (DTM) for the isolation and recognition of dermatophytes. The medium relied on the incorporation of antibacterial antibiotics and a mold inhibitor, and it indicated the growth of dermatophyte fungi by the change in color of a pH indicator from yellow to red (see color plate).

Considerable experience has now been gained in the use of this medium in the field, and the results are reported here. An opportunity to test the system under adverse conditions in the Mekong Delta of Vietnam occurred in 1968, when a field epidemiological survey team from the Walter Reed Army Institute of Research and the Department of Dermatology, University of Miami, conducted extensive studies of dermatophytosis among U.S. Army personnel, military and civilian populations of the Republic of Vietnam, and local environmental sources.

Method

Formulation of Dermatophyte Test Medium (DTM)[2]

Warning: Ingredients have been selected by clinical testing. Substitution from sources other than those indicated may change the characteristics and selectivity of the medium.

Distilled water, 1,000 ml
Phytone (BBL), 10 g
Dextrose, 10 g
Agar-agar (BBL or Difco), 20 g
Phenol red solution, 40 ml
0.8 M HCl, 6 ml
Cycloheximide (Actidione, Upjohn), 0.5 g
Gentamicin sulfate, 0.1 g of active drug = 100 μg/ml
Chlortetracycline HCl, 0.1 g = 100 μg/ml

Preparation and use of medium

1. Add phytone, dextrose, and agar to 1,000 ml distilled water and boil to dissolve agar.
2. Add 40 ml of phenol red solution while stirring. (Phenol red solution: 0.5 g Difco bactophenol red dissolved in 15 ml 0.1 N NaOH made up to 100 ml with distilled water.)
3. Add 6 ml 0.8 M HCl added while stirring.
4. Dissolve 0.5 g cycloheximide in 2 ml acetone and add to hot medium while stirring.
5. Dissolve sufficient gentamicin sulfate powder (Schering Corp., New Jersey) in 2 ml distilled water to give a final concentration in the medium of 100 μg/ml of active drug. The amount is dependent on the assay of each sample of gentamicin. A sample with activity of 500 μg/mg would, for example, require 0.2 g to be added to each liter of medium.
6. Autoclave at 12 lbs for 10 minutes and cool to approximately 47°C.

[1] Research supported by U.S. Army Medical Research & Development Command, Office of The Surgeon General, in cooperation with the Commission on Cutaneous Diseases of the Armed Forces Epidemiological Board, Contract No. DA-49-193-MD-2236.

[2] Commercially available as Dermatophyte Test Medium (DTM) from Pfizer Diagnostics Division, 300 West 43rd Street, New York, New York 10036 and as Colab Dermatophyte Medium from Colab Laboratories, Inc., 3 Science Road, Glenwood, Illinois 60425.

7. Dissolve 0.1 g chlortetracycline HCl (American Cyanamid, New York) in 25 ml sterile distilled water and add to medium while stirring.

8. Pour plates or dispense in one-ounce wide-mouthed bottles (8 ml per bottle) and cool on the slant.

9. Store under refrigeration for maximum shelf life.

10. After inoculation, it is essential to *leave caps loose* to allow adequate growth and development of indicator color change. Optimum temperature for incubation is 28° to 30°C. Excessively cool room temperature may delay growth.

11. Color change of indicator should be interpreted *not later than two weeks* after inoculation. Longer incubation may produce false positive results from slow-growing contaminant fungi and has not, in our studies, increased the recovery of dermatophytes.

Methods of sampling

Prior cleansing of the skin was not employed in these studies, except in some situations when gross dirt was removed with sterile saline-soaked gauze. On glabrous skin, the active border was scraped with sterile scrapers or scalpels. Infected hairs were plucked with sterile forceps, and, in cases where vesiculation was a feature, the vesicle top was selected for culture. In recent surveys of schoolchildren, DTM in plastic Petri dishes was used as a touch-plate method. In closed lesions seen in some cases of *T. mentagrophytes* infections in Vietnam, incision of subcutaneous nodules yielded pus from which positive cultures were obtained.

Cultures from nails have not been included in these studies because of the high rate of contamination by saprophytic fungi, some of which may be pathogenic in nails (*4*).

Results

Civilian clinic—Miami, Florida

During 1967 and 1968, 1,800 cultures were made in a civilian dermatology clinic and in schools in which tinea capitis was endemic. Table 1 shows that in this series only 54 cultures (3 per cent) produced a false color reaction. Thus, on color change alone the system was 97 per cent accurate.

In later surveys of schoolchildren with tinea capitis, we have been impressed with the massive cultures of *T. tonsurans, M. audouinii,* and *M. canis* obtained by pressing the surface DTM agar plates against the lesion on the scalp. We now prefer this method to individual plucking of hairs.

Prison study—Texas, 1969

Dr. Edgar B. Smith of the Baylor University College of Medicine used DTM in a survey of a prison population. In 444 cultures from the feet, he obtained dermatophytes from 176. In a personal report to the authors, Dr. Smith stated that the indicator system performed well, and there were no false positives within the two-week incubation recommended.

Combat troops—Mekong Delta, Vietnam, 1967

Blank and co-workers (*2*) reported on the high prevalence of *T. mentagrophytes* infections among combat troops in the Mekong Delta. During these studies, DTM was used to isolate dermatophytes from cutaneous lesions. From 91 body sites cultured among 54 combat infantrymen, dermatophyte fungi were recovered 59 times, yielding a positive diagnosis on virtually

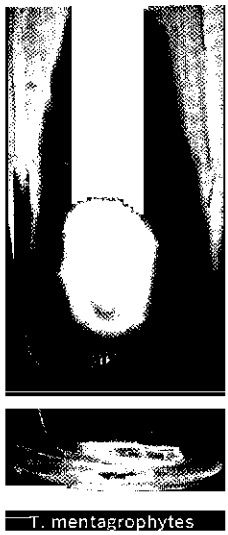
Table 1

Results of 1,800 cultures using DTM

	Dermatophyte	Contaminant fungi	Bacteria	Candida
Number of organisms isolated	688	94	22	196
Number that turned medium red	688	31[a]	8[a]	15[a]

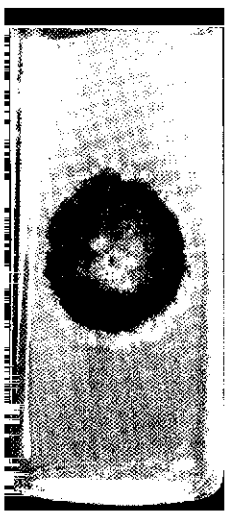
[a] 54 false reactions = 3 per cent of total cultures

T. rubrum



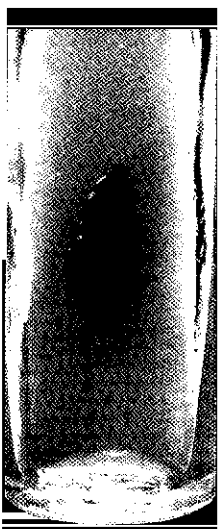
T. mentagrophytes

T. violaceum

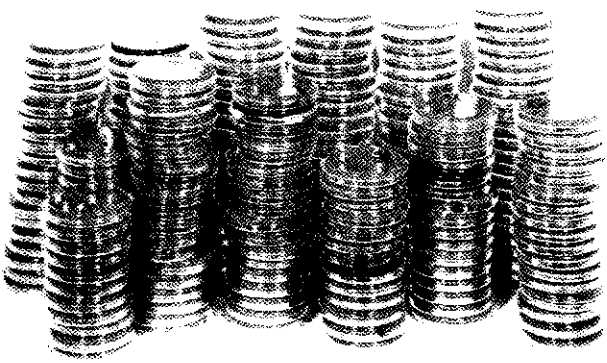


Contaminant
Aspergillus sp.

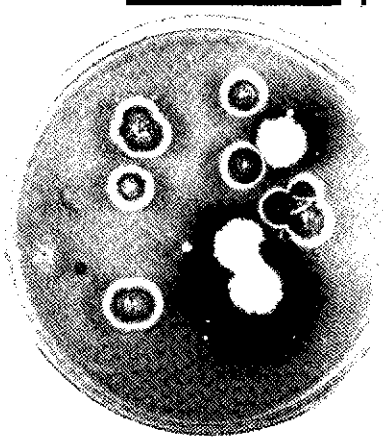
Contaminant
Paecilomyces sp.



Contaminant
Cladosporium sp.



Survey of Normal Perineal Skin Defects Carriers



Direct Plating of Pet Hair

every man. These cultures were examined within two weeks. There were no false positives, and no isolates required longer than two weeks to produce a positive color change.

Mycological survey—Mekong Delta, Vietnam, 1968

Allen and co-workers (*1*) report the value of DTM in surveys of military personnel in Vietnam. In their study, they cultured over 600 men, frequently just after return from combat operations, when they were muddy and still damp from rice paddy water. They obtained high yields of dermatophytes. No prior preparation of the skin was employed, and the cultures were interpreted by a laboratory technician unskilled in mycology, who achieved an accuracy in diagnosis greater than 97 per cent based on the color change from yellow to red in the medium. This ability to distinguish dermatophytes from other organisms enabled them to conduct extensive epidemiological studies under adverse field conditions without the support of trained mycologists. Later examination of these cultures by experts confirmed the validity of their findings.

Environmental sampling

During unreported studies of troops in training at Fort Benning, Georgia, in 1967; at Eglin, Florida, in 1967 and 1968; and in the Everglades, Florida, we have used DTM in attempts to obtain dermatophytes from heavily contaminated sources, including soil, swamp water, boots, and dirty socks. In early studies, in which gentamicin was the only antibacterial antibiotic incorporated in the medium, up to 85 per cent of the cultures were grossly contaminated with gram-negative swamp bacteria that were resistant to gentamicin. The addition of chlortetracycline almost completely eliminated the problem of bacterial contamination, but significant numbers of saprophytic fungi grew on the medium in spite of the inclusion of cycloheximide, and many produced alkaline metabolites that turned the indicator red. Under these conditions, the medium could not be relied on to identify dermatophytes by color change alone. However, it was superior to any other medium in terms of recovery rates.

We have achieved considerable success with DTM in the recovery of dermatophytes from the pelage of wild rats trapped in the inundated terrain of the Mekong Delta and from the DaNang area in Vietnam during the rainy season. We have also obtained good recovery on DTM plates of dermatophytes from artificially inoculated unsterile mud from the Everglades. All other media used were by comparison heavily overgrown with other organisms. We have also found DTM plates useful in the subculture of hairs employed as bait in the recovery of dermatophytes from soil.

Discussion

Dermatophyte Test Medium (DTM) has now been used in five separate studies involving over 3,000 subjects and has shown an accuracy ranging from 97 to 100 per cent. The investigators conducting these studies used media obtained from our laboratories, where ingredients are standardized and manufacture is carefully monitored. In developing this medium, we have selected the ingredients by comparative testing, and we would again stress that substitution of any component may alter the characteristics.

As with any antibiotic-containing medium, it should be used as fresh as possible, although, when stored at 5° to 10°C, tubed DTM appears to retain its properties for at least nine months.

Interpretation of results should be made within two weeks of inoculation, and adequate ventilation must be provided by loosening the caps of the container. In the many thousands of isolates studied, we have never encountered a dermatophyte that did not change the indicator from yellow to red, providing the cap was loose.

DTM does not interfere with the gross morphology and microscopic characteristics of dermatophytes, but reverse pigmentation is largely obscured by the intense red color in-

dicator. We have a distinct impression that pleomorphic changes occur more slowly on DTM than, for example, on Sabouraud's medium, and this view is shared by F. M. Rush-Munro in New Zealand. In the five years we have used DTM in Miami, we have rarely encountered the fluffy or "anthropophilic" form of *T. mentagrophytes*. This may be a geographic phenomenon, but *in vitro* studies suggest that the medium favors granular growth.

Although our particular combination of antibiotics enhances the recovery of dermatophytes from heavily contaminated sources such as soil and boots, the color indicator cannot be relied on alone to determine which samples are positive or negative. Nevertheless, when growth is not confluent, the indicator will often pinpoint a single colony of a dermatophyte among many contaminants. The same holds true for nail cultures, where DTM is helpful but not necessarily diagnostic on color change alone.

We have screened a large number of antimicrobial agents in an attempt to produce the perfect selective indicator medium useful under any condition. The suppression of bacterial contamination is almost total, and further improvements in this respect seem unnecessary. There is need, however, for a more efficient agent than cycloheximide for the complete selective suppression of saprophytic fungi in environmental sampling.

The most exciting aspect of DTM has been its value in large-scale epidemiology under field conditions. Within two weeks of sampling populations in the Mekong Delta of Vietnam, we were able to evaluate prevalence and carrier states, to pinpoint populations at risk, and to estimate the distribution of species. Further analysis of cultures did not significantly alter our preliminary results.

We believe DTM is particularly suitable for establishing profiles of dermatophytosis in countries where skilled mycologists are not available.

ACKNOWLEDGMENTS

The list of those who have assisted in the development of DTM is long, but we particularly wish to thank Dr. Harvey Blank, Dr. Nardo Zaias, and Gerbert Rebell for their continuous support and advice; Robert Weaver and Ray Drewry, who worked under exceptional conditions to obtain specimens in Vietnam; and the U.S. Army Medical Research and Development Command, which financed this project. Costs of color reproduction for this publication were borne by Chas. Pfizer & Co., Inc.

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ISOLATION AND IDENTIFICATION MEDIA FOR SYSTEMIC FUNGI

Howard W. Larsh

Many questions relating to the isolation and identification of the pathogenic fungi that cause systemic infections in man are still unanswered. These uncertainties have stood in the way of the establishment of realistic morbidity and mortality rates for the systemic mycotic diseases.

Progress in this field has been slowed by the shortage of artificial cultural media and susceptible field laboratory animals for all fungal agents. Moreover, far too little importance has been given to the necessary contribution of persons specially trained in this work, with the result that proficient mycology has not been done in many diagnostic clinics and hospital laboratories.

To present a list of specific media and their ingredients at a meeting of this level would be presumptuous. Most of these formulations have been known and used for many years in diagnostic laboratories. This presentation will therefore be limited to the rationale of their use and to a consideration of the advantages and limitations of the various media in the isolation and identification of organisms producing systemic mycotic infections. The observations will be based largely on personal experience in culturing hundreds of specimens, both clinical and from nature, over the past two decades.

Artificial cultural media can be conveniently classified into three main groups: primary, selective, and differential. The primary and selective types can and often have been used for purposes of isolation, whereas differential types delineate the species. The number and kinds of media employed to isolate and identify pathogenic fungi depend on the type of specimen to be examined. It is essential to appreciate this fact and to understand the advantages and limitations of each medium.

Proper collection and treatment of the specimen is a key factor in the ultimate isolation of the causal fungus. It is important that the collection procedures be exact and that the conditions be aseptic if at all possible. Specimens showing obvious contamination must be treated prior to inoculation in such a way as to assure the growth of pathogens free from contaminating bacteria and fungi. There are many effective methods for removing extraneous material and thus reducing the number of contaminating microorganisms.

The digestion of sputum species with either N-acetyl-L-cysteine or dithiothreitol gave satisfactory results in our studies at the University of Oklahoma laboratory. The specimens were liquefied rapidly, neutralized, and concentrated, without any apparent effect on the pathogenic organisms. Best results were observed when the concentration of sodium hydroxide was minimal and the pH did not exceed 8.0. Clinical specimens other than sputum that were not seriously contaminated were diluted in L-cysteine saline containing 150,000 units of penicillin and 10,000 units of streptomycin per milliliter. In each instance, approximately 1 ml of the treated specimen was plated onto an artificial cultural medium that contained the same antibiotics.

Direct isolation of pathogenic fungi from soil

or from other specimens in nature has not been achieved very frequently in the course of these studies. Treatment of specimens with antibiotics or other chemicals so as to assure inhibition or retardation of saprophytic fungi and bacteria has proved difficult. Overgrowth of the cultural plates by these organisms can mask or prevent the growth of the pathogens. It has been found that the inoculation of treated specimens into experimental animals is the most successful means of isolating pathogens from nature. Even with this procedure, however, the rate of isolation from the environment is still relatively low.

Primary isolation media

The primary isolation medium used by most mycologists has not been radically changed since the introduction of Sabouraud's formulation (*12*). There have been modifications of his original medium, however. Present ingredients have little in common with earlier ones other than that they belong to the same group of chemical compounds. It has long been impossible to obtain Fairchild's peptone or the crude maltose and honey that Sabouraud used in his original medium. Also, it should be remembered that his primary specimens were taken mostly from patients with superficial infections and that the problem of contamination was probably not serious. The dextrose-peptone medium now commonly used in medical mycology laboratories has its limitations, but it is adequate if the problem of contaminating organisms is not too great.

In the University of Oklahoma studies, it has been a relatively frequent finding that plain Sabouraud's dextrose agar will not support the growth of pathogens from primary clinical specimens, especially, in the case of *Histoplasma capsulatum*. Nevertheless, when the pathogen has first been isolated and is then transferred to this medium, luxuriant growth occurs. Knowledge that certain chemical compounds have a marked effect on the growth of specific pathogens dictates that plain Sabouraud's dextrose or some other type of plain artificial cultural medium must be included in the isolation regimen. Plain Sabouraud's agar plates have not been too successful in yielding isolates of pathogens; the usual result has been complete overgrowth by saprophytic organisms. Consequently, it has been our procedure for some 15 years to include at least one plain medium for each specimen processed. Experience with plain Sabouraud's dextrose medium in isolation attempts with specimens from nature, such as soils from chicken houses, caves, and other highly contaminated areas, has been futile.

Selective isolation media

The media for selective isolation of pathogenic fungi from contaminated specimens include specific antibiotics that either retard or inhibit the growth of other microorganisms. The commonly used antibiotics have been chloramphenicol, cycloheximide, penicillin, and streptomycin. Commercially available media contain chloramphenicol and cycloheximide. These media with antibiotics cannot be described as ideal, since various species of pathogenic fungi may be either completely or partially inhibited by their presence.

Cycloheximide is known to inhibit many isolates of *Cryptococcus neoformans* and several species of the genus Candida. In addition, *Allescheria boydii* and species of the genus Aspergillus may be partially inhibited, although in most cases only retardation occurs. Chloramphenicol is known to affect the growth of some isolates of Nocardia and Actinomyces. The concentration of this antibiotic in the commercially available dehydrated media is not inimical to most pathogenic fungi; however, Campbell (*3*) has shown some cultures of the yeast phase of *H. capsulatum* to be affected. In the Oklahoma studies the same phenomenon has been observed in a few yeast and mycelial phases of the same fungus. For this reason, we prefer to use penicillin, streptomycin, and cycloheximide in the primary isolation medium. The amount of penicillin and streptomycin is not critical.

The usual amounts added are 20 units of penicillin, 40 units of streptomycin, and 0.5 mg of cycloheximide per milliliter of specimen. No apparent inhibition was seen when 200,000 units of penicillin, 400,000 units of streptomycin, and 1.5 mg of cycloheximide per milliliter were used for a sputum specimen containing *H. capsulatum*. One must have full knowledge of the action and spectrum of the antibiotic as well as its physical properties. The indiscriminate use of antibiotics should be discouraged.

Sabouraud's dextrose antibiotic agar has proved successful for the isolation of most pathogenic fungi. Nutritionally fastidious fungi have been isolated on either brain-heart infusion or blood agar base containing 8 to 10 per cent blood, to which antibiotics have been added. Frequently in clinical specimens, especially sputum and urine, *H. capsulatum* has been recovered on the latter two media and not on Sabouraud's dextrose agar, either with or without antibiotics.

Incubation of these antibiotic-containing media should be at 25°C, as many pathogens are inhibited by cycloheximide at 37°C. An exception to this rule is *Coccidiodes immitis*, which has been recovered from clinical specimens much more rapidly at 37°C on blood agar containing antibiotics. Growth of this fungus in its mycelial phase occurs within three to five days at 37°C. At this time, there is usually a distinct metallic sheen present in the medium, with arthrospores delineated. The plates must be sealed with tape to prevent dehydration and to provide environmental conditions conducive to growth of the fungus.

In recent years, specific media have been developed that permit the isolation of *Cryptococcus neoformans*, *Candida albicans*, and other pathogenic fungi from contaminated clinical specimens. Shields and Ajello (*13*) introduced a selective isolation medium containing glucose, creatine, and an extract of *Guizotia abyssinica* (thistle seed) on which colonies of *C. neoformans* produce a brown pigment. However, other species of Cryptococcus and Candida do not produce pigmentation on this medium. Saprophytic molds may be inhibited by the addition of diphenyl in a concentration of 0.01 per cent. Botard and Kelley (*2*) modified Littman's oxgall agar by adding an extract from the seeds of *G. abyssinica*. In this medium, colonies of *C. neoformans* became brown, whereas the color failed to develop in colonies of eight other yeasts. Of particular significance was the failure of the yeast phase of dimorphic pathogens to take on the brown coloration. Vogel (*15*) recently developed a new isolation and identification medium for *C. neoformans*. This basal medium contains potato dextrose, urea agar base, and chloramphenicol. It is bright yellow in color due to the reaction at pH 3.5 of the phenol red indicator. The growth of *C. neoformans* on this medium at 37°C is definitive identification of the species. In addition, bacterial contaminants such as Proteus, Staphylococcus, and *Escherichia coli* are completely inhibited at pH 3.5. Evron and Ganor's (*5*) use of sodium taurocholate agar with the addition of Tween 80 permitted the isolation of *C. albicans* from clinical specimens, and chlamydospore formation was induced in primary cultures. The yeast extract in agar developed by Omieczynski and co-workers (*11*) was superior to Sabouraud's dextrose agar for the isolation of *C. immitis* from sputum. Sporulation was stimulated in the yeast medium, and the pour-plate technique was superior to the surface method for isolating the fungus from sputum heavily contaminated with Candida. Several other examples could be cited, but these are sufficient to show that active investigation is being done on the development of media for the isolation of pathogenic fungi.

Differential media

The successful isolation of pathogenic fungi in pure culture is usually all that is necessary for identification of the species. Characteristic colonial growth, along with the development of diagnostic and taxonomic microscopical structures, is generally sufficient for a positive identi-

fication. However, this is unfortunately not the case when the fungus is isolated from a chronic infection. Additional morphological, physiological, and sometimes serological studies are required. It is in these areas that more recent advances in diagnostic procedures have been concentrated.

The present paper does not attempt to give a complete review of the standard special and differential media used for the identification of pathogenic fungi. This subject has been covered very well in the U.S. National Communicable Disease Center's *Laboratory Manual for Medical Mycology* (*1*) and in the medical mycology textbook by Emmons, Binford, and Utz (*4*). The discussion here will deal mainly with the differential media that have been developed recently and with methods that may be required to obtain a definitive identification.

Variation among isolates has been one of the chief sources of difficulty in the identification of certain pathogenic fungi. Many if not all the systemic fungi have myriads of variants. For the most part, the variants do not produce diagnostic characteristics on the commonly used cultural media. For example, Huppert *et al.* (7) observed that numerous morphological variations occur in *C. immitis* when the organism is grown on Sabouraud's dextrose agar after eight weeks' incubation at 25°C. This finding led the authors to suggest that production of pathology and endosporulation spherules must take place before an isolated culture can be identified as *C. immitis*. Variants have also been reported to occur in *H. Capsulatum* (*10*), *C. albicans*, and *C. neoformans*. In view of the number and kinds of variations observed in the fungi causing systemic infections in man, it is necessary to use differential media and techniques to assure correct identification of the species.

The production of characteristic or diagnostic spores in culture media is a crucial step in the identification of the fungus. Many of the media in use contain too much carbohydrate, which supports vegetative growth and yields a sparse production of spores. The success of a sporulation medium is attributed at least in part to the small amount of sugar in the formulation. One of the important features in a taxonomic key is the type and arrangement of spores. There are several sporulation media that have been successfully used in the field of mycology: potato dextrose agar, chlamydospore agar, corn meal agar, and others. However, in a recent comparative study, the medium of Smith and Furcolow (*14*) produced a much greater number of spores than did the potato dextrose or Sabouraud's dextrose agars.

Jennings and co-workers (*8*) have shown that *G. abyssinica* seed agar and virulence for mice were the two most successful tools for the identification of *C. neoformans* in a routine clinical laboratory.

Fisher and Kane's (*6*) diluted oxgall agar has proved excellent for the identification of *C. albicans*. The inoculated area is protected with a coverslip and incubated at 28° C for 24 to 48 hours. Under these conditions, *C. albicans* produces an abundance of chlamydospores and few or no blastospores, whereas *C. stellatoides* produces many blastospores, long filaments, and an occasional chlamydospore.

The yeasts pose a special problem in regard to both genus and species differentiation. The cryptococci may be distinguished from the genus Candida by their ability to produce urease. This requires the use of a urea agar base. The species identification may be accomplished by utilizing the nitrate and carbohydrate assimilation media. The Candida species may be identified by the use of carbohydrate fermentation and assimilation media.

In many instances, dimorphic pathogens have to be converted from the mycelial phase to the yeast phase when definitive identification cannot be made from colonial and microscopical observations. Most isolates can be converted on artificial cultural media without difficulty. However, in isolates that do not readily convert on these media the tissue culture technique has proved efficient.

The Oklahoma experience with primary

mycelial cultures of *H. capsulatum* showed that conversion to the pathogenic phase occurred on various tissue cell lines, and HeLa cells are now being used in the tissue culture technique, according to the following procedure:

A suspension of the organism, approximately 5×10^5 mycelial fragments per milliliter, is inoculated into HeLa cells maintained in monolayers. A special conversion medium consisting of minimal essential medium (MEM) supplemented with glutamine (2 millemole/100ml) is used to bathe the cells. At 24 and 48 hours after inoculation mycelial fragments can be recognized, and they show various stages of becoming rounded or segmenting into spherical shapes. After 72 hours, a mixture of incomplete conversion is more readily seen, and shortly after this several converted forms can be observed. At this time, the monolayer is removed from the Smith bottles and spread on the surface of a blood agar tube that also contains glutamine. A yeast-phase culture of the organism is usually obtained upon a single transfer of this material after two or three days.

Stages of conversion, as they occur in the tissue culture system, are usually difficult to follow; however, most phases can be detected using fluorescent antibody techniques.

In the earlier Oklahoma studies, phagocytosis of the organism was a factor considered essential for the conversion of *H. capsulatum* (*9*). Recent findings, however, indicate that the major requirement for conversion and maintenance of yeast cultures from primary mycelial isolations is the presence of glutamine.

It has been our policy to convert all *H. capsulatum* isolates to the yeast phase. This, with other findings, will usually satisfy the most critical investigator that a definitive identification of the fungus has been made. Species clarification and identification of other diphasic pathogenic fungi have been achieved more rapidly with the use of tissue culture techniques.

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SYSTEMATICS OF YEASTS OF MEDICAL INTEREST

Donald G. Ahearn

Advances in chemotherapeutic and surgical procedures are associated not only with an increased incidence of mycoses but also with predisposition to infection by a greater variety of yeasts. For example, of yeasts received during 1968 and 1969 at the U.S. National Communicable Disease Center, Atlanta, *Candida parapsilosis* was one of the more common species associated with fungemia, and *Torulopsis glabrata* showed a high frequency of association with urinary tract infections. Relatively few clinical laboratories attempt to identify such adventitious yeasts. Typically, examinations of yeasts are limited to tests for pseudomycelium and chlamydospores characteristic of *Candida albicans,* or to tests for capsule formation, urease activity, and/or pathogenicity for mice for the identification of *Cryptococcus neoformans.* Unfortunately, the spectrum of results from these tests will not definitively identify all isolates of either species. To facilitate therapy and to elucidate the epidemiology of adventitious yeasts, rapid and specific identification of clinical isolates is necessary. This paper offers procedures and criteria applicable in the routine clinical laboratory for the identification of yeasts associated with man.

Methods

A variety of procedures and media have been described for the isolation and classification of yeasts. The media given below have been adapted mainly from Wickerham (*12*) and Lodder (*7*).

Isolation

Sabouraud's dextrose agar containing 0.5 g chloramphenicol per liter, or Wickerham's acidified yeast extract and malt extract agar, are suitable for the isolation of most yeasts from clinical specimens. The latter medium contains 3 g malt extract, 3 g yeast extract, 5 g peptone, 10 g dextrose, 10 ml lactic acid (85 per cent), 20 g agar, and 990 ml distilled water. A solution of neomycin to give a final concentration of 500 μg/ml may be substituted for or used in addition to the chloramphenicol or the lactic acid. The lactic acid and the antibiotic solutions —with the exception of chloramphenicol, which is heat stable—are sterilized separately by filtration and added to the autoclaved medium when it has cooled to about 50° C. Mycosel agar (BBL) and mycobiotic agar (Difco) are frequently used to isolate fungi from clinical specimens. These media, which contain 0.5 g cycloheximide per liter, support the growth of *C. albicans* and strains of *C. tropicalis, C. guilliermondi,* and other yeasts, but they are inhibitory to *Cryptococcus spp.* and *Torulopsis spp.*. Isolation media should be incubated at about 35° C (rare strains of *Cryptococcus neoformans* of established pathogenicity give slow, weak growth *in vitro* at 37° C, whereas some saprophytic cryptococci grow well at 37° C). All primary isolates obtained on slant cultures, regardless of gross appearance, should be considered mixed cultures. It may be necessary to streak yeast

cultures several times on agar plates to get pure cultures.

Germ tubes

Bovine or pooled human sera or Medium 199 (*8*) are all suitable media for the induction of germ tubes in *C. albicans* and *C. stellatoidea*. The sera preparation or Medium 199 is dispensed in quantities of 0.2 to 0.3 ml into clean nonsterile test tubes. The tubes may be frozen and stored for prolonged periods before use. All morphologically distinct yeast colonies are picked directly from the isolation media with Pasteur pipettes and placed into the germ tube broth. The pipettes are left immersed in the serum for two to three hours at 37° C and used to transfer one drop of the cell suspension to a glass slide. Under low magnification, the fields are examined for clumps of cells. With higher power these clumps are generally found to include cells with tangled germ tubes. Colonies that are germ tube negative are streaked to get pure cultures. Cultures of *C. albicans* and *C. tropicalis* should be used as controls in each run of unknown yeasts. The procedures for this test have been described in greater detail in a previous report (*1*).

Pseudomycelium production

Corn meal agar or yeast morphology agar (Difco) are adequate for the induction of pseudohyphae. Four to eight yeast isolates may be cut into the agar medium in a Petri dish. The cuts are made several millimeters larger than a sterile coverslip, which is placed over the cut. After two to four days' incubation at 22° to 26° C, the yeasts may be examined for mycelial production either with low magnification, through the back of the unopened plate, or with higher magnification, through the coverslip. Most of the common candidas readily produce pseudohyphae, but with slower growing forms the plates should be examined over a period of at least 10 days.

Ascosporulation

Suspect colonies on the yeast extract and malt extract isolation medium should be examined microscopically for ascospores after four to six days' incubation. Malt extract agar, 5 per cent in a 3 per cent agar solution, is a general ascospore medium for *Saccharomyces spp.* Conditions for induction of ascospores differ markedly from one genus to another (*7*).

Assimilation tests

Yeast nitrogen base and yeast carbon base (Difco), prepared according to the defined formulae of Wickerham (*12*), are recommended for the assimilation tests. The yeast nitrogen base (6.7 g) and 5 g of the appropriate carbohydrate (glucose, galactose, sucrose, lactose, maltose, raffinose, trehalose, melibiose, inositol, cellobiose, and xylose) are dissolved in 100 ml of distilled water. The yeast carbon base (11.2 g) is fortified with 0.78 g KNO_3. These concentrated bases (10×) are sterilized by filtration. To facilitate storage and handling in the clinical laboratory, the bases may be solidified in 900 ml distilled water containing 20 g of washed agar. The agar solution is sterilized separately by autoclaving. Control slants containing the base medium without the carbon or nitrogen sources should be included in the assimilation spectrum for each yeast. Inocula for the carbohydrate assimilation tests are prepared by transferring a few cells to 5 ml of the yeast nitrogen base broth containing 1.0 mg per liter of glucose. For the nitrate assimilation test, the cells are starved in a yeast carbon base broth. After 24 to 36 hours' incubation at 22° to 26° C, a 0.01 ml cell suspension is taken to inoculate the assimilation slants. The tests are incubated for at least 96 hours at 22° to 26° C. Positive reactions are based on a comparison with growth on the glucose slant.

Fermentation tests

The basal fermentation broth contains 0.55 per cent yeast extract, 0.75 per cent peptone, and sufficient brom cresol purple to give a purple

color. The fermentation base is dispensed, 4 ml per tube, into 16×125 mm screw-cap test tubes containing gas inserts and then sterilized by autoclaving. The carbohydrates, in 6 per cent solutions (except for raffinose at 12 per cent), are sterilized separately by filtration, and 2.0 ml is aseptically added to each tube. Sugars to be routinely tested for fermentation include glucose, galactose, sucrose, maltose, and raffinose. The fermentation tubes may be inoculated with a cell suspension prepared directly from the stock slant. If the same cell preparation used for inoculating the assimilation tests is employed, the inoculum should be 0.1 to 0.2 ml. The caps should be securely tightened during the 10-day incubation period at 22° to 26° C. Positive fermentation is recorded for the production of gas only. To detect false negatives due to supersaturation of the broth, all tubes giving an acid reaction should be shaken lightly and the cap vented. This operation is frequently followed by a rapid release of gas. All positive fermentation reactions with a carbohydrate are accompanied by a positive assimilation of that carbohydrate; however, sugars may be assimilated without being fermented.

Urease activity

Urea agar base (Difco), prepared according to Christensen (*3*), or with the modifications of Littman (*6*), is suitable for the detection of urease. All species of the genera Cryptococcus and Rhodotorula and a number of species of Candida and Trichosporon are urease positive.

A flow chart for the identification of clinical yeast isolates is presented in Figure 1.

Results and Discussion

About 120 species are presently described for the genera Candida, Cryptococcus, and Torulopsis. To distinguish many of these species, over 40 assimilation and fermentation tests are required. Fortunately, the yeasts most commonly associated with man may be classified with a limited spectrum of tests. The germ tube

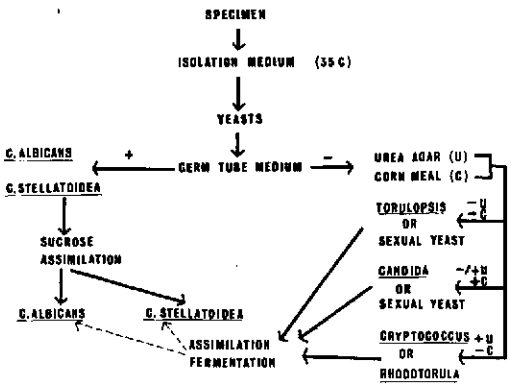

Figure 1. Flow chart for the identification of clinical yeast isolates.

and the urease tests provide a means of rapid screening for the clinical laboratory.

The germ tube method described here allows identification of over 95 per cent of the clinical isolates of *Candida albicans* directly from the primary isolation medium. *C. stellatoidea* also produces a germ tube, but this yeast is of relatively rare occurrence and may be considered an alpha-glucosidase negative variant of *C. albicans* (*4*). The sucrose assimilation test or the rapid procedure of Kamaya (*5*) may be used to distinguish the variety. The small percentage of false negative germ tube reactions usually results from using aged, nonactively growing cells, or too many cells for the volume of serum. Young cells from 24- to 96-hour cultures at concentrations from 10^6 to 10^7 cells per milliliter are the proper inoculum. In contrast to the usual pseudohyphal cell of the candidas, the germ tube is not constricted at its juncture with the parent cell (Figure 2). It should be noted that *C. albicans* produces both germ tubes and pseudohyphal cells under conditions used for the germ tube test; only the germ tubes are a specific character. Threshold conditions for the induction of germ tubes vary with the strain. Under strictly controlled conditions, tests performed with sera, Medium 199, or egg albumin appear to be definitive (*2, 4, 11*).

All germ tube negative yeasts are inoculated

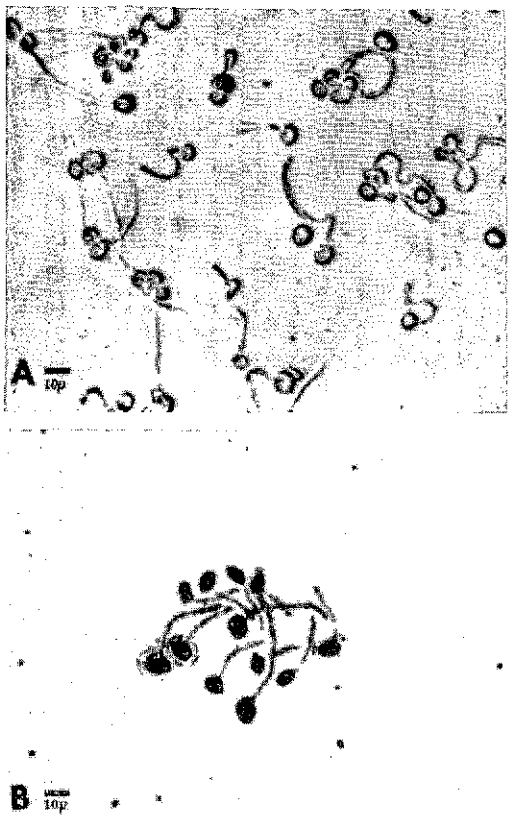

Figure 2. ***Candida albicans*** germ tubes (A) unstained after 2 hours in Medium 199, and (B) clumped germ tubes, stained with lacto-phenol cotton blue.

onto urea slants at the time they are streaked for purity. The urea slants are normally read over a five-day period, but cryptococci frequently give a positive reaction within 24 hours. The urease test is not specific, but a positive reaction accompanied by the characteristic cell morphology and clinical syndrome may justify a presumptive identification. Definitive identification is based on the assimilation tests or pathogenicity for mice. Unlike other yeasts, *C. neoformans* is always considered pathogenic when associated with a human host. Usually the cells are encapsulated, globose to oval, and they reproduce by unicellular budding. On rare occasions, a few strains produce pseudo- to true mycelium (*9*). The morphology of the mycelial stage and the high molar percentage of guanine and cytosine in the DNA of *C. neoformans* (*10*) indicate a relation to the heterobasidiomycetes. *C. neoformans* may be distinguished from *C. uniguttulatus* in the failure of the latter species to assimilate dulcitol and adonitol.

The presence or absence of pseudomycelium on corn meal agar is an important taxonomic characteristic. Species of Cryptococcus, Torulopsis, and many sexual yeasts fail to produce pseudohyphae, whereas Candida, Trichosporon, and many Pichia and Hansenula species produce profuse pseudohyphae. *Candida tropicalis* typically forms a heavy mycelium within 24 to 36 hours, often making it look like a *Trichosporon sp.* This latter genus produces arthrospores (Figure 3). The morphology on corn meal may be quite distinctive; however, even strains of *C. albicans* may form only sparse areas of pseudohyphae, and strains of *Saccharomyces cerevisiae* may appear as candidas. For this reason, a total characterization may be needed to identify some of the variants of even common species.

Positive assimilation reactions generally develop over a shorter incubation period than fermentation reactions. The assimilation tests

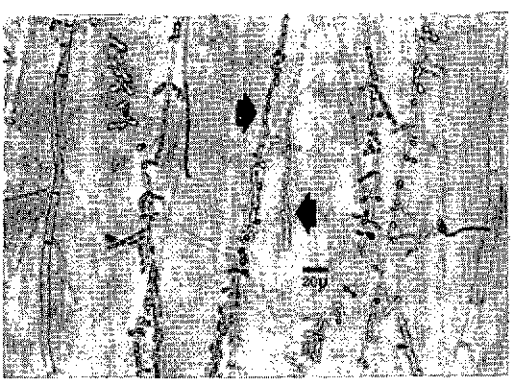

Figure 3. ***Trichosporon cutaneum*** arthrospores on corn meal agar after 24 hours.

also appear to be more stable and less influenced by incubation conditions. Common Candida species show less vigorous fermentation in defined fermentation broths and may vary from their established fermentation patterns at higher incubation temperatures. At 35° to 38° C in a yeast extract–peptone fermentation medium, some isolates of *C. albicans* ferment sucrose. The

Key to Asporogenous Clinical Yeast Isolates[1]

1.	Germ tubes positive	2
	Germ tubes negative	3
2.	Sucrose positive	*Candida albicans*
	Sucrose negative	*Candida stellatoidea*
3.	Pseudohyphae absent or sparse	4
	Pseudohyphae well developed	8
4.	Inositol positive (Cryptococcus)	5
	Inositol negative (Torulopsis)	17
5.	Potassium nitrate positive	6
	Potassium nitrate negative	7
6.	Maltose and sucrose positive	*Cryptococcus albidus*
	Maltose variable; sucrose negative	*Cryptococcus terreus*
7.	Maltose, sucrose, and dulcitol positive; lactose and melibiose negative	*Cryptococcus neoformans*
	Maltose, sucrose, and lactose positive; melibiose and dulcitol variable	*Cryptococcus laurentii*
	Maltose and sucrose positive; lactose, melibiose, and dulcitol negative	*Cryptococcus uniguttulatus*
8.	Arthrospores produced (Trichosporon)	9
	Arthrospores not produced (Candida)	10
9.	Potassium nitrate negative; lactose and melibiose positive	*Trichosporon cutaneum*
	Potassium nitrate positive; lactose positive; melibiose variable	*Trichosporon pullulans*
10.	Potassium nitrate positive	Candida I
	Potassium nitrate negative	11
11.	Lactose positive (Candida II) and fermented	*Candida pseudotropicalis*
	Lactose negative	12
12.	Raffinose positive (Candida III); melibiose positive	*Candida guilliermondii*
	Raffinose negative	13
13.	Trehalose positive	14
	Trehalose negative (Candida VI)	15
14.	Cellobiose positive (Candida IV); maltose fermented	*Candida tropicalis*
	Cellobiose negative (Candida V)	16
15.	Glucose positive; galactose, sucrose, maltose negative	*Candida krusei* complex
16.	Maltose fermented	*Candida tropicalis*
	Maltose fermentation negative	*Candida parapsilosis*
17.	Only glucose and trehalose fermented	*Torulopsis glabrata*

[1] Unless fermentation is stated, all positive reactions denote assimilation. Yeasts producing red pigments are omitted.

strong and rapid fermentation of a sugar is an important aid in diagnosis, but assimilation tests are of more fundamental value.

Most clinical yeast isolates belong to the genus Candida. This genus, like Torulopsis and Trichosporon, is both oxidative and fermenta-

tive. Fell and Meyer (unpublished observations) have divided the genus Candida into workable groups on the basis of oxidative assimilation reactions. These groups, and representative species for each, are listed below.

Group I: KNO_3 positive: *C. utilis, C. pelliculosa*

Group II: Lactose positive; nitrate negative: *C. tenuis, C. pseudotropicalis*

Group III: Trehalose and raffinose positive; nitrate, inositol, and lactose negative: *C. membranaefaciens, C. guilliermondii*

Group IV: Cellobiose and trehalose positive; nitrate, inositol, lactose, and raffinose negative: *C. tropicalis, C. viswanathii, C. zeylanoides*

Group V: Trehalose positive; nitrate, inositol, lactose, raffinose, and cellobiose negative: *C. tropicalis, C. parapsilosis, C. albicans*

Group VI: Nitrate, inositol, lactose, raffinose, and trehalose negative: *C. krusei, C. rugosa*

Once a species has been keyed to an individual group or groups (there is some overlapping among groups, *e.g.* isolates of *C. tropicalis* vary in the assimilation of cellobiose), a reduced spectrum of tests for each group permits species classification.

The physiological characterization of common clinical yeast isolates is presented in Table 1. A key for the identification of the more common isolates, based partially on the assimilation groupings for Candida, the germ tube test, and the morphology on corn meal agar is given below. The key presupposes that ascospores are not produced.

Table 1

Characteristics of Yeast Isolates

	Dextrose	Galactose	Lactose	Maltose	Sucrose	Melibiose	Cellobiose	Trehalose	Raffinose	Melizitose	Inositol [a]	Rhamnose [a]	Erythritol [a]	Xylose [a]	Urease	Hyphae	Germ tubes	KNO_3
Candida albicans	F	VF	—	F	+	—	—	VF	—	V	—	—	—	+	—	+	+	—
Candida stellatoidea	F	VF	—	F	—	—	—	V	—	—	—	—	—	+	—	+	+	—
Candida tropicalis	F	F	—	F	F	—	V	F	—	+	—	—	—	+	—	+	—	—
Candida parapsilosis	VF	+	—	+	VF	—	—	VF	—	+	—	—	—	+	—	+	—	—
Candida krusei	F	—	—	—	—	—	—	—	—	—	—	—	—	—	V	+	—	—
Candida pseudotropicalis	F	F	F	—	F	—	+	—	F	—	—	—	—	V	—	+	—	—
Candida guilliermondii	F	VF	—	+	F	VF	+	F	F	+	—	V	—	+	—	+	—	—
Rhodotorula rubra	+	+	—	+	+	—	+	+	+	+	—	—	—	+	+	R	—	—
Trichosporon cutaneum	+	V	+	V	V	+	V	V	V	V	V	V	V	+	+	+	—	—
Trichosporon pullulans	+	+	+	+	+	V	+	+	V	+	V	+	+	V	+	+	—	+
Cryptococcus laurentii	+	+	+	+	+	V	+	+	V	V	+	+	V	+	+	R	—	—
Cryptococcus neoformans	+	+	—	+	+	—	+	+	V	+	+	+	V	+	+	R	—	—
Cryptococcus terreus	+	V	—	V	—	—	+	+	—	V	+	V	—	+	+	R	—	+
Cryptococcus albidus	+	V	V	+	+	V	+	+	+	+	+	V	V	+	+	R	—	+
Saccharomyces cerevisiae	F	F	—	F	F	—	—	V	VF	—	—	—	—	—	—	RA	—	—
Torulopsis glabrata	F	—	—	—	—	—	—	F	—	—	—	—	—	—	—	—	—	—

[a] Tested for assimilation only.

F = fermentation and assimilation positive
VF = positive assimilation with variable fermentation
\+ = positive assimilation
V = strain variation in assimilation
R = occasional to rare hyphal elements
A = ascospores

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DIAGNOSTIC PROCEDURES FOR THE ISOLATION AND IDENTIFICATION OF THE ETIOLOGIC AGENTS OF ACTINOMYCOSIS

Lucille K. Georg

It has become recognized in recent years that several agents are responsible for the disease actinomycosis in man and animals. These microorganisms fall into two genera: Actinomyces and Arachnia. The members of these genera are facultative, microaerophilic, or anaerobic gram-positive organisms of bacterial size (one micron or less in diameter). They are highly variable in morphology, but they are usually either diphtheroidal or filamentous in form. Branching is characteristic, but not always easy to demonstrate.

According to recent classification schemes, the genera Actinomyces and Arachnia are classified with the bacteria in the phylum Schizomycophyta, the order Actinomycetales, and the family Actinomycetaceae. All members of the order Actinomycetales (including the Mycobacteriaceae) present an irregular morphology and occasionally produce a rudimentary type of mycelium. True aerial mycelium is found only in the Actinomycetaceae, Streptomycetaceae, and Actinoplanaceae. This mycelium differs from that seen in the Eumycetes, or true fungi, by being extremely thin (less than one micron in diameter), and in one family, Actinomycetaceae, it fragments readily into bacillary and coccoid forms.

Members of the order Actinomycetales exhibit characteristics other than morphology that substantiate their classification as bacteria. Biochemical studies of the cell walls of various members show that, unlike those of the Eumycetes, they do not contain chitin and cellulose. Instead, the cell walls, similar to those of other Schizomycetes, consist of sugars, glucosamines, and amino acids.

In learning to isolate and identify the agents of actinomycosis, it is essential to recognize that these organisms are true bacteria. The author has found that bacteriological techniques, now well standardized for the isolation and identification of the anaerobic bacteria, are directly applicable to the study of the agents of actinomycosis.

The disease actinomycosis is a chronic infection characterized by the development of a suppurative reaction and by the presence in the exudate of granules consisting of clusters of gram-positive, filamentous, branched organisms. The individual filaments are frequently encased in a hyalin material producing structures known as clubs. The laboratory diagnosis depends on the demonstration of such organisms in exudates and/or their isolation in culture.

Etiologic agents [1]

Actinomyces israelii (Kruse) Lachner-Sandoval, 1898

This is a normal inhabitant of the human

[1] Cited in the order of their importance in human actinomycosis.

mouth and throat, where it exists as a commensal. It is also the common and most important cause of the disease known as actinomycosis in humans (*4, 17, 21*). *A. israelii* apparently is not commonly found in animals. Only two isolates from bovine actinomycosis, "lumpy jaw," have been identified by biochemical and serological criteria as this species. Its pathogenicity can be readily demonstrated by experimentally inoculating pure cultures into animals.

Arachnia propionica (Buchanan and Pine) Pine and Georg, 1969

This organism, originally described as *Actinomyces propionicus,* has recently been placed in a new genus, Arachnia, because of important differences in cell wall composition and metabolic products (*19*). Recent reports indicate that *A. propionica* is an important agent of human actinomycosis. The organism was first described from a case of lacrimal canaliculitis by Buchanan and Pine in 1962 (*6*). In 1967, Gerencser and Slack (*13*) reported on three further isolations from human infections. In the past five years, the Mycology Section at the U.S. National Communicable Disease Center, Atlanta, has identified 11 additional isolates from human disease. These were from both localized and systemic infections. Of the systemic infections, at least three are known to have been fatal. Clubbed granules, composed of branched filamentous organisms, were observed in the suppurative exudates from a number of these cases. The clinical picture produced by *A. propionica* can be identical to that seen in actinomycosis due to *A. israelii*. Like *A. israelii, A. propionica* is probably a normal inhabitant of the human mouth and throat, since most infections appear to originate in this area; however, sufficient data are not yet available to determine this. The pathogenicity of *A. propionica* may be demonstrated by inoculating pure cultures into experimental animals. The resulting lesions are comparable to those produced by *A. israelii*.

Actinomyces naeslundii Thompson and Lovestedt, 1951

Until recently, this organism has been considered a saprophytic component of the normal flora of the human mouth. It was probably first observed in 1925 by Naeslund, who described a group of "facultative Actinomyces-like organisms" from human dental tartar. Thompson and Lovestedt described similar isolates from the oral cavity in 1951 and proposed the name *Actinomyces naeslundii* (*23*). Further characterization and a comparison to *A. israelii,* which it resembles, were made by Howell *et al.* in 1959 (*16*). All these workers considered *A. naeslundii* a harmless saprophyte commonly occurring in the oral cavity of man.

Recent studies by Coleman and co-workers, however, indicate that this organism has pathogenic potential. These authors described eight isolates from pathological clinical materials. One of these isolates had been obtained in pure culture from a case of empyema of the gall bladder and another from a case of lacrimal canaliculitis (*7*). The pathogenic potential of *A. naeslundii* was further supported by animal inoculation studies. In a study of the comparative pathogenicity of *A. naeslundii* and *A. israelii* by Coleman and Georg (*8*), *A. naeslundii* produced significant lesions in 89.7 per cent of the mice inoculated. In comparison, with the same methods, *A. israelii* produced infections in 95.8 per cent. In many instances, lesions produced by *A. naeslundii* were as severe as those produced by *A. israelii*.

Actinomyces odontolyticus Batty, 1958

This organism was described by Batty as a new Actinomyces species from the oral cavity in 1958 (*1*). Although it was morphologically and physiologically similar to other known members of the genus Actinomyces, it had greater tolerance to oxygen and produced colonies that developed a dark red color after several days' growth on blood agar. All of Batty's 200 isolates were either from scrapings of carious teeth or from saliva. Although the organism seemed

to be associated with dental caries, there was no evidence of its etiological relationship to this disease. Brown and Georg (*5*) reported recently on the isolation of this species from extra-oral sources: from blood in two cases and from various pathological lesions in eight other instances. The latter included an isolation in pure culture from a gangrenous appendix. The organism had little ability to produce lesions in mice when inoculated intraperitoneally, however. Its true pathogenic potential remains to be established.

Actinomyces bovis Harz, 1877

This organism is the common cause of actinomycosis in cattle. For many years *A. bovis* and *A. israelii* were believed to actually represent the same organism, and since the name *A. bovis* had priority, many workers used it for the etiologic agent of both animal and human actinomycosis. However, in 1940, Erikson (*9*) carried out a careful comparative study of bovine and human isolates in Scotland and presented evidence that they were not the same species. More recent studies (*23*) in the United States confirm Erikson's work. It is now accepted that they are indeed two distinct species. The name *A. bovis* is reserved for the species usually isolated from bovine infections and *A. israelii* for the species usually isolated from human infections. The two species are not host-specific, however. *A. israelli* has been isolated at least twice from bovine infections, although *A. bovis* is not known to have been isolated from human clinical material. The distinguishing characteristics of *A. bovis* have been defined by Pine *et al.* (*20*).

Actinomyces viscosus (Howell *et al.*) Georg, Pine, and Gerencser, 1969 (*11*)

This organism, which has been isolated from the oral cavities of both man and animals, is similar morphologically and biochemically to *A. naeslundii*. It differs chiefly in being catalase positive and more aerobic. Differences in cell wall constituents and antigenic factors have also been demonstrated. A review of the distinguishing characteristics of *A. viscosus* has been made by Gerencser and Slack (*14*). To date, no clear relationship to human or animal infections has been established. However, the fact that it is capable of producing suppurative lesions when inoculated into mice (*10*) leads us to believe that it should be considered a potential agent of human or animal disease. Also, because of similarities to the previously mentioned organisms, it should be considered in the differential identification of Actinomyces and Arachnia species.

Actinomyces eriksonii Georg *et al.*, 1965 (*12*)

The taxonomic position of this organism is presently in doubt. On the basis of the similarity of end products from glucose fermentation and of cell wall constituents, it could be considered a species of Bifidobacterium.

Collection of materials for examination and culture

The clinical material is usually pus, except in early thoracic cases, where sputum or pleural fluid may be available. In closed subcutaneous lesions, sterile syringes may be used to withdraw pus. If draining sinuses are present, pus may be collected in a sterile tube at the edge of the lesion or by washing or curetting the walls of the sinus tract. Often, if free-flowing pus with granules is not seen, a gauze pad applied over the sinus opening may trap pus and granules exuded over a period of time.

Steps in laboratory diagnosis

Direct examination of clinical material

Exudates: Sputum or suppurative exudates are poured into a Petri dish and searched for granules. These are white or yellowish grains up to 5 mm in diameter and generally firm in consistency. Although the formation of granules is characteristic of actinomycosis, they are not always present.

Fresh preparation of a granule: The granule is placed in a drop of water, KOH, or clear lactophenol and pressed out gently with a coverslip. It is then examined for delicate filaments and clubs. (Clubbing is not always present.)

Stained smears from clinical material: If granules are found, they are crushed and smeared. If no granules are found, smears are made of pus, sputum, centrifuged pleural fluid, and other such materials. The smear is then stained with Gram and acid-fast stains (acid-fast stain as for *Nocardia asteroides*) and examined under oil immersion for gram-positive, non-acid-fast filaments or pleomorphic, occasionally branched forms of bacillary size.

Isolation of the etiological agents of actinomycosis

If gram-positive, non-acid-fast diphtheroidal or filamentous branched forms are observed in stained smears, or if actinomycosis is suspected on the basis of clinical data, then cultures should be made.

Preparation of inoculum: When granules have been obtained, the chance of isolating an Actinomyces species is excellent. The granules are washed in several changes of sterile saline, then most of the saline is drained off and they are crushed with a loop or sterile glass rod to make a suspension before using it as inoculum. If granules are not present, as is usually the case with sputum sent for culture, small bits of secreted material or concretions are selected, washed of adherent saliva in sterile saline, and then used as inoculum.

Culture media and conditions of incubation: All Actinomyces and Arachnia species—with the exception of *A. viscosus,* which is facultative—grow best under conditions of reduced oxygen tension. The addition of CO_2 stimulates the growth of most isolates. The optimal temperature for incubation is 37° C. Methods for anaerobic incubation in the presence of CO_2 are given in the Appendix. All species require enriched media, such as those containing brain or heart infusions, tryptose, or casitone, for good growth. Some isolates are serophilic. (None of these organisms will grow on such simple media as nutrient broth, Sabouraud dextrose agar, or Czapek agar.)

Isolation broth: For primary isolation, an enriched thioglycollate broth, such as thioglycollate with TST (see Appendix), is recommended. This should be freshly boiled and cooled before use. The broth culture should be monitored daily for gram-positive organisms morphologically suggestive of Actinomyces or Arachnia species. If growth is sparse, the addition of 0.2 ml sterile rabbit serum per 10 ml of the broth medium may be helpful.

Purification of broth cultures: When adequate growth is obtained in broth culture, the isolation of pure cultures of Actinomyces species usually depends on the repeated streaking of solid media and the transfer of isolated colonies to fresh tubes of broth. Brain-heart infusion (BHI) agar plates, without blood, are usually most satisfactory. This medium should be freshly prepared and poured before use. Plates are incubated under anaerobic conditions (see Appendix). The use of inhibitors in broth medium may help to eliminate some of the contaminating organisms. Phenethyl alcohol (PEA) in a concentration of 0.25 per cent has been found useful in eliminating gram-negative contaminants. For the inhibition of gram-positive contaminants (anaerobic diphtheroids), the addition of 2μg/ml of tetracycline to broth media is helpful in some instances. It should be used concurrently with media without this antibiotic.

Examination of plate cultures: The BHI agar plates should be removed from jars after 24 or 48 hours' incubation. The plates are examined open under a stereoscope, with magnifying glass, or under the low-power objective (10×) of the microscope. Examples of colonies of each type observed (unless an obvious contaminant) should be selectively transferred to thioglycollate broth for further study. It is well to keep in

mind that although the microcolony of Actinomyces and Arachnia species is usually characterized as a filamentous "spider" type, smooth, entire colonies are occasionally produced by certain species.[2] If difficulty is encountered in obtaining a subculture from a microcolony, serum is added to the thioglycollate broth and the thioglycollate tube is incubated in an anaerobe jar, or under an anaerobe seal, to improve anaerobic conditions. Once pure cultures have been obtained, both microcolonies and macrocolonies (obtained by incubating plates for 7 to 10 days) should be studied in order to characterize the organism morphologically.

Identification of cultures

Differential morphological characteristics: *Actinomyces* and *Arachnia* species, like many other bacteria, have two basic growth forms that are usually described as rough or smooth (R or S). These descriptions reflect the degree of filamentation that the organism develops. A filamentous microcolony is referred to as a "spider form"; a rough macrocolony, as a "molar tooth" colony. Although the different species can vary from isolate to isolate, or even from one subculture of the same isolate to the next, each species has a degree of roughness or smoothness that is quite characteristic (Tables 1 and 2).

Differential physiological characteristics: Methods used in anaerobic bacteriology are applicable in most tests. Best results are obtained by using standardized techniques and adequate inocula of actively growing organisms. For poorly growing isolates, the addition of 0.1 to 0.2 ml sterile rabbit serum to the seed culture may be helpful. Serum may also be added to test media such as nitrate broth or fermentation broths to obtain adequate growth. Special methods and media applicable to this group of organisms are listed further below.

Determination of oxygen requirements: Measured inoculum (usually one small drop from

[2] *A. bovis, A. odontolyticus,* and occasional isolates of *A. israelii* and *A. naeslundii* form smooth colonies without filaments.

Table 1

Frequency of Actinomyces and Arachnia species exhibiting rough "R type" morphology

	Thioglycollate broth		BHIA colonies	
	(Gross)	(Microscopic)	24–48 hrs. (Microscopic)	7–10 days (Gross)
Species	Granular, broth clear	Filamentous, branched organisms	Filamentous, "spider" colony	Heaped, lobulated, "molar tooth" colony
Actinomyces bovis	Very rare	Very rare	Very rare	Very rare
Actinomyces israelii	Common	Common	Common	Common
Actinomyces naeslundii	Rare	Occasional	Common, with dense centers	Rare
Actinomyces odontolyticus	Very rare	Very rare	Very rare	Very rare
Actinomyces viscosus	Never	Rare	Occasional, with dense centers	Not observed
Arachnia propionica	Common	Common	Common	Occasional

Common = usual form for this species
Occasional = about 50% of the isolates have this character
Rare = unusual isolates have this character

Table 2

Frequency of Actinomyces and Arachnia species exhibiting smooth "S type" morphology

Species	Thioglycollate broth		BHIA colonies	
	(Gross)	(Microscopic)	24–48 hrs. (Microscopic)	7–10 days (Gross)
	Diffuse	Diphtheroidal, rarely filamentous	Flat, granular, entire	Smooth and convex
Actinomyces bovis	Common	Common	Common	Common
Actinomyces israelii	Rare	Rare	Very rare	Occasional
Actinomyces naeslundii	Common	Occasional	Rare	Common
Actinomyces odontolyticus	Common	Common; coccoid forms also	Common	Common
Actinomyces viscosus	Common, usually viscous	Common	Occasional	Common
Arachnia propionica	Rare	Rare, occasional spheroplasts	Rare	Occasional

Common = usual form for this species
Occasional = about 50% of the isolates have this character
Rare = unusual isolates have this character

the tip of a capillary pipette) from an actively growing broth culture is streaked with a single stroke from the base to the top of each of eight BHI agar slants. Pairs of inoculated slants are incubated at 37° C under the following conditions: aerobically (cotton plugs only), with a "CO_2 seal," and with an "anaerobic + CO_2" seal. (For the preparation of the "CO_2 seal," and "anaerobic + CO_2" seal, see Appendix.) Growth is recorded by gross comparison as 0 to 4+ after 7 to 14 days. The pair of slants showing heaviest growth is considered 4+. If replicate tubes do not give similar readings, the test is repeated (Table 3).

Catalase production: This is the most useful test for separating the important Actinomyces

Table 3

Production of catalase and relationship to O_2 in Actinomyces[a] and Arachnia species

Species	Catalase	O_2 requirements
Actinomyces viscosus	+	Facultative (good growth with or without CO_2)
Actinomyces naeslundii	0	Facultative (in the presence of CO_2)
Actinomyces bovis	0	Microaerophilic to anaerobic
Actinomyces israelii	0	
Actinomyces odontolyticus	0	
Arachnia propionica	0	

[a] The growth of all Actinomyces species is stimulated by CO_2.

species (with the exception of *A. viscosus*, which is catalase positive) from many gram-positive, catalase-positive bacteria. It is particularly useful in separating *Propionibacterium* (*Corynebacterium*) *acnes* from these organisms. The most reliable method is to use the growth on a BHI agar slant. If the culture has been grown under an anaerobe seal, the seal should be removed and the culture allowed to stand 30 minutes before testing. Then a 3 per cent H_2O_2 preparation is poured over the growth on the slant and a stream of small bubbles is watched for (Table 3).

In regard to the fermentation tests, a thioglycollate fermentation base medium is recommended for microaerophilic or anaerobic isolates, and a meat extract-peptone fermentation base medium for facultative or aerobic isolates (see Appendix).

The following additional biochemical tests and media are recommended:

- *Indole production and nitrate reduction*
  These reactions are demonstrated according to standard procedures after maximal growth is reached (usually two days) on an indole-nitrite medium (BBL).[3]

- *Milk reactions*
  Nonhomogenized whole milk with added iron filings is used to study milk reactions. Acidity is determined by adding 0.1 per cent bromcresol purple (aqueous) after 14 days' incubation.

- *Gelatin liquefaction*
  Thiogel (BBL) is used to demonstrate liquefaction of gelatin. Tests are read after 7, 14, and 21 days of incubation.

- *H_2S production*
  To detect H_2S production, lead acetate paper strips are inserted in the top of a streaked heart infusion (HI) agar slant and a streaked and stabbed triple sugar iron (TSI) agar slant. Slants are incubated in an anaerobe jar.

- *Esculin hydrolysis*
  Hydrolysis is detected in infusion broth containing 0.1 per cent agar and 0.1 per cent esculin. A few drops of 1.0 per cent aqueous ferric citrate are added to a seven-day-old culture. A brown-black color indicates that esculin has been hydrolyzed.

- *Urease production*
  This is demonstrated in the fermentation base medium with added urea broth (1 ml of Seitz-filtered urea broth (Difco) added to 8 ml autoclaved fermentation base). It should be read after 14 days' incubation for development of an alkaline reaction.

- *Starch hydrolysis*
  Starch plates (nutrient agar plus 0.5 per cent soluble starch) are inoculated in duplicate with a single streak of the culture across the center of the plate. One plate is incubated in a CO_2 jar and the other, anaerobically with CO_2. Tests for hydrolysis are done by flooding the plate with Gram's iodine after seven days' incubation at 37° C. The test is particularly useful for distinguishing *A. bovis*. This organism produces a wide zone of hydrolysis. Other Actinomyces species produce only a narrow zone, or no hydrolysis at all. As a standard procedure, all liquid media are boiled for 10 minutes and cooled rapidly before inoculation. The inoculum for all tests is taken from a 48-hour enriched thioglycollate broth culture. If good growth is not obtained in the test media, all media are supplemented with sterile rabbit serum (0.2 ml per tube).

Production of propionic acid: Determination of the ability to produce propionic acid as a

[3] Baltimore Biological Co., Baltimore, Md. Use of trade name is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health, Education, and Welfare.

metabolic end product of glucose fermentation is useful in the identification of *Arachnia propionica*. A practical method for determining this volatile acid in broth medium has been described by Li and Georg (*18*). A simple gas chromatograph apparatus useful for steroid determination may be employed.

Detection of DAP: The detection of DAP is useful in the identification of *Arachnia propionica*. A practical procedure in which whole-cell hydrolysates and simple paper chromatography are used may be carried out without special equipment according to the method of Boone and Pine (*3*). This consists of washing the cells obtained from broth cultures (about 1 ml of packed cells is required) and hydrolyzing 2 hrs in 2 to 6 ml of 6N hydrochloric acid. The soluble hydrolysate is evaporated to dryness and resuspended in a minimal amount of distilled water. Ten microliter aliquots are spotted on a No. 1 Whatman filter paper, and the chromatogram is migrated at 37° C in the ascending manner using the solvent described by Hoare and Work (*15*) (about three hours are required). After drying overnight, the paper is developed in a solution of ninhydrin in acetone and dried in a 100° C oven for two minutes. The development of a characteristic greenish grey spot indicates DAP. The physiological characteristics of Actinomyces and Arachnia species are listed in Tables 4 and 5, as are those of *Propionibacterium* (*Corynebacterium*) *acnes* and *Corynebacterium pyogenes*. These two organisms must be distinguished from morphologically similar Actinomyces species.

Use of fluorescent antibody (FA) techniques:

Table 4

Biochemical characteristics of Actinomyces, Arachnia, Corynebacterium, and Propionibacterium species

Biochemical Reactions[a]	Actinomyces bovis	Actinomyces israelii	Actinomyces naeslundii	Actinomyces odontolyticus	Actinomyces viscosus	Arachnia propionica	Coryne-bacterium pyogenes	Propioni-bacterium acnes
Catalase	O	O	O	O	+	O	O	+
Esculin hyd.	+	$+^{O}$	$+^{O}$	V	+	O^{+}	O	O
Gelatin hyd.	O	O	O	O	O	O^{+}	+	+
Indole	O	O	O	O	O	O	O	$+^{O}$
Milk	A^{OC}	A^{C}	A^{OC}	A^{OC}	A^{OC}	A^{OC}	P	A^{C}
Nitrate red.	O^{+}	V	$+^{O}$	+	+	+	O	$+^{O}$
Starch hyd.[b]	+	O^{+}	O^{+}	O	O^{+}	O^{+}		O
Urease	O	O	O	O	O	O	O	O
Reaction on blood agar HIBA[c]	NH	NH	NH	Red colonies after 3-5 days	NH	NH	Beta hemolysis	NH
Prod. of propionic acid[d]	O	O	O	O	O	+	O	+
Diamino-pimelic acid (DAP) in cell walls[e]	O	O	O	O	O	+	O	+

[a] Reactions read at 7 days.
Symbols: A = acid, a = weak acid, C = coagulated, D = digested, H = hemolytic, NH = non-hemolytic, P = peptonized, V = variable, superscripts = occasional reactions.

[b] Starch hydrolysis: + = wide zone of clearing; 0^{+} = negative, or a narrow zone of clearing (less than 10 mm D).

[c] HIBA = heart infusion agar + 5% rabbit blood

[d] Propionic acid may be determined by gas chromatography (*18*).

[e] DAP may be determined by paper chromatography (*3*).

Table 5

Fermentation of carbohydrates by Actinomyces, Arachnia, Corynebacterium, and Propionibacterium species

Reactions[a]	Actinomyces bovis	Actinomyces israelii	Actinomyces naeslundii	Actinomyces odontolyticus	Actinomyces viscosus	Arachnia propionica	Coryne-bacterium pyogenes	Propioni-bacterium acnes
Adonitol	O	O	O	O^A	O	A		V
Arabinose	O^A	V	O^A	V	O	O	O^a	O
Dulcitol	O	A^O	O	O	O	O		O
Glucose	A	A	A	A	A	A	A	A
Glycerol	O^A	O	V	A^O	A^O	O^A	a^O	A^O
Inositol	A^O	V	A^O	V	A^O	O^A	a^O	O
Inulin	O	O^A	V	O	A^O	O		O
Lactose	A	A^O	A^O	A^O	A^O	A	A	O
Maltose	A^O	A	A^O	A	A	A	A	O
Mannitol	O^A	V	O	O	O	A	O	V
Raffinose	O	V	A^O	V	A^O	A		O
Rhamnose	O^A	O^A	O^A	V	O	O		O
Salicin	O^A	V	V	A^O	A^O	O^A	O	O
Sorbitol	O	O^A	V	O	O	A		V
Starch	A	O^A	a^O	A	A	A^O	A	O
Sucrose	A^O	A	A^O	A^O	A	A	A	O
Trehalose	V	V	A^O	A^O	V	A		O
Xylose	V	A^O	O	V	O	O	A	O

[a] Reactions read at 7 days by removing aliquots and adding indicator. Late reactions read at 10 to 14 days in culture medium.

Symbols: A = acid (yellow with bromthymol blue), a = weak or late acid, O = negative, V = variable, superscripts = occasional reactions.

FA tests are currently used as an aid in the detection and identification of Actinomyces and Arachnia organisms in clinical materials and cultures (*2, 17*). Reagent FA conjugates are not yet available.

Animal inoculation: These inoculations are not routinely done as an aid in the identification of Actinomyces or Arachnia species. However, pathogenicity may be demonstrated in mice.

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APPENDIX

Thioglycollate with TST [1]

Thioglycollate	29.5 g (BBL [2] with indicator & dextrose)
Trypticase soy broth (BBL)	1.5 g
Tryptose broth (Difco) [3]	1.25 g
Distilled water	1,000.0 ml

Dispense in 10 ml amounts in screw-capped tubes. Autoclave 15 lbs. for 15 minutes. Boil and cool just before using.

[1] Some isolates are serophilic and require the addition of serum to produce good growth. In this case, add 0.2 ml sterile rabbit serum per 10 ml to either of these broth media.

[2] Baltimore Biological Laboratories, Baltimore, Md.

[3] Difco Laboratories, Detroit, Mich.

Actinomyces maintenance broth (AM broth)[1]

Available as "actinomyces broth" from BBL. Dispense in 10 ml amounts in screw-capped tubes. Autoclave 15 lbs. for 15 minutes. Boil and cool before using, and after inoculation, seal with "anaerobic + CO_2 seal."

Thioglycollate fermentation media for actinomyces

Use for microaerophilic or anaerobic species.

Fermentation base: thioglycollate without dextrose or indicator (BBL #01–397 or Difco B-432 are satisfactory).

For each 100 ml of medium add:

Yeast extract (without dextrose—Difco #B127 is satisfactory) 2 g bromthymol blue (1 per cent aqueous)

Dispense 8 ml in 15 × 125 mm tubes with fermentation vials. Autoclave 15 lbs. for 15 minutes. Add aseptically to each 8 ml autoclaved fermentation base 0.5 ml Seitz-filtered 10 per cent aqueous solutions of the following sugars: glucose, mannitol, lactose, sucrose, maltose, salicin, glycerol, soluble starch, xylose, arabinose, and inositol.

Meat extract-peptone fermentation base

Use for facultative or aerobic Actinomyces and related genera, *Actinomyces viscosus*, and *Rothia dentocariosa*.

Meat extract (Difco beef)	3.0 g
Bacto peptone	10.0 g
Na Cl	5.0 g
Distilled water	1,000.0 ml
Andrade's indicator	10.0 ml

Adjust to pH 7.4. Add 4.5 ml to 15 × 125 mm cotton-plugged tubes. Do not use inserts. Autoclave 15 lbs. for 15 minutes. Add aseptically 0.5 ml Seitz-filtered sugars to each tube to give a final 1 per cent concentration of lactose, maltose, xylose, glucose, sucrose, mannitol, and soluble starch, and a final 0.5 per cent concentration of salicin, glycerol, arabinose, and inositol.

Methods for cultivation under microaerophilic and anaerobic conditions

Tubed media:

(1) *Tube cultures with anaerobic seals ("anaerobic + CO_2 seal")*

Anaerobic conditions may be obtained in test tube cultures by using pyrogallol-carbonate seals. Prepare as follows: Clip off that portion of the non-adsorbent cotton plug that extends above the end of the tube. By a rotary motion, push in the remainder of the plug, leaving a space of about ½" to ¾" at the top of the tube. Insert a small wad of absorbent cotton into this space. Add 5 drops each of pyrogallol solution [4] and 10 per cent Na_2CO_3 solution. Plug with rubber stopper.

(2) *Tube cultures under microaerophilic conditions ("CO_2 seal")*

Use same procedure as above, except in place of adding pyrogallol and Na_2CO_3, add 5 drops each of KH_2PO_4[5] and 10 per cent Na_2CO_3.

Plate cultures:

(1) *Anaerobic conditions*

Anaerobe jar without catalyst (such as a Thomas or Case jar). Use a mixture of 95 per cent N_2 and 5 per cent CO_2.

Anaerobe jars with catalyst: Use Brewer jar, electrically heated 10 minutes to reactivate catalyst, with a mixture of 80 per cent N_2, 10 per cent H_2, and 10 per cent CO_2; Torval jar (room temperature catalyst) with the same mixture as for Brewer jar; or Gaspac anaerobe jar (BBL 06–200) with disposable H_2-CO_2 generator envelopes (BBK 06–112)—by far the simplest and most practical method currently available.

(2) *Microaerophilic conditions*

Candle jars are most satisfactory for incubation of plates under microaerophilic conditions. The burning of the candle supplies a small amount of CO_2.

[4] Pyrogallol solution: 100 g pyrogallol (pyrogallic acid) + 150 ml H_2O.

[5] KH_2PO_4 (1M or 136 g per 1,000 ml H_2O).

DISCUSSION

Chairman Restrepo: Discussion of the papers presented thus far is now open.

Dr. Carbonell: The medium discussed by Mr. Taplin seems to be very good for epidemiological surveys. Is it available commercially?

Mr. Taplin: Yes, it is. It may be obtained from Pfizer Diagnostics Division, 300 West 43rd Street, New York, and from the Colab Laboratories, 3 Science Road, Glenwood, Illinois.

I should like to add that gentamicin is a remarkably good antibiotic in mycology. It is water soluble, highly stable, and apparently quite innocuous to yeast and fungi up to 100 μg/ml. This comes from the Schering Corporation in Bloomfield, New Jersey. I believe that if there is enough demand for it, they will produce it for nonhuman uses, so we might all do ourselves a favor by requesting such material from the Department of Microbiology at the Schering Corporation. We think it has a great deal of use in mycology, not just for dermatophytes, but for deep fungi and yeasts as well.

Dr. Kaplan: Another question for Mr. Taplin. Do the nonpathogenic Trichophytons, e.g. *T. terrestre* and other nonpathogenic keratinophilic fungi, react positively on your DTM medium?

Mr. Taplin: Yes, they do. Under the term dermatophytes we include such things as Chrysosorium species, whether or not they are pathogenic to man.

Dr. Mariat: I should like to stress our great interest in the paper presented by Mr. Taplin. The proposed medium will be very useful for epidemiologic studies. This brings to mind the technique we proposed (*Ann Inst Pasteur,* 1967) to take samples from large areas of scalp and skin and also from other surfaces, such as the skin of wild animals, walls, etc. It is a modification of existing methods, including that of MacKenzie. A 5 x 5 cm piece of autoclaved carpet is kept in a paper bag. To take the sample, one has only to brush strongly against the area of interest. It is possible to inoculate plates of Sabouraud's medium containing antibiotics immediately after the sample is taken, or, on the other hand, to wait days or weeks before inoculating the plates. This technique showed the presence of dermatophytes (*T. soudanense*) on the scalp in 15 per cent of the apparently tinea-free African Negroes living in Paris. We are already using this method in our department at the Pasteur Institute for routine studies and also for epidemiologic and etiologic investigations. We have now to use Mr. Taplin's medium for even better results.

Mr. Taplin: We employed the touch plate technique in tinea capitis using plates of DTM. It is excellent. We prefer it over plucking hairs. You can, for example, walk through a school cafeteria while the children are having lunch, press a plate on everyone's scalp, and obtain massive cultures. I am sure, Dr. Mariat, that you will find the method will work very well.

Chairman Restrepo: This method is also good for rubbing inanimate surfaces, and it helps to isolate dermatophytes from swimming pools or showers. It is very easy to use, and one can recover large quantities of organisms.

Dr. Negroni: Did Mr. Taplin get the same number of positive results with microscopic examination as with his own culture medium?

Mr. Taplin: We have compared this medium with currently available media such as Mycosel. We have always had an increased recovery on our own medium. As far as correlation with KOH direct microscopic results is concerned, this was very good in Vietnam. In our clinic at Miami we do not recover as many dermatophytes as we see positive microscopics. When we split up the data, however, we find that many of the ones that did not produce dermatophytes yielded *Candida albicans,* so we think that some of the people examining the preparations are seeing the hyphae of Candidas and are calling them positive for fungi. I am sure we lose some, but it is always

difficult to know about the cultures you do not isolate.

Dr. Mayorga: It is a well-known fact that in cases of nail fungus infection about 50 per cent of them give negative cultures when Mycosel is used. I should like to ask Mr. Taplin if his medium would recover some of the pathogens that we lose by using the regular media.

Mr. Taplin: I would like to pass this question on to my colleague, Dr. Nardo Zaias, who is one of the world's experts in the isolation of pathogens from nails.

Dr. Zaias: I think your recovery rate from nails is about correct. I have just published a report on cultures of 100 abnormal toenails from a random hospital population. I ground these nails carefully and plated them out in various media such as Sabouraud's yeast with antibiotics in an attempt to recover all yeasts and fungi. The results were somewhat surprising: out of 100 nails, I only recovered dermatophytes from 25 per cent; from 50 per cent I recovered fungi and yeasts other than dermatophytes; and from the remaining 25 per cent I did not recover any fungi or yeasts. So far as toenails are concerned, there are many more organisms that may be responsible for or associated with abnormal nails than dermatophytes.

Dr. MacKenzie: I have four questions for Dr. Ahearn. First, in identifying yeasts in general—for example, *Candida albicans*—what is your opinion of the use of differential media?

Second, what about chlamydospore production?

Third, with regard to *Cryptococcus neoformans,* is it necessary to inoculate animals to obtain definitive diagnosis?

And finally, how many species of yeasts do you think should be included in the repertoire of a hospital diagnostic laboratory?

Dr. Ahearn: Most screening media, such as corn meal with Tween 80, will probably permit identification of 95 per cent or more of the primary isolates of *C. albicans,* which is a relatively easy species to identify. Some strains, however, may fail to produce chlamydospores, and other strains may even fail to produce pseudomycelium. The germ tube test permits identification of over 95 per cent of the isolates of *C. albicans* directly from the isolation media; nearly 100 per cent accuracy may be obtained, depending on how much care is taken in conducting the test. With the procedure employing controlled inocula, we are obtaining this degree of accuracy.

Accuracy above 95 per cent may also be achieved with crude and nonsterile methods. For example, the colonies may be picked with a straw or Pasteur pipette directly from the isolation medium, and nonsterile serum or tissue culture medium 199 may be used. This is why I think the germ tube test is an excellent diagnostic tool.

Cryptococcus neoformans may be identified on the basis of the sugar assimilation tests.

As to how many yeasts should be included in the routine clinical laboratory's spectrum, I would suggest approximately 15 species. These would include as the principal ones *Candida parapsilosis, Candida tropicalis, Cryptococcus albidus, Rhodotorula rubra,* and *Torulopsis glabrata.* Many of these have been recently recognized as adventitious, mainly when associated with the predisposing factors discussed this morning.

Dr. Huppert: I would like to ask Dr. Georg what happened to the *Actinomyces eriksonii* strains.

Dr. Georg: The classification of *A. eriksonii* is presently in question. It will most likely be removed to another genus, probably Bifidobacterium.

Dr. Borelli: In regard to Dr. Lash's presentation, I should point out that often in Latin American laboratories we do not have all the selective media at our disposal, so that very frequently we resort to animal inoculations in order to obtain isolates. For example, *Nocardia brasiliensis* from open lesions, *Paracoccidioides brasiliensis* from ulcers of the mouth, and severely contaminated materials sometimes must be inoculated into mice, preferably small mice

weighing about 10 g. When we think there is a lot of bacterial infection, we do it peritoneally, or else intradermally, in order to obtain strains of *Nocardia brasiliensis*. Otherwise, we cannot isolate it at all.

With *Sporothrix schenckii*, it is sufficient to inoculate the tail or foot of a domestic rat using a needle contaminated with material from the suspect lesion in order to obtain an infection from which to isolate the strain.

Turning to the paper of Dr. Ahearn, I agree that we need the constant cooperation of zymologists. However, I would like to stress the need for the diagnosis of Candidas in the original culture, sown directly with clinical material that will produce chlamydospores in 24 to 48 hours in a number of strains. Of course, this does not preclude the usefulness of taxonomic study of all the human yeast strains that are being isolated.

Dr. Drouhet: We have also been interested in the rapid identification of yeasts and have tried several methods and techniques. We studied the following approaches at the same time: (1) filamentation on a potato-carrot-bile agar medium, which gives filaments for the Candida group and chlamydospores for *C. albicans* within 24 hours; (2) sensitivity to actidione on Sabouraud actidione (0.5 g ‰) agar to separate sensitive species (*C. tropicalis*) from nonsensitive strains (*C. albicans*); (3) reduction of tetrazolium to separate species not reducing this salt (*C. albicans*) from species that reduce it strongly (*C. tropicalis*), giving red-violet colonies; (4) assimilation of sugars; (5) fermentation of sugar in tubes containing soft agar 6 g ‰ with peptone (10 g ‰) and a pH indicator, to which the sugar solutions are added. The response on acidification and gas formation is obtained in 24 hours.

With all these methods we can obtain a diagnosis within 24 to 48 hours of seven or eight principal species of Candida that can be pathogenic (Table 1).

In the case of *Cryptococcus neoformans*, growth at 37°, the positive urease test, the presence of a capsule, and the absence of assimilation of lactose are the most important criteria. The pathogenic power for mice is an additional criterion.

Dr. Ahearn: The definitive identification of yeasts is now dependent on an expanded spec-

Table 1

Rapid identification of principal Candida species

	Response in four hours	Response in twenty-four hours													
		Media			Sugar assimilation						Fermentation				
	Serum germ tubes [a]	PCB agar medium [b]	Sabouraud + 0.5‰ actidione [c]	Sabouraud + 0,1‰ tetrazolium	Glucose	Maltose	Lactose	Sucrose	Galactose	Raffinose	Glucose	Sucrose	Maltose	Lactose	Raffinose
C. albicans	+	+	+	white	+	+	0	+	+	0	AG	A	AG	0	0
C. stellatoidea	±	0	+	rose	+	+	0	0	+	0	AG	A	AG	0	0
C. tropicalis	0	0	0	red-violet	+	+	0	+	+	0	AG	AG	AG	0	0
C. pseudotropicalis	0	0	+	rose	+	0	+	+	+	+	AG	AG	0	AG	AG
C. guilliermondii	0	0	+	red	+	+	0	+	+	+	AG	AG	0	0	AG
C. krusei	0	0	0	white	+	0	0	0	0	0	AG	0	0	0	0
C. parapsilosis	0	0	0	rose-red	+	+	0	+	+	0	AG	0	0	0	0
C. zeylanoides	0	0	+	white	+	0	0	0	0	0	0	0	0	0	0

[a] + = filamentation at 37°
[b] + = appearance of chlamydospores
[c] + = growth

A = acid
G = gas

trum of assimilation tests. There has been no up-to-date monograph available for general laboratory use. I believe that in June of this year a new edition of *The Yeasts,* edited by Lodder, will be published. This should simplify a number of the present difficulties. Still, the identification of yeasts will be quite complex, and there is no simple scheme or technique that works for all of them.

Dr. Seabury: Going back a number of years to the simpler time of the identification of yeasts, I would warn all of you who have to do your own work and do not have a battery of scientists available for this purpose that doing fermentation and assimilation tests—particularly fermentation tests—for the separation of the yeasts just by the method of Wickerham is not simple. Unless you are well trained technically and can do this in triplicate and forgive a few errors, you will find that it is much, much simpler to send isolates of this type off to a regional identification laboratory, if any is available.

I would also like to add my comment and commendation to Dr. Borelli, since in the past my colleagues and I have had a few words to say about the use of tissue culture for conversion and identification of organisms, particularly the dimorphic fungi, when it is so simple and so inexpensive to do primary inoculation into cheap laboratory animals. We are fortunate here in being able to have cesarean-born, barrier-raised mice such as the Charles River strain, which are extremely susceptible to inoculation. Whether you use these or other animals, and particularly when you are dealing with contaminated specimens, I think it is much cheaper, much faster, and on the whole it gives you a higher yield. It may not be so fancy—and it does have hazards to the animal caretaker.

Chairman Restrepo: If there are no other comments, I would like to remark on Dr. Larsh's presentation. The yeast extract medium, when used for the primary isolation of *P. brasiliensis,* is far superior to the regular mycological media. In our laboratory we have increased the percentage of isolation by 30 per cent with the use of this medium.

THE FLUORESCENT ANTIBODY TECHNIQUE IN THE DIAGNOSIS OF MYCOTIC DISEASES

William Kaplan

The value of the fluorescent antibody (FA) technique as a diagnostic and research tool in microbiology has been amply documented. It can be used for the rapid visualization and identification of microorganisms, both viable and nonviable, in culture as well as in clinical and environmental specimens. In modified form, immunofluorescence can also be used to detect and measure antibodies in sera and other types of clinical materials.

Attracted by the potentialities of this versatile technique, a number of workers have investigated the possibility of using the FA procedure in medical mycology. For the most part, these investigators have stressed practical diagnostic applications. Their studies have dealt with the development of sensitive and specific reagents and effective methods for demonstrating mycotic disease agents in culture and in clinical materials. Some workers have also used immunofluorescence to detect and measure fungal antibodies in sera. By now, the FA technique has been applied to nearly all the important mycoses. A review of what has been accomplished shows that immunofluorescence has been developed to a practical level for the diagnosis of a number of mycotic diseases. However, for other mycoses much additional work remains to be done.

The present paper is not meant to be a comprehensive review of all that has been published on the applications of immunofluorescence in medical mycology; instead, it covers those diagnostic applications considered to be of greatest value at this time. These applications concern the diagnosis of the following diseases: blastomycosis, candidiasis, coccidioidomycosis, cryptococcosis, histoplasmosis, paracoccidioidomycosis, sporotrichosis, and actinomycosis.

Blastomycosis

The FA technique has been developed to a practical level for the diagnosis of blastomycosis. A reagent specific for the detection and identification of the yeast form of *Blastomyces dermatitidis* has been developed, and its efficacy for diagnostic use appears to have been confirmed.

Gordon (*7*) was the first worker to use the FA procedure to stain the yeast form of *B. dermatitidis*. In a preliminary communication, he reported that fluorescein isocyanate-labeled globulins obtained from rabbits experimentally infected with *B. dermatitidis* brightly stained the yeast form of this fungus. Although this reagent also cross-reacted with cells of *Candida albicans*, it showed only slight cross-staining or none at all with cells of *Paracoccidioides brasiliensis* and *Histoplasma capsulatum*. These preliminary findings pointed to the possibility of preparing an FA reagent specific for the yeast form of *B. dermatitidis*, and such a product was subsequently developed by Kaplan and Kaufman (*15*). Conjugates were prepared from antiglobulins of rabbits that had been immu-

nized with formalinized *B. dermatitidis* yeast-form cells. In addition to staining the yeast and mycelial forms of *B. dermatitidis*, the labeled antiglobulins also cross-stained the yeast and mycelial forms of *H. capsulatum*, *P. brasiliensis*, and cells of numerous other heterologous fungi. These reagents could be rendered specific for the yeast form of *B. dematitidis* by adsorptions with yeast-form cells of *H. capsulatum* and *Geotrichum candidum*. The resulting products were effective for the detection and identification of the tissue form of *B. dermatitidis* both in culture and in clinical materials, including formalin-fixed tissue sections (*16*), but they did not react with the mycelial form of *B. dermatitidis*. Thus, FA still cannot be used to identify the mycelial form of this fungus.

Candidiasis

A number of workers have explored the possibility of using immunofluorescence for the detection and identification of *Candida albicans* and other Candida species. In all cases, the FA reagents that have been developed stained the homologous organisms brightly in culture and in clinical materials. However, these products cross-reacted with other members of the genus and also with other heterologous fungi. All attempts to produce species-specific conjugates for the identification of the Candidas have failed. As yet, no single specific reagent is available for diagnostic use. However, some workers have successfully used a combination of FA reagents to identify some of the Candida species. Gordon, Elliott, and Hawkins (*9*) reported that the specific identification of *Candida albicans* types A and B, *C. tropicalis*, *C. stellatoidea*, and *Torulopsis glabrata* could be accomplished by using a pair of fluorescein-labeled antiglobulins directed against *C. albicans* type A and against *T. glabrata*. The former conjugate, when adsorbed with cells of *C. stellatoidea*, differentiated *C. albicans* type A–*C. tropicalis* and *C. albicans* type B–*C. stellatoides* from all other yeast-like organisms tested. The latter conjugate, when adsorbed with *C. albicans* type A cells, separated the two pairs of organisms into their respective components and permitted the identification of *T. glabrata*. The results obtained with immunofluorescence using these adsorbed reagents agreed with results from conventional identification methods in 95 per cent of 585 yeast-like fungus specimens.

Although species-specific fluorescent antibodies for the Candidas have not been produced, Candida FA reagents are of great value in experimental studies and in screening clinical materials where the mere detection of organisms is required.

Several investigators have reported that the indirect FA technique can be used to detect and measure antibodies to *C. albicans* in sera. Lehner (*27*) examined sera from 96 patients with clinical candidiasis, 99 individuals classified as Candida carriers, and 80 noncarrier control subjects. He reported that sera from control subjects commonly had fluorescent antibody titers of up to 1:8, whereas sera from carriers had titers of up to 1:16. In contrast, with few exceptions, sera from patients with clinical candidiasis had titers greater than 1:16. On the basis of these findings, he concluded that the indirect FA technique can be used as an aid in the serological diagnosis of candidiasis. In an independent study, Esterly (*3*) essentially confirmed the work of Lehner. She examined sera from 47 patients with clinical candidiasis and 122 normal individuals and Candida carriers. The results showed that 96 per cent of the 122 "normals" and carriers had serum titers of 1:64 or less, whereas 26 of the 47 patients with clinical candidiasis had titers greater than 1:64. It is noteworthy that two thirds of the patients with candidiasis who were older than six months of age had titers of 1:128 or greater. The conclusion was that the indirect FA test is a simple, rapid, and reproducible method for detecting serum antibodies to *C. albicans* and that it can be used to advantage as a diagnostic tool. Esterly attributed the differences in mean titers in the two studies to the interpretation of the staining endpoint.

Coccidioidomycosis

The FA technique has been developed to a practical level for the detection and identification of the tissue form of *Coccidioides immitis* and also for the detection of antibodies to this organism in serum. Kaplan and Clifford (*11*) were the first to prepare specific conjugates for the identification of the tissue form of *C. immitis*. They found that antiglobulins produced by rabbits infected with *C. immitis* and by rabbits immunized with formalin-killed arthrospores could be used for this purpose. Conjugates prepared from these antiglobulins stained endospores and spherule contents. However, they lacked specificity and cross-reacted with *H. capsulatum*, *B. dermatitidis*, and other heterologous fungi. Specificity for the tissue form of *C. immitis* was achieved by adsorption of these reagents with yeast-form cells of *H. capsulatum*. The preparation from the antiglobulins of infected rabbits could also be rendered specific by dilution. A study was carried out to evaluate the specific conjugates for diagnostic use. Using the reagents, it was possible to stain the walls of endospores and the contents of spherules in smears of lungs from mice that had been inoculated intraperitoneally with suspensions of 42 different isolates of *C. immitis* and in smears of clinical materials from 16 of 21 culturally confirmed cases of coccidioidomycosis in humans. These conjugates also stained *C. immitis* in sections of formalin-fixed tissues.

With the collaboration of Dr. Milton Huppert, the studies mentioned in the paragraph above were extended in order to develop an FA inhibition test for the detection of antibodies to *C. immitis* in sera (*13*). The specific conjugates produced by Kaplan and Clifford were used in this investigation. A total of 106 sera were examined using the FA inhibition procedure, and the results were compared with those obtained by the complement fixation (CF) test. Of the 106 sera tested, 91 had been obtained from confirmed coccidioidomycosis cases and 15 from individuals affected with other diseases. All these patients had resided in a coccidioidomycosis endemic area for varying periods of time. Of the 91 coccidioidomycosis case sera, 76 were positive by both the FA inhibition and the CF tests, and 5 were negative by the two methods. Of the 15 sera obtained from patients with other diseases, 9 were negative and 4 were positive by the two tests. These data show good over-all agreement between the FA and CF tests in this series. The efficacy of the FA inhibition test for the detection of tube precipitin (TP) positive sera was also studied. These early coccidioidomycosis case sera were negative by the CF test. Of 11 TP positive sera examined, 10 were positive by the FA inhibition procedure. To obtain information on the specificity of the FA inhibition test, sera from cases of blastomycosis, cryptococcosis, and histoplasmosis were tested. Nearly half of the histoplasmosis sera were positive, whereas the blastomycosis and cryptococcosis sera were negative. The high degree of correlation between the results from both the CF and TP tests and the FA inhibition procedure indicates that the latter technique can be used for the rapid detection of antibodies to *C. immitis* in sera. The FA inhibition test should not be used, however, in lieu of the routinely employed conventional serologic procedures. Instead, it should be regarded as a supplementary diagnostic tool for the testing of anticomplementary sera and for the detection of antibodies in sera when rapid results are required.

No FA reagent has been developed for the reliable identification of the mycelial form of *C. immitis*. The conjugate specific for the tissue form of this fungus does not react with elements of the mycelial form. However, the specific conjugate can be used to some advantage indirectly in identifying cultures of the mycelial form. The identification of *C. immitis* in culture necessitates the use of animal pathogenicity tests to demonstrate conversion to the tissue form. The specific FA reagent can be used to detect and identify endospores and spherules of *C. immitis* in tissues of inoculated animals when conventional methods are unsuccessful.

Cryptococcosis

The FA technique has many practical applications in the diagnosis of cryptococcosis. It can be used to detect and identify *Cryptococcus neoformans* in culture and clinical materials. It can also be employed to demonstrate cryptococcal antibodies in sera and other body fluids.

Among the early reports on the use of immunofluorescence in medical mycology is the one by Eveland and his associates (*5*). They successfully used unadsorbed fluorescein-tagged *C. neoformans* antiglobulins to study the distribution of *C. neoformans* and its polysaccharide breakdown products in formalin-fixed tissues. Subsequently, Marshall and his co-workers (*29*) stained sections of tissue from human cases of cryptococcosis with fluorescent antibodies and compared the results with those obtained using Mayer's mucicarmine technique. They reported that their conjugate, although nonspecific for *C. neoformans,* stained the organism more intensely and rapidly than mucicarmine. In 1960, Kase and Marshall (*17*) used the FA technique to identify *C. neoformans* in culture. Of the 92 isolates of this fungus tested, 91 were stained with labeled rabbit antiglobulins prepared against strains of *C. neoformans* identified as Evans type A and Neill type 5. The isolate that did not react belonged to Evans *C. neoformans* serotype C (*4*). Kase and Marshall stained this isolate with conjugates prepared from antiglobulins against Evans *C. neoformans* serotypes B or C. They also reported that their labeled antiglobulins did not cross-react with 23 different species of heterologous yeasts belonging to genera other than Cryptococcus. However, their reagents did stain four isolates of *C. neoformans* var. *innocuous,* which many workers consider to be a distinct species, *C. diffluens.* Kase and Marshall did not attempt to stain any of the other members of the genus Cryptococcus.

Kaufman and Blumer (*18*) investigated the possibility of producing specific FA reagents for the identification of *C. neoformans.* Their labeled *C. neoformans* antiglobulins brightly stained 27 different isolates of *C. neoformans.* These, however, cross-reacted with other Cryptococcus species and various members of the genus Candida. They found that consecutive adsorptions with yeast cells of *C. neoformans* var. *uniguttalatus, Candida albicans,* and *C. curvata* rendered the conjugate specific for *C. neoformans.* However, the adsorbed reagent failed to stain a number of strains of *C. neoformans*—a fact that limits its diagnostic usefulness. This failure to stain led Pidcoe and Kaufman (*30*) to attempt to develop a specific FA reagent of greater sensitivity. They prepared such a conjugate by adsorption of fluorescein-labeled *C. neoformans* antiglobulins with cells of *C. diffluens* and *C. krusei.* It did not cross-stain 30 heterologous fungi belonging to the genera Blastomyces, Candida, Coccidioides, Cryptococcus, Histoplasma, Rhodotorula, and Torula. In tests with 34 isolates of *C. neoformans,* all but one stained brightly with this reagent. Furthermore, the adsorbed conjugate was successfully used to identify *C. neoformans* in smears of lesion exudates and in tissue sections from infected individuals. Pidcoe and Kaufman concluded that their reagent can be effectively used for the accurate and rapid diagnosis of cryptococcosis.

Immunofluorescence has an important application in the serological diagnosis of cryptococcosis. The indirect fluorescent antibody (IFA) technique is currently used for this purpose. Vogel and his associates (*37, 38*) first showed that the IFA test could be used to detect cryptococcal antibodies in sera. Vogel (*36*) continued these studies and recently reported that sera from 80 per cent of patients with cryptococcosis were positive by the IFA test. Low-level cross-reactions were obtained with sera from some individuals who did not have this disease. However, no cross-reactions were noted with sera from patients with blastomycosis, histoplasmosis, or sporotrichosis.

Recently, Kaufman and Blumer (*20*) evaluated the IFA test along with other tests for the serological diagnosis of cryptococcosis. They reported that for maximal diagnostic coverage

three serological tests should be used concurrently: the latex agglutination test, for detection of cryptococcal antigens, and the tube agglutination and the IFA tests, for *C. neoformans* antibodies. In their hands, the IFA test permitted the detection of cryptococcal antibodies in 38 per cent of patients with cryptococcosis. Some cross-reactions were obtained with sera from patients with blastomycosis and histoplasmosis. Although the IFA test was not entirely specific, they considered it a valuable tool for the serological diagnosis of cryptococcosis, particularly when specimens are negative by the latex and tube agglutination tests.

Histoplasmosis

Immunofluorescence can be used to great advantage in the diagnosis of histoplasmosis. It can be employed for the detection and identification of the tissue form of *H. capsulatum* in culture and in clinical materials. In addition, it can be used to demonstrate *H. capsulatum* antibodies in serum.

The earliest report on the application of the FA technique to histoplasmosis is by Gordon (*8*). He successfully stained yeast-form cells of *H. capsulatum* with fluorescein-labeled globulins obtained from rabbits infected with *H. capsulatum*. This conjugate, however, cross-stained *B. dermatitidis* isolates and other heterologous fungi. Attempts to eliminate the cross-reactions by dilution of the reagent were only partially successful.

Following Gordon's report, Kaufman and Kaplan (*23*) initiated studies to develop an FA reagent specific for the tissue form of *H. capsulatum*. They produced such a conjugate by adsorbing fluorescein-labeled *H. capsulatum* antiglobulins with yeast-form cells of *B. dermatitidis*. The specific conjugate was used to identify tissue-form cells of *H. capsulatum* in culture and in impression smears. In an extensive investigation, Porter, Thomas, Furcolow, and Varga (*33*) examined 800 clinical specimens with a conjugate produced in accordance with the procedures of Kaufman and Kaplan and with a reagent produced by multiple adsorption with tissue and *Candida sp.* powders. They reported the former reagent to be effective, easy to prepare, and more specific than the latter.

In a further evaluation of the specific conjugate, however, a number of isolates of *H. capsulatum* were encountered that failed to stain or stained weakly (*31, 19*). These isolates were shown to belong to one of the five recognized serotypes of *H. capsulatum*, designated type 1:4.

The failure of the specific conjugate developed by Kaufman and Kaplan to react with the 1:4 serotype limited its diagnostic usefulness. Therefore, Kaufman and Blumer (*21*) carried out a study to produce an FA reagent that would specifically demonstrate *H. capsulatum* regardless of its antigenic makeup. Their approach was to prepare labeled antiglobulins against the most complete serotype of *H. capsulatum*. Adsorption of this conjugate with cells of *C. albicans* eliminated all cross-reactivity except that with *B. dermatitidis* and *H. duboisii*. However, the *C. albicans*-adsorbed reagent brightly stained all known serotypes of *H. capsulatum*. Attempts to eliminate the residual cross-staining by adsorption with either *B. dermatitidis* or *H. duboisii* resulted in the conjugate's loss of staining reactivity for both the homologous and heterologous organisms. Despite the presence of cross-reacting antibodies, the *C. albicans*-adsorbed conjugate can be used for diagnostic purposes. Usually *H. capsulatum* can be differentiated from *B. dermatitidis* on the basis of morphology. When required, these two organisms can be differentiated with FA by using a conjugate specific for the yeast form of *B. dermatitidis*. This latter reagent stains *B. dermatitidis* but does not react with *H. capsulatum*. Conventional mycological methods would have to be used to differentiate *H. capsulatum* from *H. duboisii*.

A number of workers have investigated the value of Candida-adsorbed fluorescein-labeled *H. capsulatum* antiglobulins for demonstrating *H. capsulatum* in human clinical materials. They found that their conjugates still cross-reacted to some degree with *B. dermatitidis*

yeast-form cells. Several workers have explored the possibility of using the reagent with human sputum. On the basis of studies with 84 sputum samples from 28 patients with confirmed or suspected chronic (cavitary) pulmonary histoplasmosis, Lynch and Plexico (*28*) concluded that Candida-adsorbed conjugates can be used to advantage for the rapid screening of sputa for the presence of *H. capsulatum*. They reported that better results were obtained with smears made from the centrifuged sediment of trypsin-digested sputa than with undigested material. In an independent study, Carski, Cozad, and Larsh (*2*) essentially confirmed the work of Lynch and Plexico. Carski and his associates concluded that the FA procedure, performed with Candida-adsorbed labeled antiglobulins, can be a valuable adjunct to cultural and clinical methods in the diagnosis of pulmonary histoplasmosis. Because of persistent cross-reactions of their conjugates with some strains of *B. dermatitidis* and several possible false positive results, these investigators urged caution in using immunofluorescence as the sole method for diagnosing this disease. Porter and her co-workers (*32*) explored the possibility of using Candida-adsorbed conjugates to demonstrate *H. capsulatum* in tissue impression smears. Tissues from 372 animals, representing 16 species, were examined by the FA procedure, and the results were compared with those obtained by culture and histopathology. The findings indicate that the FA technique is very useful for demonstrating *H. capsulatum* in impression smears of naturally infected animal tissue. However, none of the three methods alone—FA, histopathology, or culture—was completely reliable for the diagnosis of such infections. For maximal diagnostic coverage, the use of all three in combination was recommended.

Several workers have investigated the applicability of immunofluorescence to the detection of *H. capsulatum* in fixed tissue sections. In studies on the pathogenesis of experimental histoplasmosis in the mouse, Procknow, Connelly, and Ray (*34*) showed that it was feasible to use immunofluorescence for demonstrating *H. capsulatum* in picric acid–alcohol–formalin–fixed tissue sections. Yamaguchi, Adriano, and Braunstein (*39*) successfully applied the FA technique to the demonstration of *H. capsulatum* in sections of human lung and lymph node tissue that had been fixed in either 10 per cent formalin or Bouin's fluid. They reported that they were able to detect stained yeast cells in deparaffinized sections of 22 of 24 tissues previously found by the Grocott staining procedure to be infected with organisms morphologically consistent with *H. capsulatum*. Unadsorbed fluorescein-labeled *H. capsulatum* antiglobulins were used in both these studies. Kaplan and Kraft also found the FA procedure very useful in demonstrating *H. capsulatum* in paraffin sections of formalin-fixed tissues showing active disease processes. It was reported that digestion of deparaffinized sections in 1 per cent trypsin for one hour at 37° C before the conjugates were applied considerably enhanced the staining of *H. capsulatum* and also all other pathogenic fungi. In addition, it was found that the FA procedure would stain *H. capsulatum* and the other fungi in tissue sections that had been previously stained by the H & E, the Brown and Brenn, and the Giemsa techniques. It was not possible, however, to stain *H. capsulatum* or other fungi in sections previously stained by the Gomori methenamine-silver nitrate, the PAS, or the Gridley procedures. Unabsorbed fluorescein-labeled *H. capsulatum* antiglobulins and conjugates that had been adsorbed with *C. albicans* were used in these studies.

A number of researchers have studied the possibility of employing the FA procedure to demonstrate *H. capsulatum* antibodies in sera. Kaufman, Schubert, and Kaplan (*24*) performed studies with a modification of Goldman's one-step FA inhibition test, using the specific conjugate developed by Kaufman and Kaplan for improved accuracy. These workers examined 53 sera from suspected and confirmed cases of histoplasmosis by the FA inhibition test and compared the results with those obtained by the

complement fixation and immunodiffusion tests. They found the FA inhibition test to be a simple and effective procedure for the rapid detection of antibody against whole yeast cells of *H. capsulatum*. It was not effective, however, for detecting antibody to histoplasmin. In a follow-up study, Kaufman, Brandt, and McLaughlin (*22*) used the FA inhibition test and the agar gel precipitin test to qualitatively examine 127 anticomplementary sera from humans and dogs clinically suspected of having histoplasmosis. Whenever possible, these results were compared with those obtained by the CF test in repeat nonanticomplementary sera. The results obtained with the FA inhibition and immunodiffusion tests showed a 97 per cent correlation with those obtained by the CF test. The concurrent use of these two tests, therefore, can provide a rapid serological diagnosis when sera are anticomplementary and cannot be tested by the CF procedure.

Recently, Hook and Fife (*10*) investigated the possibility of using soluble antigens to detect antibodies to *H. capsulatum* by immunofluorescence. Soluble antigens from both the yeast and mycelial forms of *H. capsulatum* were purified by gel filtration, fixed onto paper discs, and used in an indirect FA procedure to detect antibodies in sera from patients with histoplasmosis. Evaluation of this soluble antigen FA procedure indicated adequate sensitivity for serodiagnosis. However, sera from culturally confirmed cases of blastomycosis, coccidioidomycosis, and cryptococcosis also reacted with the soluble antigens. Consequently, in terms of specificity, the technique offered no advantages over the standard serological tests for histoplasmosis. In view of its rapidity and relative simplicity, however, Hook and Fife concluded that it could be profitably used as a screening procedure for the serodiagnosis of histoplasmosis.

As yet, there are no FA reagents for the specific identification of the mycelial form of *H. capsulatum*. Immunofluorescence cannot, therefore, be used for this purpose.

Paracoccidioidomycosis

At the present time, the diagnosis of paracoccidioidomycosis by immunofluorescence is still in the experimental stage. Nevertheless, a review of what has been done suggests that FA may become a useful tool for this disease.

Silva and Kaplan (*35*) were the first workers to investigate the possibility of applying the FA technique to the diagnosis of paracoccidioidomycosis. Conjugates were prepared from two lots of rabbit antisera produced against the yeast form of two strains of *P. brasiliensis*. Both reagents brightly stained elements of the yeast and mycelial forms of 15 isolates of *P. brasiliensis*. In addition, they cross-reacted with the tissue forms of *H. capsulatum, B. dermatitidis, Sporothrix schenckii,* and other heterologous fungi. Adsorption of one lot of labeled antibodies two times with yeast cells of *S. schenckii* and once with cells of the mycelial form of *C. immitis* rendered this conjugate specific for the tissue form of *P. brasiliensis*. Adsorption of the second conjugate once with mycelial growth of *C. immitis,* once with yeast cells of *H. capsulatum,* and once with cells of *Rhodotorula sp.* rendered it specific for the tissue form of *P. brasiliensis*. In a limited evaluation, the two specific reagents enabled the detection and identification of *P. brasiliensis* in sputum from three culturally confirmed cases of pulmonary paracoccidioidomycosis and in purulent exudate from an infected lymph node. These preliminary results suggest that immunofluorescence may become a valuable tool for the rapid diagnosis of paracoccidioidomycosis. Further evaluation is needed, however, before FA can be routinely used with confidence for the accurate determination of this disease.

Silva and Kaplan reported that their specific conjugates did not react with the mycelial form of *P. brasiliensis*. Apparently in the mycelial form the specific factor or factors present in the yeast form are either lacking or too attenuated to be detected. Additional studies are required before FA can be used to identify the mycelial form of *P. brasiliensis*.

Sporotrichosis

The FA technique is an excellent procedure for the rapid diagnosis of sporotrichosis. Reagents and methods for the specific identification of the tissue form of *Sporothrix schenckii* have been developed and are in use at several mycological centers.

The earliest report on the application of the FA technique to sporotrichosis is by Kunz (*25*). In 1959, he labeled rabbit *S. schenckii* antiglobulins with fluorescein isocyanate and successfully stained this fungus in culture, in frozen tissue sections, and in smears from experimentally infected animals. None of 18 heterologous fungus species reacted with the labeled antibodies.

In 1959, Kaplan and Ivens (*14*) also carried out studies on the application of immunofluorescence to the identification of *S. schenckii* in culture and in clinical materials. Conjugates were prepared from globulins of rabbits that had been immunized with formalin-killed *S. schenckii* yeast-form cells. These reagents stained both the yeast and mycelial forms of the fungus. Conjugates diluted 1:4 to 1:12, depending on the lot tested, brightly stained the fungus in routine tests. Furthermore, the diluted reagents did not cross-stain any of 47 strains of 21 heterologous fungus species representing 12 different genera. These workers also demonstrated the presence of *S. schenckii* cells in impression smears of infected mouse testes and in sections of formalinized tissues from a mouse and a rat that had been experimentally infected. Kaplan and González Ochoa (*12*) evaluated these labeled specific antiglobulins for the diagnosis of sporotrichosis in man. Smears of lesion exudates from 34 patients suspected of having sporotrichosis were examined, and the results were compared with those obtained by culture. The FA technique revealed *S. schenckii* cells in smears from 24 (89 per cent) of 27 culturally positive individuals. Of seven culturally negative individuals, one was found positive by FA. This FA positive patient responded favorably to potassium iodide therapy. Work on the evaluation has continued, and sputa and fixed tissues have also been tested. The results to date are summarized in Table 1. It can be seen that the latest findings confirm the earlier favorable indications. Of 41 culturally positive cases studied, 37 (90 per cent) were positive by FA; of 28 culturally negative cases, 27 (96 per cent) were FA negative.

Table 1

Comparison of fluorescent antibody (FA) and culture methods for demonstration of *Sporothrix schenckii* in clinical materials from suspected cases of sporotrichosis

Culture results	Fluorescent antibody results		Totals
	FA positive	FA negative	
Culture positive	37	4	41
Culture negative	1	27	28
Totals	38	31	69

The conjugates that are specific for the tissue form of *S. schenckii* also stain the mycelial form of this fungus. Additional evaluation is necessary, however, before the reagents can be relied on to identify the mycelial form.

Actinomycosis

Immunofluorescence is an excellent method for the detection and identification of the principal etiologic agents of actinomycosis in man. FA reagents for the specific staining of *Actinomyces naeslundii* and the two recognized serotypes of *A. israelii* have been developed by Lambert, Brown, and Georg (*26*). These conjugates can be effectively used to detect these organisms either in culture or in smears of tissue and lesion exudates (*1*). Gerencser and Slack (*6*) have prepared a conjugate for the specific staining of *Arachnia* (*Actinomyces*) *propionica* both in culture and clinical materials. The development of these reagents has greatly simplified the diagnosis of actinomycosis, since the isolation and identification of the etiologic agents by conventional methods is both time-consuming and difficult.

Comments

An objective review of what has been accomplished clearly indicates that immunofluorescence is a valuable and practical tool with wide application in the diagnosis of mycotic diseases. The technique has been developed to a practical level for the diagnosis of most of the principal mycoses and is currently in routine use in some medical mycological centers. All the workers who have employed immunofluorescence have been favorably impressed with its speed, simplicity, and versatility. However, the proper use of the FA technique requires no less skill, experience, and judgment than any other mycological procedure. Training must be made available to those who plan to use it for diagnostic purposes.

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SEROLOGY: ITS VALUE IN THE DIAGNOSIS OF COCCIDIOIDOMYCOSIS, CRYPTOCOCCOSIS, AND HISTOPLASMOSIS

Leo Kaufman

Extensive studies by numerous investigators have clearly demonstrated that coccidioidomycosis, cryptococcosis, and histoplasmosis occur in Central and South America and that they constitute public health problems. The exact magnitude of these problems remains to be determined. This may be attributed in large part to the fact that distinct and differential clinical characteristics are frequently lacking in the systemic mycoses. Because the symptoms of these diseases are not pathognomonic, clinical diagnoses cannot always be made with certainty. Frequently physicians do not consider these diseases in making a differential diagnosis and fail to perform the mycologic, histologic, and immunologic studies necessary to establish a definitive determination of disease. Such determination depends on isolation and identification of the infecting fungus, or histological demonstration of the pathogen—procedures that require mycological training and experience and that may not even be possible to carry out in some cases.

Clinicians have found that immunologic tests frequently provide them with the earliest evidence of a fungus infection. Positive immunologic data can lead to increased and often successful efforts to isolate the etiologic agent. In the critically ill individual, the rapid establishment of a presumptive diagnosis by serology, coupled with immediate follow-up therapy, often makes it possible to save the patient's life. In addition, thoroughly evaluated and standardized immunologic tests can provide information on the severity and extent of the illness, the effects of therapy, and the epidemiologic aspects of the disease.

Coccidioidomycosis

Infection with *Coccidioides immitis* elicits an immunological response in the host. Conversion from a negative to a positive skin test with coccidioidin is usually the earliest immunologic response to infection. Because nearly every infected individual will develop a hypersensitivity to coccidioidin, the skin test is considered a valuable screen for serological testing. Smith and his co-workers (*20*), for example, never observed positive serologic results in patients with primary coccidioidal infections in the absence of a positive skin test.

The complement fixation (CF) and tube precipitin tests are invaluable serological aids in the diagnosis and prognosis of coccidioidomycosis (*20*). The soluble antigen coccidioidin, a pool of culture filtrates derived from multiple strains of *C. immitis,* is used in both tests. Heating the pooled filtrate at 60° C for 30 minutes destroys the antigen responsible for the complement fixation activity but has no effect on the precipitinogens (*10, 18*). The two tests measure at least two different antigen-antibody systems. The precipitin test is most effective in detecting early primary infection or cases undergoing an exac-

erbation of existing disease. Precipitins are seldom detected six months after infection. A positive tube precipitin reaction is indicative of early active disease and becomes evident in many cases during the first week of clinical illness. The CF test may also become positive early in the disease, but its reacting antibodies are detectable for longer periods of time than those in the tube precipitin test. The CF titer tends to parallel the severity of the infection (*20*), rising as the patient's condition deteriorates and declining as the patient improves.

These tests are very specific and demonstrate no cross-reactions with sera from viral, rickettsial, bacterial, or most other mycotic infections. Smith *et al.* (*20*) found that the combination of the CF and tube precipitin tests yielded positive results in over 90 per cent of the primary symptomatic coccidioidomycosis infections. Any precipitin or CF titer with coccidioidin should be presumptive evidence for *C. immitis* infection. CF titers greater than 1:16 on successive specimens usually indicate disseminated disease. Low titers, such as 1:2 and 1:4, have been found to be indicative of early, residual, or meningeal coccidioidomycosis (*20*). However, positive reactions at these low dilutions have also been noted in sera from patients known not to have coccidioidomycosis. Therefore, caution must be exercised in making a diagnosis on the basis of tests with such concentrated sera. Experience has shown that with titers of 1:2 and 1:4 a diagnosis of coccidioidomycosis must be based on subsequent serological tests, and preferably mycological studies.

Other screening tests that yield results comparable to those of the tube precipitin or CF tests are available for laboratories that are not able to perform the classical procedures.

One, the latex particle agglutination test, uses latex particles sensitized with coccidioidin heated at 60°C for 30 minutes. This test is more sensitive than the tube precipitin method and yields a higher percentage of positive responses. It has the additional advantage that results can be obtained in a few minutes (*12*).

Another technique is the immunodiffusion test (*10, 11*), which gives results that correlate with those of the CF test. The antigen is a heat-labile toluene extract of the mycelial growth. The combination of the latex particle and agar gel tests has permitted the detection of 93 per cent of the serum specimens from proven coccidioidomycosis cases. Huppert and co-workers (*12*) recommend that sera positive by either of these techniques be further analyzed with the standard CF and tube precipitin tests in order to better determine their clinical significance.

Cryptococcosis

Until recently, individuals suffering from cryptococcosis were considered to be immunologically inert. Thus, diagnosis had to be limited to time-consuming cultural and biochemical procedures. Continued investigation of serological procedures for cryptococcosis, however, has resulted in the development of diagnostically and prognostically useful tests. The most effective procedures are an indirect fluorescent antibody (IFA) technique (*22*) and a tube agglutination (TA) test, both for *Cryptococcus neoformans* antibodies (*6*), and a latex agglutination (LA) test for cryptococcal antigens (*2*). Although reagents for these tests are not yet commercially available, a number of laboratories in the United States are preparing their own products and performing one, two, or all three procedures.

In the Mycology Section at the U.S. National Communicable Disease Center, Atlanta, it has been found that maximal serologic diagnosis of cryptococcosis can be made by using the three tests concurrently (*14*). Both the IFA and tube agglutination tests are used, because their ability to detect cryptococcal antibodies in proven case sera varies. It is not uncommon for sera that are negative for antibodies with the IFA test to be positive with the tube agglutination test and vice versa.

Our studies have shown that, regardless of the clinical type of cryptococcosis, some patients show an antibody response only, others give

an antigen response only, and still others have both.

In an evaluation by the author, sera from 66 culturally proven cases of cryptococcosis were studied. Of these, 29 per cent were positive for antigen only, 47 per cent for antibody only, and 19 per cent for both antigen and antibody. Sera from only three patients (5 per cent) yielded negative results in serologic tests. These analyses would have permitted a presumptive diagnosis of cryptococcosis in 63 (95 per cent) of the 66 proven cases. No single test provided this level of sensitivity.

Of the three tests, the latex agglutination is most useful for detecting cryptococcal meningitis. Of 21 proven case cerebrospinal fluid specimens, 71 per cent were positive with the latex agglutination test. In contrast, less than 0.5 per cent were positive with the tube agglutination test, and none with the IFA tests for antibody.

The latex agglutination test is highly specific. A false positive was rare and occurred only with sera from patients suffering from severe rheumatoid arthritis. Cross-reactions occurred with the tests for antibody, particularly the IFA test, which demonstrates a 79 per cent specificity. Most of the cross-reactions occur with sera from patients with blastomycosis and histoplasmosis.

Positive tube agglutination and IFA reactions are considered presumptive evidence for cryptococcosis. However, a positive reaction, particularly with the IFA test, could also reflect a cross-reaction or past infection. The latex agglutination test is diagnostically and prognostically applicable. Any latex agglutination titer is diagnostic. Increasing titers indicate progressive infections, and declining titers signify response to chemotherapy and improvement in the course of the disease.

Histoplasmosis

Humans infected with *Histoplasma capsulatum* usually develop a positive reaction to a histoplasmin skin test within two weeks after exposure (*5*). The skin test is useful in defining endemic areas of histoplasmosis. However, it has limited value as a diagnostic tool, since it does not distinguish between past or present infections. In general, a positive reaction is of diagnostic significance only if the skin test was negative before the onset of clinical symptoms.

It has been adequately demonstrated that the level of complement-fixing antibodies, precipitins, and agglutinins to *H. capsulatum* antigens may be significantly increased in histoplasmin-sensitized subjects after a single histoplasmin skin test (*1, 4, 16*). Clearly, the laboratory worker and clinician should be fully cognizant of any factor or factors other than disease that might bear on the serologic findings. In a recent study of 114 histoplasmin-sensitized but clinically well subjects, slightly less than 12 per cent developed complement-fixing antibodies after a single histoplasmin skin test. The majority of the antibody responses were in the 1:8 to 1:16 range, but a few showed a maximum titer of 1:32 (*16*). Of the same 114 hypersensitive subjects, 17 (15 per cent) produced precipitins in response to a single histoplasmin skin test. In most cases, complement-fixing and precipitating antibody responses were detected in serum specimens drawn 15 days after skin testing. Preferably, blood for serological studies should be drawn before skin testing, but the patient can usually be bled within two or three days after the skin test without induced antibodies being detected in the serum. The induced serum reactions are primarily with the histoplasmin antigen, although antibody to the yeast-form antigen of *H. capsulatum* has also been elicited (*17*). A single histoplasmin skin test produces no serological responses in nonsensitized individuals.

Frequently serologic evidence is the factor responsible for intensive histologic and cultural studies that permit the subsequent definitive diagnosis of histoplasmosis. This serologic evidence is usually obtained through the CF, immunodiffusion (ID), and LA tests, used either alone or in combination. Of these procedures, the most widely used is the CF test. Properly performed, it can yield information of diag-

nostic and prognostic value. In general, antibodies to *H. capsulatum* are detected within a month after infection and are present for varying periods of time.

At the NCDC (*21*) and other institutions, two antigens are used in the CF test: one, a suspension of intact yeast cells of *H. capsulatum* (*19*), and the other, histoplasmin, the soluble mycelial filtrate. Sera from culturally proven cases of histoplasmosis may react to only one of the antigens; consequently, for adequate diagnostic coverage the two are used in combination (*8, 15*). In many cases both antigens may be positive. In a recent study of 220 serum specimens from proven human histoplasmosis cases, 182, or 83 per cent, were positive when the histoplasmin antigen was used alone, and 206, or 94 per cent, were positive when only the yeast-form antigen was used. However, 212 (96 per cent) were positive when both antigens were employed.

The interpretation of test results can sometimes be difficult because these antigens commonly cross-react with sera from individuals with other systemic mycotic infections. They can also react with sera from clinically normal individuals, from persons who have had a mycotic infection but are presently well, or from patients suffering from nonmycotic infections. In such cases, titers are usually 1:8 or 1:16 and are mainly observed with the yeast-form antigen. However, the sera from culturally proven cases of histoplasmosis can often demonstrate these same titers. Consequently, such levels are taken to be presumptive evidence of histoplasmosis. Higher titers are of more diagnostic significance (*3*), but they cannot be relied on as the sole means of diagnosis (*13*). Since a serologic reaction is not always indicative of active infection, CF titers must be interpreted taking into account the total clinical picture, including radiological findings. Changes in titer are of diagnostic significance, and fourfold titer fluctuations in either direction are usually of prognostic value. The absence of a specific immunological response does not exclude histoplasmosis, particularly when only a single specimen has been tested and when the clinical picture strongly suggests pulmonary mycotic disease. In such situations, it is recommended that serial serum specimens taken three to four weeks apart be tested for antibodies. In disseminated or terminal histoplasmosis a state of anergy frequently exists and immunologic responses are negative.

The ID test using concentrated histoplasmin may be employed as an adjunct or screening procedure in the diagnosis of histoplasmosis. Two precipitin bands have diagnostic value (*7*). One, the H band, is rarely influenced by skin testing. Patients with active and progressive histoplasmosis usually demonstrate H antibody. The H precipitin may be detectable one to two years after apparent clinical recovery. The second precipitin band, the M, is found in sera of patients with acute and chronic histoplasmosis and may appear in sensitized normal individuals after skin testing with histoplasmin. The demonstration of only an M band may be indicative of active infection, past infection, or recent skin testing. Although the H band is usually associated with the M band, proven case histoplasmosis sera containing only the H precipitin have been seen.

Accurate interpretation of the ID test requires that the physician know whether the patient was recenly skin tested. The presence of M antibody when there has been no recent skin test may indicate an early infection, since this antibody appears before the H precipitin. The disappearance of the H precipitin is of prognostic value. The M precipitins will eventually disappear, but more slowly than the H. We have found the ID test to be useful in interpreting the cross-reactions so often encountered with the CF test and also in examining anticomplementary sera.

The latex test (*9*) has particular application for studying early case specimens and for the testing of anticomplementary sera. It is not a substitute for the CF test; it should be regarded, rather, as an adjunct that is easy to perform.

In summary, properly evaluated and standardized serologic tests are available for the diagnosis of coccidioidomycosis, cryptococcosis, and histoplasmosis. It is believed that widespread use of the CF, ID, IFA, LA, and TA tests can contribute to the rapid and accurate detection of these diseases and to their proper treatment.

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SEROLOGIC PROCEDURES IN THE DIAGNOSIS OF PARACOCCIDIOIDOMYCOSIS[1]

Angela Restrepo M. and Luz H. Moncada F.

The clinical manifestations of paracoccidioidomycosis are not always classical. Signs and symptoms of the severe pulmonary form are seldom so typical that the etiology can be determined without laboratory assistance. In most cases, the combined use of direct examination, biopsy, and culture makes it possible to recognize the causative agent. These procedures, however, may not always be feasible. Some patients do not raise adequate samples, and others do not exhibit external lesions that can be studied in the laboratory. Hence, the indirect evidence furnished by serologic tests is important in determining the presence and the degree of activity of the infection.

Three historical periods can be considered in the development of serologic procedures for the diagnosis of paracoccidioidomycosis.

The first began when Moses (*12*) employed complement fixation (CF) in 1916 and recorded positive results in eight of the ten patients studied. Sporadic attempts were subsequently made by other Brazilian authors (*5*), but it was only in 1949 that Lacaz (*8*) began to use complement fixation procedures on a regular basis and demonstrated that most of the patients had detectable antibody titers.

The work of Lacaz linked the first period to the second, during which Fava Netto's investigations were carried on. In 1955, Fava (*4*) studied the values of different types of antigens and selected and standardized a polysaccharide yeast cell preparation. Quantitative CF and tube precipitin tests were performed on 100 patients with the disease. His studies revealed that 98.4 per cent of the cases had circulating antibodies at the time of diagnosis. By 1961 he had analyzed 220 cases and found that it was possible to determine the course of infection by serial serologic tests. Fava Netto, Ferri, and Lacaz (*7*) showed that the CF test was better than tube precipitation as a means of determining the activity of infection. Precipitins were found to disappear sooner than CF antibodies. This second period can be ascribed to Fava Netto because of the fundamental nature of his studies.

The third period comprises a series of different studies. In 1960, Maekelt (*11*) established a serology section for the diagnosis of deep-seated mycoses and prepared antigens from both the mycelial and the yeast phases of *Paracoccidioides brasiliensis*. In 1962, Lacaz, Ferri, Fava Netto, and Belfort (*9*) used immunodiffusion and immunoelectrophoresis to compare antigens from *P. brasiliensis* and *P. loboi* and determined that antibodies to the former were located in the gamma globulin fraction of human serum. In 1966, López and Fava Netto (*10*) reported the results of a serologic follow-up of 33 patients treated with a new sulfonamide. In 1966 and 1967, Restrepo (*15, 16*) employed immunodiffusion techniques in diagnosis and compared

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the results with those of complement fixation. In one of the experiments, comparisons of the activities of yeast and mycelial filtrates were also made, and it was found that the yeast form was more potent. In 1968, Negroni and Negroni (*13, 14*) published the findings of a comparative study carried out with various types of antigens. A filtrate from shaken yeast cultures was found to be superior for both the immunodiffusion and the CF techniques. The authors also performed quantitative agar gel precipitin inhibition tests and found this procedure to be more sensitive than others. Pollak, too, has also used agar gel techniques for the detection of serum antibodies in patients with paracoccidioidomycosis (personal communication, February 1967). The various types of antigens and serologic procedures are summarized in Table 1.[2]

[2] Although efforts were made to cite all papers dealing with the use of serologic procedures in the diagnosis of paracoccidioidomycosis, the authors are aware that some reports published in journals of limited circulation may have escaped attention. They apologize for the involuntary omissions and would be grateful to receive reprints of any articles missed.

The present paper reviews the findings reported by the authors in earlier publications and presents new data on serologic behavior in 61 cases of paracoccidioidomycosis.

Method and materials

A total of 1,038 patients with suspected mycotic infections of the lungs, the mucous membranes, and/or the reticuloendothelial system were studied over a five-year period. The diagnostic procedures included both mycological and serologic tests. In all the patients, diagnosis was established by microscopic observation (direct KOH mounts, biopsies) and/or by isolation of the causative agent in cultures, using the techniques already described (*17*). Patients were bled at the time of consultation, immediately before skin testing. An effort was made to repeat the serologic tests at varying intervals during the course of treatment.

Two kinds of serologic procedures were used: complement fixation and agar gel immunodiffusion. The antigens were obtained from both *P. brasiliensis* and *Histoplasma capsulatum*. For

Table 1

Types of serologic procedures and antigens employed in the diagnosis of paracoccidioidomycosis

First author and year of publication	Serologic procedures							
	Complement fixation				Tube precipitation	Agar gel immunodiffusion		
	Yeast Extract/Filtrate		Mycelium Extract/Filtrate		Yeast Extract	Yeast Extract/Filtrate		Mycelium Extract/Filtrate
Moses (1916)			X					
Gomes (1924)			X					
Fonseca (1927)			X	X				
Basgal (1931)				X				
Lacaz (1945)				X				
Fava Netto (1955)								
(1959)								
(1961)	X				X			
Maekelt (1960)	X							
Lacaz (1962)	X	X	X	X	X	X		X
Lopes (1966)	X							
Restrepo (1966)								
(1967)		X		X			X	X
Negroni (1968)	X			X				X

the former, three different strains in the yeast phase were grown separately in submerged shaken cultures in a specially designed dialysate medium. Cultures were incubated at 35°C for four weeks, at the end of which time they were centrifuged and the culture fluids dialyzed, concentrated by pervaporation, and pooled (*15, 18*). A single batch of antigen was used for all the tests. The antigens derived from *H. capsulatum* were the soluble mycelial antigen, histoplasmin,[3] and a whole yeast cell preparation (*1*). All antigens were box-titrated against known human-reactive sera. Serologic tests were carried out using standard techniques (*3, 19*).

Results

Paracoccidioidomycosis was demonstrated in 61 of the 1,038 patients studied, and histoplasmosis in 12. In 32 additional cases the results of the serologic tests were suggestive of a mycotic disorder. The remaining cases were not considered to be of mycotic origin.

At the time of diagnosis, 58 of the 61 patients with paracoccidioidomycosis (95.0 per cent) had precipitin bands, and 48 (78.6 per cent) were reactive in the CF study when tested with *P. brasiliensis antigen*. Three sera were anticomplementary.

A comparison of the two serologic procedures revealed that both tests were simultaneously reactive in 48 cases, or 78.6 per cent, and nonreactive in three, or 4.9 per cent (Table 2). The immunodiffusion test gave positive results in seven patients (11.4 per cent) whose CF tests were nonreactive. The contrary was not observed. Three patients (4.9 per cent) gave precipitin bands, but their sera were anticomplementary.

The number of precipitin bands varied from one to three, and the CF titers ranged from 1:8 to 1:4,096. As can be seen in Table 3, almost half the patients showed two precipitin bands and CF titers above the level of 1:64. No relationship was found between the number of bands and the height of the CF titers: there were sera with three precipitin bands and a CF

[3] Kindly supplied by the U.S. National Communicable Disease Center, Atlanta, Georgia, and NCDC, Kansas City, Kansas, USA.

Table 2

Comparison of serologic results obtained with immunodiffusion and with complement fixation procedures in proven cases of paracoccidioidomycosis [a]

Serologic procedures (*P. brasiliensis* yeast culture filtrate)	Cases	
	Number	%
Immunodiffusion and complement fixation REACTIVE	48	78.6
Immunodiffusion and complement fixation NONREACTIVE	3	4.9
Immunodiffusion REACTIVE and complement fixation NONREACTIVE	7	11.4
Immunodiffusion NONREACTIVE and complement fixation REACTIVE	0	—
Immunodiffusion REACTIVE and complement fixation ANTICOMPLEMENTARY	3	4.9
Totals	61	100.0

[a] At the time of diagnosis

Table 3

Serologic procedures in proven cases of paracoccidioidomycosis grouped by number of precipitin bands and highest complement fixation titer [a]

Serologic procedures (*P. brasiliensis* yeast culture filtrate)		Cases	
		Number	%
Immunodiffusion bands	0	3	4.9
	1	22	36.0
	2	28	45.9
	3	8	13.1
Totals		61	100.0
Complement fixation: highest titer [b]	N [c]	10	15.5
	8–32	10	15.5
	64–256	22	36.0
	512 +	16	26.2
	Anticomplementary	3	4.9
Totals		61	100.0

[a] At the time of diagnosis
[b] Reciprocal of dilution
[c] No fixation at 1:8 dilution

Table 4

Results of serologic procedures in patients with proven paracoccidioidomycosis according to degree of illness

Degree of illness	Number of cases	Serologic procedures (*P. brasiliensis* yeast culture filtrate)[a]			
		Immunodiffusion		Complement fixation	
		Number reactive	% reactive	Number reactive	% reactive
Minor	16	14	87.5	12	75.0
Moderate	23	22	95.0	18	78.2
Severe	22	22	100.0	18	81.8
Totals	61	58	95.0	48 [b]	78.6

[a] At the time of diagnosis

[b] Three sera were anticomplementary.

titer as low as 1:8, and sera with only a single band and a high CF titer.

The initial figures were not significantly altered when the group of patients was subdivided according to degree of illness (Table 4). In all stages, precipitin bands were detected in at least 87 per cent of the cases, while more than 75 per cent were reactive in the CF test. Nor were large differences found when correlating the serologic results with the time of onset of the disease (Table 5). Twenty-six patients were tested during the first six months of illness, 14 before the end of a year, and 21 after a year. Immunodiffusion was positive in 90 to 100 per cent, and CF tests gave significant titers in 71 to 84 per cent of the patients.

Some relationship did appear to exist, however, in regard to the particular organs involved (Table 6). The largest number of precipitin bands was obtained with the sera from patients showing either lung involvement (30 cases) or disseminated disease (five cases). The highest CF titers were also observed in patients with lung involvement. Patients having reticuloendothelial compromise were poor producers of CF antibodies.

Table 5

Results of serologic procedures in patients with proven paracoccidioidomycosis according to time of onset of the disease

Time of onset	Number of cases	Serologic procedures (*P. brasiliensis* yeast culture filtrate) [a]			
		Immunodiffusion		Complement fixation	
		Number reactive	% reactive	Number reactive	% reactive
Less than six months	26	26	100.0	22	84.6
Seven to twelve months	14	13	92.8	11	78.5
More than twelve months	21	19	90.4	15	71.4
Totals	61	58	95.0	48 [b]	78.6

[a] At time of diagnosis

[b] Three sera were anticomplementary.

Table 6

Relationship between organs involved and results of serologic procedures in proven cases of paracoccidioidomycosis

Organs involved	Number of cases	Serologic procedures (*P. brasiliensis* yeast culture filtrate)[a]						
		Immunodiffusion bands			Complement fixation: highest titer[b]			
		None	1	2–3	N[c]	8–32	64–256	512+
Lungs alone, lungs and mucous membranes	46	2	14	30	3	5	19	16[d]
Mucous membranes	8	1	6	1	4	2	2	—
Reticuloendothelial system	2	—	2	—	2	—	—	—
Disseminated disease	5	—	—	5	1	4	—	—
Totals	61	3	22	36	10	11	21	16

[a] At the time of diagnosis
[b] Reciprocal of dilution
[c] No fixation at 1:8 dilution
[d] Three sera in this group were anticomplementary.

Follow-up studies were possible in 50 patients for varying periods of time. The three initially negative patients were bled monthly for nine, six, and five months, respectively, but in all instances the serologic tests remained negative. The results of the follow-up studies in the other cases are summarized in Table 7.

It can be seen that the immunodiffusion test remained positive in all the 26 cases followed for less than a year, in 12 of the 13 observed for one to two years, and in seven of the eight studied for longer periods. CF antibodies were detected in fewer cases, although more than 70 per cent of the patients were still reactive at the end of two years of observation.

Of the 36 patients who initially had multiple precipitin bands, 19 were included in the follow-up studies. In four patients, all the bands that were present in the beginning continued to persist in subsequent serum samples. In 13 cases, one of the bands disappeared, and in the remaining two cases, two of the three bands were lost. Figure 1 shows the position of the bands. The one persisting is located closer to the central antigen reservoir. The various patterns of serologic reactivity are illustrated in Figure 2.

Table 7

Follow-up serologic studies in patients with proven paracoccidioidomycosis undergoing treatment

Follow-up period	Serologic procedures (*P. brasiliensis* yeast culture filtrate)					
	Immunodiffusion			Complement fixation		
	No. of patients initially reactive	Detectable bands		No. of patients initially reactive	Detectable titers	
		No. patients	%		No. patients	%
Less than a year	26	26	100.0	26	22	84.5
One to two years	13	12	92.2	7	5	71.4
Over two years	8	7	88.8	7	5	71.4
Totals	47	45	95.7	40	32	80.0

Figure 1. Agar gel immunodiffusion test. Central well: *P. brasiliensis* yeast phase culture filtrate. Peripheral wells: sera from different patients with paracoccidioidomycosis. The internal band is the one persisting during treatment.

Thirty of the patients experienced a marked decrease in CF titers after initiation of therapy, in a fashion similar to cases 37, 39, and 45. Case 53 showed a different behavior, with a sharp rise in antibody titers right after the beginning of treatment. Nine patients had similar curves. Case 53 also showed a second peak much like the one observed in case 45, corresponding to a period of clinical relapse. Such peaks were noticed in six patients. In the rest of the cases, the CF titers remained stationary.

As stated before, antigens derived from *H. capsulatum* were also used. At the time of diagnosis, 16 of the 61 patients with paracoccidioidomycosis (26.2 per cent) reacted with these heterologous antigens. Of the 12 proven histoplasmosis cases, eight cross-reacted with *P. brasiliensis* antigen (Table 8). Five of the paracoccidioidomycosis patients showed precipitin bands with histoplasmin, and 13 had CF titers either with histoplasmin or with *H. capsulatum* yeast-phase antigen. During the follow-up study,

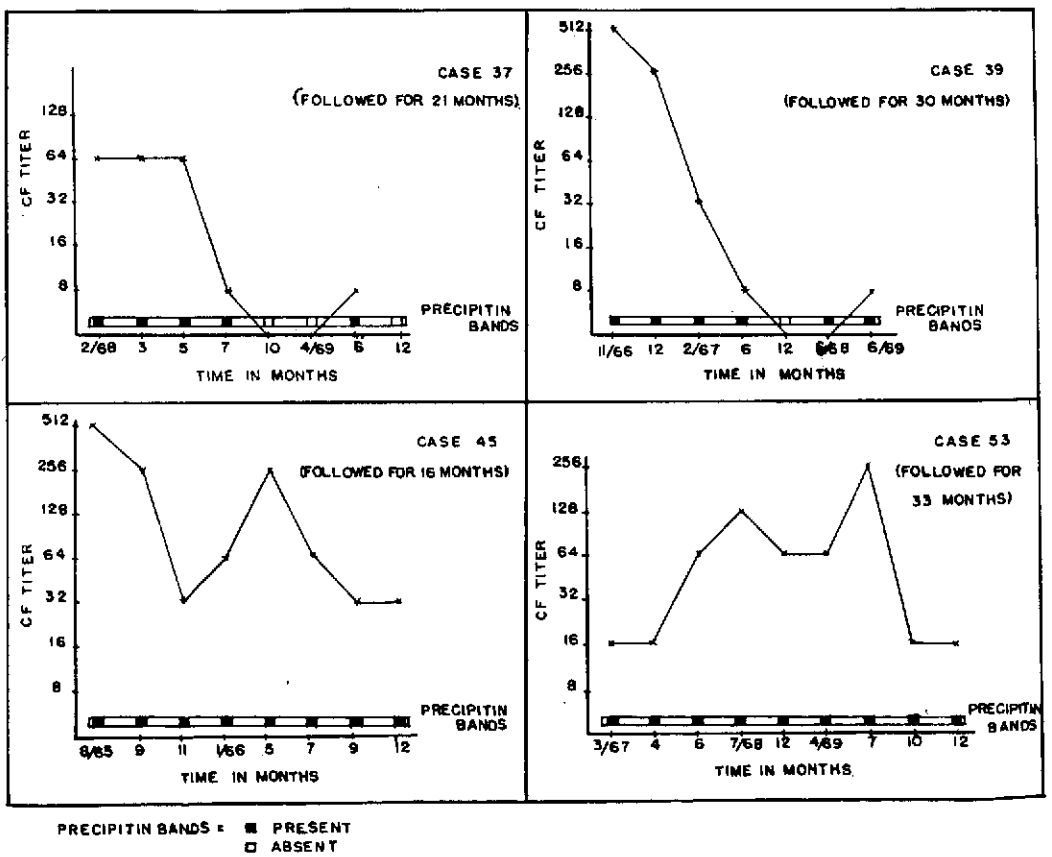

Figure 2. Serologic patterns exhibited by paracoccidioidomycosis patients undergoing treatment.

Table 8

Results of serologic procedures in patients with proven paracoccidioidomycosis and histoplasmosis when employing *P. brasiliensis* and *H. capsulatum* antigens

Disease and number of cases	Serologic procedures [a]				
	Immunodiffusion bands		Complement fixation titers [b]		
	P. brasiliensis (yeast filtrate)	Histoplasmin	*P. brasiliensis* (yeast filtrate)	Histoplasmin	*H. capsulatum* (yeast cells)
Paracoccidioidomycosis					
3	2	1	1,024	N [c]	N
2	2	1	512	16	16
8	2	None	512	16	32
3	2	None	512	16	N
Totals 16					
Histoplasmosis					
2	1	1	8	16	128
4	None	1	N	32	32
2	None	None	16	128	8
Totals 8					

[a] At the time of diagnosis
[b] Reciprocal of dilution
[c] No fixation at 1:8 dilution

Table 9

Results of serologic procedures in patients with suspected but unconfirmed deep-seated mycosis

Number unconfirmed cases	Serologic procedures [a]				
	Immunodiffusion bands		Complement fixation titers [b]		
	P. brasiliensis (yeast filtrate)	Histoplasmin	*P. brasiliensis* (yeast filtrate)	Histoplasmin	*H. capsulatum* (yeast cells)
No other mycologic studies done [c]					
12	2	None	512	N [d]	N
8	2	None	1,024	8	16
4	1	1	64	8	32
Totals 24					
Cultures and direct examinations negative					
3	1	None	64	N	N
3	None	1	16	64	128
2	None	None	8	64	16
Totals 8					

[a] At the time of diagnosis
[b] Reciprocal of dilution
[c] Only serum was available for examination.
[d] No fixation at 1:8 dilution

eight additional patients developed either precipitin band or CF antibodies against this fungus. The heterologous titers were lower than the homologous ones, however. Cross-reactivity in the patients with histoplasmosis was very similar.

Apart from the proven cases, 32 suggested cases of mycotic infection were revealed in the serologic tests (Table 9). In 24 of these, the evidence was based on only one or two serum samples, since the patients lived in different parts of the country. In another eight patients it was possible to conduct all types of studies (repeated direct preparations and cultures), but they remained unconfirmed, since neither *P. brasiliensis* nor *H. capsulatum* was seen or recovered from clinical specimens.

Discussion

Fava Netto, in his classic paper (*14*), stated that the combined use of two serologic procedures, tube precipitation and complement fixation, established the presence of antibodies in 98.4 per cent of the patients with paracoccidioidomycosis. The results of the present study come very close to this percentage and demonstrate the importance of serologic procedures in the diagnosis of this disease. At the time of diagnosis, most of the patients had circulating antibodies that were detectable by either the agar gel immunodiffusion test or by complement fixation. Although both tests were simultaneously reactive in 78.6 per cent of the cases, immunodiffusion proved to be superior in that it revealed antibodies in roughly 16 per cent of the cases that had negative or anticomplementary CF tests. The agar gel immunodiffusion test has properties that are desirable for the screening of patients with possible *P. brasiliensis* infection. It is not only a simple and easily performed technique, it is also reliable, since it detects antibodies in 95 per cent of the cases. The time-honored CF test keeps its importance, however, for its quantitative nature correlates well with the patient's clinical condition and with his response to treatment.

The serologic data did not show significant differences in patients with disease of long or short duration. This was also observed by Negroni and Negroni (*14*) in their serologic study of 26 patients with paracoccidioidomycosis. However, Fava Netto's large series of cases (*5*) reveals a close relationship between circulating antibodies and the time of onset of the disease. The present authors noticed that the patients with lung involvement had the highest CF titers and also had multiple precipitin bands. Low CF titers and multiple bands were observed in patients with disseminated disease, but the number of cases of this kind was hardly sufficient to permit valid comparisons.

It is noteworthy that precipitating antibodies, as detected by the immunodiffusion test, were of long duration. Such antibodies were present in all the 26 patients followed for a year, in all but one of the 13 followed for two years, and in seven of the eight studied for periods of three to four years. In general, despite clinical improvement, more than 85 per cent of the patients were still reactive after several months of treatment. Actually, only two of the 47 patients followed became precipitin negative, and only 15 lost one of the initially detected bands. This is different from what Fava Netto found with the tube precipitin technique (*4, 7*). According to his report, precipitins were the first antibodies to appear in the blood and also the first ones to be lost during the course of successful treatment. In histoplasmosis and in coccidioidomycosis (*2*), these antibodies are considered to be of short duration. The present authors originally suggested (*16, 17*) that the persistence of precipitin bands in patients undergoing treatment could be a sign of fungal activity and an indication to continue therapy. The chronic nature of the disease and the fungistatic activity of the drugs in use may still support such a suggestion, but it will be necessary to carry on studies for much longer periods before the relationship between precipitating antibodies and the development of the mycotic process can be determined.

In regard to CF antibodies, 30 of the 36 patients in the follow-up study exhibited a marked decrease in their titers after therapy was begun. Increased antibody titers were observed in other patients. These titers were of short duration, however, and could have reflected stronger antigenic stimulation, probably due to drug-released fungal products. Clinical relapses were also accompanied by an increase in circulating antibodies. Some patients had stationary CF titers, which were interpreted by others (*10, 14*) as "serologic scars" and not always indicative of fungal activity.

The dialyzed culture prepared from the yeast phase of *P. brasiliensis* proved to be both a reliable and a versatile antigen—reliable in that it detects antibodies in 78 to 95 per cent of the cases, depending on the technique, and versatile in that it can be used in both the immunodiffusion and the CF tests (undiluted and diluted at 1:32, respectively). Moreover, the antigen is also relatively specific. The authors processed a large number of sera from normal persons (roughly 3,000 samples) and found only one positive immunodiffusion test.[4] As expected, there were cross-reactions with histoplasmosis in some of the patients. Precipitin bands occurred in the sera of two of these patients, and low CF titers were detected in four. A similar pattern of reactivity was observed when sera from patients with paracoccidioidomycosis were tested against the *H. capsulatum* antigens. Here, however, the homologous titers were much higher that the heterologous. The cross-reactions (about 26 per cent) are not of such a level as to preclude the use of serologic tests in the diagnosis of paracoccidioidomycosis.

In cases that pose diagnostic problems, the serologic tests make it possible to focus attention on paracoccidioidomycosis. Five of the patients studied were suspected of having this disease when the serologic results were first reported. *P. brasiliensis* was then found in clinical specimens. The authors were not as successful with three other cases, however, which had antibodies only against this fungus. Some of the unconfirmed out-of-town patients could have had the disease also, but it was not possible to process clinical specimens to determine the presence of the causative agent.

There are currently five centers in South America [5] that perform serologic tests for the diagnosis of paracoccidioidomycosis. The antigens and the techniques, to some extent, vary from place to place.

It is believed that for the benefit of the patients the use of serologic tests should be expanded. It would be desirable to standardize the approach through the use of similarly prepared antigens and reference sera if meaningful correlations are to be established between the serologic response and the development of the mycotic process. Only in this way will solid progress be achieved in the near future.

[4] Unpublished observations from work carried out during the National Morbidity Survey, 1966–1967.

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DISCUSSION

Chairman Restrepo: We are now ready for discussion of the second part of this session. The floor is open.

Dr. Drouhet: In collaboration with Dr. Restrepo, we studied sera from 30 patients with paracoccidioidomycosis. Using the agar immunodiffusion technique and Grabar's immunoelectrophoretic analysis, we were able to obtain precipitins in 28 cases. From one to five lines of precipitation were obtained, and five groups could be distinguished. The precipitation lines were obtained with the yeast antigen (100× concentrated culture filtrate). The mycelial antigen gave slight reactions (one or two lines) with a few sera. The number of lines was greater in severe cases and in cases where the onset of the disease occurred much earlier. In several cases, however, five lines were seen at the beginning of the disease, so we cannot make a general rule. No C substance was present in these antigens. Cross-reactions were not observed with the *P. brasiliensis* antigens that we used with sera from 10 cases of histoplasmosis diagnosed in France, or with sera from cases of candidiasis, aspergillosis, or cryptococcosis. The position of precipitin lines appears to us to be of importance in the specific diagnosis of paracoccidioidomycosis.

Dr. Furcolow: I have a few questions regarding Dr. Restrepo's paper. First, what was the concentration of the immunodiffusion antigen that you made?

Second, I do not see the relationship between determining the specificity and sensitivity of an antigen and detecting the maximum number of reactions in proven cases. It is the degree of cross-reactions and the degree of reactions in normal people that counts. Is it true that you only had 12 cases of histoplasmosis as controls?

Third, how many cases of other chest disease did you have, and what was their reaction?

Fourth, did I understand you to say that you tested 5,000 normal people and got no reactions? I am not clear on these points.

Chairman Restrepo: In answer to your first question, the antigen is concentrated 10 times by pervaporation.

The second question was about the specificity of the antigen. Did you say that specificity cannot be determined because the number of cases we have studied is small?

Dr. Furcolow: It related to the number of histoplasmosis cases in which you determined the degree of cross-reactions.

Chairman Restrepo: We had only 12 proven cases of active histoplasmosis. We see many patients who have either histoplasmomas or calcified lesions, and these do not usually react in the serologic test. The 12 cases to which I referred were confirmed by culture.

The number of normal persons tested is close to 4,000. In a morbidity survey carried out in Colombia in 1967 some 3,000 sera were collected, and these sera were sent to our laboratory for testing. There was only one specimen that was positive in the immunodiffusion test; all the others were negative. In the CF test we got some positive low titer reactions with Histoplasma antigens, and a good number of patients gave an H precipitin band, but we only saw one patient who gave a precipitin band with *P. brasiliensis* antigen in the immunodiffusion test, as I indicated.

Dr. Furcolow: And what was the number of positive reactions in other chest diseases where you could not prove the diagnosis?

Chairman Restrepo: I do not have the exact figures, but many of the 1,000 cases we examined had tuberculosis. This is a very prevalent disease in our country. We did not diagnose other mycotic infections of the lung, although we had a considerable number of Candida isolations from sputum specimens. In the absence of serologic tests, it is difficult to prove that the Candida species isolated are the cause of the disease.

Dr. Huppert: First, Dr. Kaufman mentioned that the skin tests in coccidioidomycosis are a good screening tool to detect people who will give positive serological reactions. As reported originally by the late Dr. C. E. Smith, this is true. But it should be kept in mind that Dr. Smith routinely used a 1:100 dilution of coccidioidin for the skin test, and he found when he retested the negatives with a 1:10 dilution that he picked up an additional 10 per cent positives. The 1:100 may not pick up all the serologically reactive people.

My second point is addressed to Dr. Restrepo. The immunodiffusion precipitin line that you get in the agar gel test for coccidioidomycosis is not the same as what you get with the old precipitin test. We are dealing with a heat-labile antigen in the immunodiffusion test, whereas we are working with a heat-stable antigen in the tube precipitin test. It is the antibody response to the heat-stable antigen that disappears early in the disease, but this does not occur in the immunodiffusion reaction. Essentially what you find with the precipitin line in immunodiffusion is that it persists in coccidioidomycosis just as it does with paracoccidioidomycosis.

With regard to the specificity of the antigen, I wondered if you have taken advantage of the opportunity that you have with immunodiffusion to set up reference systems for paracoccidioidomycosis to demonstrate whether you get lines of identity or nonidentity showing the presence of antigens specific for paracoccidioidomycosis as opposed to antigens that may be shared commonly by other fungi.

Chairman Restrepo: We are working along these lines. Right now I do not have the data. The findings are not yet complete.

Dr. Pollak: I would like to show you our results with paracoccidioidomycosis tests. Table 1 shows our findings with 29 culturally proven cases. Of the total, 20 cases were positive by both complement fixation and immunodiffusion, four cases were positive by complement fixation, and four cases were positive by precipitin alone. There was only one case that did not react with either complement fixation or precipitin.

Table 1

Serology in 29 proven cases of paracoccidioidomycosis

CF and Pr	20
CF	4
Pr	4
Negative	1

CF = complement fixation
Pr = precipitation in agar gel

Table 2 shows the distribution of positive serology tests in 150 unproven cases of paracoccidioidomycosis or cases in which the culture results were unknown. There were 45 cases positive by both complement fixation and immunoprecipitin, 89 by complement fixation alone, and 16 by the precipitin test. There were three anticomplementary reactions, and these were positive by the precipitin test.

Of the 29 cases of paracoccidioidomycosis, six gave a cross-reaction with Histoplasma in the complement fixation test and two did so in the precipitin test; two cross-reacted with coccidioidin in the complement fixation test (Table 3).

In the 150 unproven cases and/or cases with unknown cultural results, there were 33 cross-reactions with *H. capsulatum* CF antigen and one cross-reaction with *H. capsulatum* in the precipitin test; there were also two cross-reactions with *C. immitis* in the CF test (Table 4).

Table 2

Positive serology in unproven cases of paracoccidioidomycosis or in cases with culture unknown

CF and Pr	45
CF	89
Pr	16
Total	150
AC and Pr	3

CF = complement fixation
Pr = precipitation in agar gel
AC = anticomplementary

Table 3

Cross-reactions with other antigens in 29 cases of paracoccidioidomycosis

Histo CF	6
Histo Pr	2
Cocci CF	2

Histo = cross-reactions with *H. capsulatum* antigen
Cocci = cross-reactions with *C. immitis* antigen

It is interesting to point out that the proportion of positive females was very low. Among the cases of paracoccidioidomycosis at our institute in Venezuela, the ratio was 1 to 20, and among the positive complement fixations in the group of unproved cases, the ratio was 1 to 30.

Another interesting fact is that there were many cases with small pulmonary x-ray findings in which all the bacteriological and mycological cultures were negative. I suppose these could be subclinical cases of paracoccidioidomycosis. Of course, very little is known about this disease in its subclinical form. I would like to emphasize that all the 150 cases had pulmonary lesions. The total number of patients with pulmonary lesions tested serologically was 750.

Chairman Restrepo: I am somewhat concerned about Dr. Pollak's high number of nonspecific reactions—150 patients with either precipitin bands or complement fixation titers. I had just said that the serologic tests are relatively specific, and I think he has tried my antigen.

Dr. Negroni, have you experienced the same difficulty? Do you have a large number of patients who give precipitin reactions or who have complement fixation titers and are not cases of paracoccidioidomycosis?

Table 4

Cross-reactions in 150 cases unproven but serologically positive for paracoccidioidomycosis

Histo CF	33
Histo Pr	1
Cocci CF	2

Histo = cross-reactions with *H. capsulatum* antigen
Cocci = cross-reactions with *C. immitis* antigen

Dr. Negroni: We only had specific reactions in 20 culturally confirmed cases of histoplasmosis, and only two cases of cross-reaction. The serologic tests were positive by mycological examination. We usually obtained only 50 per cent positive results in patients with histoplasmosis. During treatment, however, as they improved, we very often got conversion to positive skin test reactions.

Chairman Restrepo: I am tempted to believe that Dr. Pollak is seeing something very interesting in Venezuela. He might be observing patients in the early stages of paracoccidioidomycosis. At this point the disease might not be clinically obvious, or the number of organisms present in the specimens might not be adequate for their isolation. Thus, diagnosis remains unconfirmed. I am worried because I know the quality of the work being carried out at the Tuberculosis Institute. Do you have any comments on this Dr. Pollak?

Dr. Pollak: Part of this material came from our institute, and we were not able to demonstrate paracoccidioidomycosis by culture. The other part came from outside, and I was unable to obtain any cultural data from the sources that supplied it. Of our own patients, a considerable number had minimal pulmonary lesions, and their mycological and bacteriological examinations generally gave negative results. I suppose, therefore, that quite a few of these cases may be minimal forms of paracoccidioidomycosis.

Dr. Lazo: We are carrying out intensive studies on the systemic mycoses in Ecuador. One of our patients, with manifest pulmonary lesions, had had 36 negative TB inoculum tests and gave a negative result in the skin test for histoplasmosis five consecutive times over the period of a year. When a precipitin test was performed, taking the blood sample before the skin test, the result was a very weak positive for histoplasmosis. Dr. Schubert of NCDC

and Dr. Heiner, who had been in correspondence with us, also obtained positive sera from the patient. Even with the positive results for histoplasmosis with the precipitin test, there was no change in the response to the skin test. The patient's second precipitin test was negative and remained negative despite the skin test.

For the South American blastomycosis precipitin test we prepared an antigen with five strains of *P. brasiliensis*. The antigen was standardized with 24 proven cases of this disease. In all the cases, the reaction was positive with precipitin bands, and in a correlation study I observed seven positive cases in the histoplasmosis skin test. Only one of the 24 cases positive to the paracoccidioidomycosis antigen was also positive to the histoplasmosis antigen. In studying the titer, I first carried out a precipitin test with each strain without concentrating the antigen. I observed precipitin bands in only one of the five strains used in this test. All five strains of *P. brasiliensis* gave precipitin bands at concentrations of 1:10. Over all, the results showed that our strain must have had powerful antigenic capacity to obtain these immunological reactions.

Dr. Negroni: I would like to ask Dr. Restrepo if she used the skin test on her South American blastomycosis patients, and, if so, what value she thinks it has.

Chairman Restrepo: We do skin tests on all our patients, but only after the serologic tests have been performed. Here again one sees something similar to what has been reported in histoplasmosis and in coccidioidomycosis: the reaction is positive in most of the patients whose state of health is good, whereas those who have a disseminated type of disease do not react.

Personally, I do not believe that the cutaneous test can be used as a diagnostic tool, and I do not believe that it has a prognostic value either, because with amphotericin B therapy even those patients with advanced disease show some skin sensitivity once they have received the drug. I know that skin test correlations are used in other diseases, but I do not feel I have enough experience to do this for paracoccidioidomycosis.

Dr. Furcolow: I think that your suggestion, Dr. Restrepo, about the standardization of the methods and the antigen in studies, particularly studies that would attempt to make comparisons, should be carried out in the five centers that you mentioned.

With regard to the histoplasmin serologic situation for the clinicians, I should like to point out that I have had no success whatever with the latex agglutination test. The first six cases we had in children were proved by precipitin; they had been negative by latex agglutination.

The question of skin testing affecting antibody response is of very little importance to the clinician. I do not know whether Dr. Kaufman emphasized this point sufficiently. The stimulus is almost entirely to the histoplasmin antigen. In our experience, that is the one that reacts the least often. Hence, from the clinical point of view the interpretation of a positive serology as being significant can be pretty well assumed without worrying about the possibility of cross-reactions, which are not very common.

Dr. Kaufman: I tend to agree with Dr. Furcolow. We do not use the latex test on a routine basis. The antibodies are very transitory. We see many nonspecific reactions, and we find that the complement fixation test detects both acute and chronic disease.

I would also like to make a comment along the lines of what Dr. Huppert said earlier. On the basis of the experience we have had in our laboratory with immunodiffusion tests, I think it is important, when they are employed for diagnosis, that they be used with reference sera that have been well defined as to specific and nonspecific precipitin bands. This is particularly important if we wish to establish reproducible and uniform procedures that give comparable results regardless of where the test is performed. Standard reference sera must be used in every test, and a diagnosis must be based on demonstration of lines of identity.

Dr. Greer: For the last year I have been using Dr. Restrepo's antigen and control serum to study the epidemiology of paracoccidioidomycosis. To date, 33 confirmed cases have been seen. All had at least one band in the immunodiffusion test with *P. brasiliensis* antigens and about 50 per cent had two bands. Only two cases cross-reacted with histoplasmosis antigen and showed bands to both antigens.

Immunodiffusion tests have also been done on family members of these patients and on members of matched control families. The results from 287 individuals, whether they had contact or not with a paracoccidioidomycosis patient, were negative. You can see, therefore, that similar results were obtained from the same antigens even though used in another mycology laboratory.

I wish to thank Dr. Restrepo for these materials, which allowed me to begin the studies earlier than I had anticipated.

Chairman Restrepo: I should like to point out that the patients Dr. Greer is talking about are not included in the 61 cases I just reported.

Dr. Muchmore: I would like to ask Dr. Kaufman and Dr. Kaplan a question regarding the indirect fluorescent antibody tests they described. They spoke mostly about the results in patients, and I would like to ask for some information on the results in normal sera—that is, serum from subjects who have no clinical disease. Does the fluorescent antibody technique for histoplasmosis pick up subjects who are negative by gel diffusion and vice versa? For example, is there a correlation between the H and M bands and immunofluorescence?

Dr. Kaufman: We are using the IFA test to detect antibodies to *C. neoformans* and the FA inhibition test to detect antibodies to the yeast form of *H. capsulatum*. Now, you are asking whether or not normal and H and M precipitin-positive sera are reacting in the IFA test for cryptococcosis. Is this correct?

Dr. Muchmore: I am asking about the incidence of IFA positive tests in sera from normal subjects against *C. neoformans* cells on the slide, and the same thing for *H. capsulatum* cells on the slide for IFA and immunodiffusion.

Dr. Kaufman: The IFA test as used in our laboratory (with a 1:20 serum dilution) does not show reaction with sera from individuals who are "normal," i.e. individuals apparently free of mycotic disease, regardless of whether we are using *H. capsulatum* or *C. neoformans* antigens. I do not have any information as to the reaction of H and M precipitin-containing sera in IFA tests, but I do realize that the IFA tests cross-react with sera from individuals who have candidiasis, histoplasmosis, cryptococcosis, or blastomycosis; therefore, I would assume that H and M precipitin positive sera would also react.

I employ a good deal of caution in interpreting the results of the IFA tests. I consider them to be the least specific of all the tests I use. Since they involve the binding of human serum antibody to fungus antigens, regardless of whether the binding is due to a specific or nonspecific antibody, they must be adequately controlled.

Dr. Mayorga: It has been said here that the indirect fluorescent antibody technique is one of the most nonspecific tests for determining a fungal infection, and it is considered that a lot of individuals have antibodies against *Candida albicans* in their blood. I would like to ask Dr. Drouhet about his experiences at the Pasteur Institute with semiquantitative titration by the indirect fluorescent antibody technique in pulmonary candidiasis.

Dr. Drouhet: In normal subjects, we have seen a titer of fluorescent antibodies against *Candida albicans* type A at 1:10 and 1:20, but in subjects with *C. albicans* in the digestive tract or with minor mucocutaneous candidiasis, this titer is 1:40 and occasionally as high as 1:80. In patients who have had septicemia for more than 10 days, the titer can be as much as 1:180 to 1:640, or more. Such titers are also seen in patients with pulmonary candidiasis. Thus, it is very important to know that titers of less than 1:20 are normal. We found this titer for

Aspergillus fumigatus fluorescent antibodies in apparently normal persons, whereas in patients with aspergillosis the titer ranged from 1:40 to 1:640.

Dr. Furcolow: In the *New England Journal* we reported the number of cases in which we attempted to analyze the significance of negative versus strongly positive reactions to the CF tests and the skin tests. We found that patients with a strongly positive skin test had a little worse prognosis than those with negative ones. The same was true with the serologic tests: patients with higher titers had a poorer prognosis than those with low titers. The difference was not very marked. This theory that we could select anergic people did not hold up when the cases were followed clinically.

Dr. Pappagianis: I think Dr. Furcolow is specifying histoplasmosis when he refers to the possible lack of correlation between anergy and prognosis. With coccidioidomycosis we can be quite secure in relating failure to develop higher sensitivity response to the skin test and the outlook for the patient.

Of course, we must also recall that approximately 3 per cent of patients with pulmonary residuals, either cavities or coccidioidomas, may fail to give a positive skin test response even with 1:10 coccidioidin. This concentration should be used before one concludes that the patient is anergic.

In most cases in which anergy develops, one can recognize dissemination, and the titer will be accordingly high in the complement fixation test. Again, one cannot use a magic figure of 1:16 in every case of dissemination. One must qualify whether there has been widespread development of lesions or whether there are single extra-pulmonary foci in the bone, for instance, or in the skin—in which case the titer may be relatively low.

Session III

Wednesday, 25 February 1970, 9:00 a.m.

THERAPY

Chairman

Edouard Drouhet

Rapporteur

H. B. Levine

THE TREATMENT OF SUPERFICIAL MYCOSES

Nardo Zaias

The superficial mycoses are perhaps the most common dermatological conditions affecting human beings in the tropical and subtropical areas of the world. By and large, they can be divided into two major categories: (1) those causing pigmentary skin disorders with little, if any, symptomatology, e.g. tinea versicolor produced by the yeast *Malazzesia furfur*, and (2) those causing discomfort, often disabling the patient. Examples of the latter are infections by members of the genera Trichophyton, Microsporum, Epidermophyton, the so-called "ringworm fungi," and the yeast *Candida albicans*. The observations in this paper will be restricted mainly to the second category, since these conditions are symptomatic.

In order to rationally treat these infections, one must be aware of the following: (1) the characteristics of the horny layer in which these fungi live; (2) the physicodynamics of the horny layer; (3) the host tissue reaction evoked by these organisms; (4) the pharmacology and mechanisms of action of the antifungal agents used; (5) the various routes of administration of antifungal agents; and (6) the response of the fungi to the specific antifungal agents.

An awareness of all these associated factors and the correction of any irregularities in them will lead to cure of the disease, provided, of course, that the antimycotic agent is effective. Often, disregard for one or more of these factors will result in failure of cure, even though an effective therapeutic agent is used.

The ringworm fungi usually inhabit the dead horny outer layer, or stratum corneum, of the skin. They exhibit characteristic hyphae, which are long filamentous fungal elements, and they seldom, if ever, produce other structures by which they can be specifically identified. Their growth into the living portions of the skin, the stratum malpighii, is apparently inhibited by serum factors produced by the host (*3*). When invasion of the epidermis and dermis has occurred, it has been associated with a decrease in the inhibitory serum factor (*12, 13*) and with immunological abnormalities the nature and extent of which are as yet undetermined.

The dead horny cells of the stratum corneum are constantly being shed in glabrous skin and in follicular orifices, the turnover rate being two to three weeks (*14*). Organisms living in this layer then have to continually grow into the deeper portions to prevent being cast off. This fact may account for the recurrence of ringworm lesions that have been treated for less than four weeks.

A special situation exists in hair and nail infections. Scalp hair invasion by ringworm fungi involves the dead portions of the hair from the keratogenous (root) zone outward. Since the rate of hair growth is just about 1 mm per week and the hair root usually is approximately 3 to 4 mm from the surface of the skin, it would require a minimum of four to six weeks of growth for all the fungal particles to be shed. Fingernails grow at a slower rate than hair—0.7 to 1 mm per week—and toenails at an even slower rate. Therefore, the time involved in shedding fungus from nails may be as long

as four months for fingers and nine months for toes.

Prior to 1959, the treatment of superficial mycosis was dependent principally on topical agents. Most of these preparations were strongly fungicidal *in vitro* but not effective on the surface of the skin. There were also "keratolytic" chemicals that were designed to increase the "shedding off" rate of the horny layer and therefore cause the fungi to be cast off more rapidly. X-ray therapy was also used on affected areas of the scalp, which resulted in an effluvium of the infected hairs. In many instances, radiation led to disastrous complications later (*1*). All therapeutic efforts had failed completely with onychomycosis and tinea pedis.

In 1958, Dr. Harvey Blank and his coworkers at the Department of Dermatology, University of Miami School of Medicine, showed conclusively for the first time in humans that griseofulvin was a uniquely successful systemic therapeutic agent against superficial ringworm infections, exclusive of yeasts (*4*). When applied topically, however, this drug had no clinical effect. Twelve years after its introduction, oral griseofulvin has shown remarkable efficacy and a surprisingly low incidence of side effects and toxic reaction.

The precise mechanism of action of griseofulvin is not completely clear, but it seems to disrupt the mitotic spindle, and possibly other cellular microtubules, leading to multiple nuclei and DNA accumulation (*8*). Griseofulvin also affects the fungal cell wall, resulting in abnormal curling and disturbance of the hyphae. Young or nearly formed hyphae are stunted, while the older already formed hyphae are spared (*2*).

Griseofulvin permeates and remains in all living cells of the epidermis. As they mature to become horny cells, they will form a continuous layer of griseofulvin-containing cells for as long as the drug is being taken. This, in effect, creates an antifungal barrier under the fungi that remains until the normal shedding-off process eliminates the fungus (*11*). Treatment schedules should therefore take this principle into account and be adjusted according to the skin or adnexae involved.

Griseofulvin

This antibiotic is available in micro-particle form, which is absorbed in greater amounts from the gastrointestinal tract than the large particle size. Absorption is further enhanced by the presence of fatty substances in the gut (*6*). Reports of toxicity and side effects have been minimal. Except for headaches, all other effects, including skin rashes, photodermatitis, and exacerbation of acute intermittent porphyria, are rare (*5*).

So far, pertinent information has been presented on anatomy; growth; shedding rates of the dead layer of skin, hair, and nails; living habits of the fungi; metabolism; and facts known about the pharmacodynamics of griseofulvin. What remains to be discussed is the reaction of the fungi to this drug. Certainly, over the 12 years that griseofulvin has been used, the reports of resistant fungal strains to the antibiotic have been infrequent. There have been observations with *Trichophyton rubrum* (*10*), *T. tonsurans* (*9*), *T. violaceum* (*7*), *Epidermophyton floccosum* (*7*), and *Microsporum canis* (*10*).

It seems clear that if the infection is produced by a fungus susceptible to the concentrations of the drug obtained in blood levels when taken under optimal conditions, such as with the microparticle drug size and with optimal gastrointestinal absorption, then the infection *will* be cured if the drug is taken for a long enough period of time.

Treatment schedules for various forms of ringworm infection are given in Table 1.

Glabrous skin (tinea capitis and tinea cruris)

In tinea corporis and tinea cruris, pruritus may significantly decrease or completely stop soon after griseofulvin therapy is started. Clinical lesions such as scaling and vesicles may disappear entirely by the tenth to fourteenth day of therapy. Nevertheless, administration of the drug must be continued for 28 to 30 days, or else relapse may occur.

Table 1

Dose schedule of microcrystalline griseofulvin for various clinical ringworm forms

Condition	Dose	Duration
Tinea capitis		
Fluorescent type	3 g (88% cure, repeat again in 6 weeks if needed)	Stat. dose
Nonfluorescent type	15–25 mg/kg/day	6 weeks
T. corporis	1 g daily	30 days
T. cruris	0.5–1.0 g daily	30 days
T. pedis		
Without onychomycosis	1 g daily	6–8 weeks
With onychomycosis	See onychomycosis	
Onychomycosis [a]		
Fingers	1.5–2.0 g	4–6 months
Toes	1.5–2.0 g	8–12 months

[a] Avulsion of nails is recommended.

Tinea pedis

Uncomplicated tinea pedis, without onychomycosis or toe-webb candidiasis, requires at least six to eight weeks of griseofulvin therapy. Relapses are common. The reason for the high relapse rate is not precisely known, but the tropical environment of the shoe-clad foot, the greater epidermal thickness of the sole, excessive sweating, and reinfection may play a role. The best therapeutic regime includes administration of oral griseofulvin for eight weeks and the maintenance of a topical antifungal agent thereafter.

In complicated tinea pedis, that is with onychomycosis and/or simultaneous inter-toe-webb cutaneous candidiasis, each of the conditions must be treated separately with specific medication.

Tinea capitis

Three distinct clinical types exist: fluorescent, nonfluorescent, and favus. All conditions are cured by the daily administration of griseofulvin in doses of 15 to 25 mg/kg for four to six weeks. Moreover, fluorescent tinea capitis produced by species of Microsporum can be successfully cured (88 per cent) with a single 3 g dose (*15*).

Onychomycosis

Although most patients will respond to 1 g microcrystalline griseofulvin daily, some will require 1.5 g or even 2 g daily. The latter patients may have absorption abnormalities.

Fingernails usually clear up in four to five months. Toenails take as long as 18 months.

There is a definite advantage in avulsion of the nail involved. This procedure speeds up the process of nail growth, substantially diminishing the time required to complete griseofulvin therapy.

Topical antifungal agents

An effective topical antifungal agent is very much needed. It should be (1) a proven fungicidal compound that works at the surface of the skin and is not inactivated by the chemical environment operating on the surface of the skin; (2) an agent that penetrates the entire depth of the horny layer and preferably is retained there as a depot; (3) a preparation that does not produce topical irritation and is nontoxic if absorbed systematically.

Even though there are many clinically available topical antifungal agents, none have been critically evaluated with proper follow-ups. Often the "good results" reported are reflections of the self-limiting nature of localized ringworm lesions.

Cutaneous candidiasis

Candida albicans, like the ringworm fungi, inhabits the dead horny layer of the skin. Preferentially, Candida thrives in intertriginous areas. Unlike the ringworm fungi, however, Candida can live in the buccal and vaginal mucosae, and in 30 to 40 per cent of normal people it inhabits the gastrointestinal tract, which may, in fact, be the human reservoir.

The cutaneous lesion of candidiasis can be diagnosed because of its clinically characteristic

lesions: small red pustules that coalesce to form large red denuded or scaly plaques. The peripheral satellite pustules are usually diagnostic.

It is apparent that cutaneous candidiasis lesions last longer when treated with anti-Candida agents alone than when they are treated with a combination of anti-Candida agents and an adrenocorticosteroid preparation. This observation led Maibach and Kligman to show that the organism *Candida albicans*, even when dead, produced clinical signs and symptoms on the skin, which responded well to corticosteroids alone. Anti-Candida agents are necessary, however, to prevent further extension of the lesions.

Treatment

The polyene antibiotics have proved to be uniquely efficacious against yeasts and specifically against *Candida albicans*.

In topical application, the greatest concentration that is made to come in contact with the yeast will result in the most rapid cure. The polyene antibiotics combine chemically with the vital-membrane sterols in the yeast cell membrane, producing a permeability defect in the cells and subsequently their death.

By and large, the polyene antibiotics are rather unstable and are poorly absorbed from the gastrointestinal tract. Systemic administration must be performed by trained and experienced personnel, since kidney and hematologic complications may result. The topical agents most commonly used are nystatin, amphotericin B, pimaricin, and trichomycin.

Future trends

It is extremely fortunate that griseofulvin is both highly effective and nontoxic. However, a similar agent that could be used topically is greatly needed. For conditions such as tinea pedis and onychomycosis, the addition of a good topical fungicide would be of great importance.

Many new agents belonging to the nitroimidazole and benzimindozole group have shown promise. They need adequate clinical and scientifically evaluated trials.

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THE PREVENTION AND TREATMENT OF SUBCUTANEOUS MYCOSES

Antonio González Ochoa

The conventional term "subcutaneous mycoses," used in contrast to cutaneous and visceral mycoses, commonly includes rhinosporidiosis, chromomycosis, sporotrichosis, and mycetomas. However, the term is not entirely appropriate, since the last two of these mycoses can be located at deep, visceral sites, thereby changing both the prognosis and, to some extent, the treatment. The cutaneous, hematogenous type of sporotrichosis also frequently produces nodular elements in the bones, lungs, liver, and other organs, in addition to the subcutaneous nodes. With mycetoma, there is early involvement of the bones. When the actinomycetic type occurs in the chest, the site of 11 per cent of the cases caused by *Nocardia brasiliensis,* it frequently penetrates the lungs; when it occurs in the abdominal wall, it penetrates the peritoneal cavity and attacks the viscera.

In view of these considerations, and of the fungi's special selectivity in attacking the tegument, particularly the skin, the author has proposed (*2*) a classification of the mycoses based on the manner in which the different fungi attack the skin. Accordingly, the term "exclusively tegumentary mycoses" is used for the so-called cutaneous mycoses, "initial tegumentary mycoses" for the so-called subcutaneous mycoses, and "secondary tegumentary mycoses" for the visceral mycoses. The members of the first group do not, except in unusual cases, go deeper than the uppermost layers of the epidermis; they also attack the hair and nails. With those in the second group, the infection begins in the skin, but the involvement goes deeper than the skin and attacks the subcutaneous tissues; in the case of some fungi, such as the causative agents for sporotrichosis and mycetoma, the infection can attack the muscles, bones, joints, and even the viscera. With those in the third group—that is, the secondary tegumentary mycoses, which are the equivalent of the visceral mycoses—the disease starts in the deep structures, particularly the lungs, and *a posteriori,* as a function of the fungus' and the patient's survival, it appears in cutaneous locations. Since this classification attempts to group biological phenomena together, there are bound to be exceptions, such as nocardiasis, which does not spread to the tegument.

The prevention of these diseases is impossible to even envisage at this time. For one thing, the etiologic agents are entrenched in soils and plants from which they cannot be eradicated. Moreover, the diseases themselves are sporadic, and there are no predisposing factors, other than an individual's occupation, for acquiring them.

Rhinosporidiosis

The only treatment for rhinosporidiosis is destruction of the lesions, for which purpose an electric bistoury is recommended. Although some drugs have been tried, as of now there is no agent that cures this mycosis.

Sporotrichosis

Many different drugs and resources have been suggested for sporotrichosis, but potassium iodide continues to be the best specific treatment for the lymphangitic and fixed types of cutaneous sporotrichosis. The prognosis for these conditions is generally very good, and spontaneous cures have been reported. Potassium iodide is less effective for the hematogenous type, and it is not adequate when the disease attacks the bones or viscera. In such instances it should be used in association with griseofulvin or, in severe cases, with amphotericin B. Potassium iodide's lack of toxicity, economy, and ease of administration make it the agent of choice. In these respects it compares favorably to the other drugs that have been suggested for the treatment of sporotrichosis, including the sulfonamide compounds, sporotrichin therapy, diethylstilbesterol, antimony salts, X-5079C antibiotic, griseofulvin, and amphotericin B, and to nonpharmacological resources such as radiotherapy and steam treatments.

The author recommends administering potassium iodide with a teaspoon, starting with 0.50 g for adults and increasing the dose by this same amount every day up to 3 g. The daily dosage of 3 g should then be continued for three or four weeks after cure. In the author's studies, no iodism phenomena intense enough to warrant suspending treatment have been observed. This may be attributed to the care taken to be sure the preparation is chemically pure and also to the fact that the spoonfuls are given in a little milk after each of the three main meals. A great deal has been said about the lack of knowledge of how potassium iodide acts, since *Sporothrix schenckii* develops in culture media containing 10 per cent of the drug. A study recently appeared showing that there is a greater sensitivity to potassium iodide in the yeast phase than in the mycelial phase. It contends that the drug's usefulness in treating this mycosis lies in the fact that the yeast phase is the parasitic stage. However, although there is greater sensitivity to the iodide in this stage, the agent cannot be having a fungistatic action, since the concentrations needed to produce inhibition are not attained with the usual therapeutic doses. It is interesting that *S. schenckii* has not acquired resistance to potassium iodide, and it has often been observed that patients who suspend treatment and suffer a relapse are benefited again when treatment is renewed.

As stated above, in hematogenous cases of cutaneous sporotrichosis and in cases where the site is in the bones, lungs, liver, or other organs and there is no response to iodide alone, griseofulvin—and in severe cases, amphotericin B as well—should be added to the potassium iodide.

Mycetomas

In the mycetoma syndrome—that is, a fistulous tumor in which the causative agent, either an actinomycete or a mold, takes the form of cumulus mycelia or microcolonics—a different treatment should be used. With the actinomycetic mycetoma, particularly the frequent type caused by *Nocardia brasiliensis,* a cure can often be achieved with medical treatment. A paper presented by the author at the VI International Chemotherapy Congress in July 1967 (*3*) cites the results obtained by treating this kind of mycetoma with 4-sulfanilamido-5,6-dimethoxypyridine (Fanasil, Roche). The preliminary trials with this drug begun in 1961 seemed to indicate some superiority over diaminodiphenylsulfone (DDS), which had been in use in Mexico since 1947. The general evaluation of the results with both drugs showed that cure was effected in approximately 30 per cent of the cases after periods of two to four years of treatment, marked improvement was achieved in 60 per cent of the cases, and failure was recorded in 10 per cent. These variations in the results were related to the extent and duration of the lesions and, more important still, to the presence or absence of bone involvement.

In 1966, studies were begun with trimethoprim in laboratory animals and in the clinic. The results showed transadditive clinical effectiveness when this agent was associated with the sulfa

drugs. That is to say, there was reciprocal potentialization of the trimethoprim and of the different sulfonamides used, particularly sulfamethoxazole. Trimethoprim, or 2,4-diamino-5-(3, 4,5-trimethoxybenzy-pyrimidine), is a bactericidal substance with antifolic action. Sulfamethoxazole corresponds to 5-methyl-3 sulfanilamidoisoxazole. The association of these two drugs (Ro 6-2580/11) interrupts two successive stages in the metabolic process that is essential to the life of the susceptible germs. The drug, known as Bactrim, first inhibits the enzyme responsible for synthesizing dihydrofolic acid and then later inhibits the enzyme charged with reducing this substance to tetrahydrofolic acid. Both inhibitions block the formation of DNA and RNA in the microorganisms, thereby not only making it impossible for them to reproduce but also causing their death (*1, 8*). These successive actions are strongly synergistic, so their effectiveness increases ten times over that which would be produced by inhibiting each enzyme separately. Bactrim's action covers a broad range of gram-positive and gram-negative bacteria, including Salmonella, Neisseria, *Escherichia coli,* Proteus, and many others.

Although *in vitro* studies on trimethoprim's action against *N. brasiliensis,* alone and in association with Fanasil, produced no interesting results, the author decided to use this drug in treating mycetoma caused by actinomycetes (since what happens *in vitro* does not always run parallel to what happens *in vivo*). The preliminary results of this therapeutic trial, published a few months ago (*5*), and the results being presented here, despite the short experimentation period (two to sixteen months) and the limited number of cases (20 patients), are very encouraging. In this period of two to sixteen months, clinical cures were effected in 50 per cent of the patients, marked improvement was achieved in 45 per cent, and in only one patient was no change observed. In this last case it became necessary to withdraw the drug temporarily after two months' treatment because of the appearance of leukopenia; the treatment was renewed two months ago, and there has been no recurrence of this side effect to date.

The drug, which is available in the form of tablets containing 80 mg of trimethoprim and 400 mg of sulfamethoxazole, was given in accordance with the following two treatment schedules: Schedule 1 calls for one tablet every 12 hours from the start of treatment until clinical cure is achieved; Schedule 2 provides for one tablet every 24 hours beginning at the time clinical cure is effected in order to prevent a relapse. In general, this latter schedule is continued for four to twelve months, and it has maintained the cure so that the few patients who have completed the treatment have not suffered any relapse.

In all, 20 cases were treated. Examples of a few different patients will be presented to give an idea of the excellent results obtained in such a short time.

Case 1 was a mycetoma of the foot with bone involvement, which had existed for nine years and had been treated for three years with DDS and sulfa drugs without much improvement. After two tablets of Bactrim daily for ten months, a clinical cure was achieved, and a dosage of one tablet a day was continued for four months more. Four months have now passed since the drug was withdrawn, and no relapse has occurred.

Case 2 was a mycetoma of the ankle with no bone lesion, which had been treated with DDS for nine months with only slight improvement. After two tablets of Bactrim daily for five months, a clinical cure was achieved, and the patient is still taking one tablet a day.

Case 3 was a mycetoma located in the right forearm with no bone involvement, which had existed for nine months. After five months of treatment with two tablets of Bactrim a day, a clinical cure was achieved. The patient is continuing to take one tablet a day.

Case 4 was a mycetoma located on the dorsum of the right foot with minimum bone involvement, which had existed for six months. A clinical cure was achieved after four months' treat-

ment with two tablets of Bactrim a day, and the patient is still taking one tablet a day.

Undesirable side effects observed were leukopenia, which appeared at an early stage in some patients on Schedule 1. When they were switched to Schedule 2, normal figures, or an increase to over 5,000, were obtained, and the patients were then able to continue the treatment under close control. Cases requiring withdrawal of the drug were rare.

Chromomycosis

There is no effective treatment for chromomycosis. Isolated cures have been reported with many different drugs and therapeutic resources, but the results are so random that a treatment that was successful for one patient can be a failure with the next. Cures have been reported with electrocoagulation of the lesions, surgical removal followed by grafts, iontophoresis with copper sulfate, x-ray, potassium iodide, heavy metals, sulfonamides, vitamin D_2 in association with potassium iodide, amphotericin B applied intralesionally, and finally thiabendazole. The last, used by Solano (*9*), appeared to be promising; however, although noticeable improvements were obtained, none of the 14 patients in the series were cured.

A recent report on a new antimycotic agent, 5-fluorocytosine (5-FC), has demonstrated the usefulness of this drug in experimental infections of mice produced by *Candida albicans* and *Cryptococcus neoformans* (*6*). In December of last year the author presented a note at the VII Central American Dermatology Congress (*4*) on cures achieved in two cases of cryptoccosis and two cases of chromomycosis. No reports of this drug's usefulness in treating the latter disease had been made previously.

Fluorocytosine is an antimetabolite that acts on the cytosine of certain fungi while at the same time apparently having no effect on the cytosine of human cells. It is available in the form of 500 mg tablets, and the conventionally recommended dose is approximately 100 mg/kg, with a treatment duration of four weeks.

In the two cases of chromomycosis that were cured with this drug, the patients, each weighing approximately 60 kg, received 10 tablets a day, three after breakfast, three after lunch, and four after dinner. The treatment was continued until clinical cure was obtained, which in spite of the recommendation was prolonged in one case for 12 weeks and the other for 31 weeks, although in both instances the drug was withdrawn for eight weeks. One of the patients, whose pretreatment picture showed verrucous lesions that had existed for 20 years, took 1,100 g of the drug, and clinical cure was achieved after 31 weeks. In another case, clinical cure was effected by the time the patient had received a total of 400 g.

In view of the absence of side effects and collateral clinical alterations, biochemical or hematic, the number of cases treated should be increased. Although the drug proved to be completely innocuous in the author's experience with two cases of chromomycosis, two cases of cryptococcosis, and one case of moniliasis, Tassel and Madoff (*10*) mention a case of pancytopenia that occurred after three weeks' treatment at 4 mg a day, and Mayorga *et al.* (*7*) mention another case in which severe leukopenia also appeared at an early stage.

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THE TREATMENT OF COCCIDIOIDOMYCOSIS, CRYPTOCOCCOSIS, AND HISTOPLASMOSIS

John H. Seabury

Coccidioidomycosis, cryptococcosis, and histoplasmosis are all treated with the same major medicament: amphotericin B. Although 5-fluorocytosine shows promise in the treatment of cryptococcosis, and saramycetin in the management of histoplasmosis, neither agent is available for general evaluation. No attempt will be made to assess these latter agents in the present communication.

The patients with histoplasmosis and cryptococcosis summarized in Table 1 have all been treated directly by the author and kept under post-treatment interval observation for at least one year. The longest period of observation has been thirteen years in a patient with cryptococcal meningitis.

Table 1

Treatment with amphotericin B

Diagnosis	Results	No. of patients
Histoplasmosis (30 cases)	Primary cures	23
	Death from Rx	1
	Living relapses	2
	Late deaths	4[a]
Cryptococcosis (30 cases)	Relapse (retreated successfully)	1
	Primary cures	24
	Primary failures, < 22 days Rx	5
	Death from other causes	4
		3[b]
Coccidioidal meningitis (Winn) (31 cases)	Fully recovered	13
	Outpatient activity	6

[a] 2 deaths from histoplasmosis; 2 deaths from other causes but with culturable Histoplasma.

[b] See text for explanation.

The author's experience with coccidioidomycosis since the availability of amphotericin B is too limited to be meaningful. The patients with coccidioidal meningitis reported by Winn (9) have been included in Table 1 because coccidioidal meningitis appears to be the most difficult form of the disease to treat, and because the quality of his studies is very high.

Symptomatic infections with *Histoplasma capsulatum* and *Cryptococcus neoformans* are seen with about equal frequency in New Orleans, Louisiana. Pulmonary cryptococcosis has been recognized more often since improved clinical pathological and anatomical pathological techniques have been applied to sputum and lung biopsies. In the present author's studies, pathogenic cryptococci have not been isolated from sputum or bronchial washings of patients without demonstrable pulmonary lesions.

Of the patients with histoplasmosis listed in Table 1, half had acute or chronic disseminated disease. Initial treatment failed in 20 per cent of the cases. The patient who died from treatment is excluded for reasons that will become apparent. All the deaths were among patients with disseminated disease, although only half died from histoplasmosis. Nevertheless, the two deaths from other causes had culturable *Histoplasma* at autopsy and should be regarded as treatment failures. All deaths with culturable *H. capsulatum* had adrenal involvement.

Both the living patients with relapses have

been retreated with at least temporary success. One was first treated in 1960–61 and relapsed microbiologically in 1964. Extensive pulmonary spread occurred in 1966. The patient was retreated in 1967 and remains negative, although he has developed pulmonary tuberculosis. The second patient with primary relapse was first treated for fibrocavitary pulmonary disease in 1961. He relapsed and was treated with saramycetin in 1963. He had still another relapse two weeks later and was retreated with amphotericin B in 1964 and again in 1965, having shown x-ray evidence of renewed activity and a sputum positive for Histoplasma. Both patients had underlying chronic obstructive lung disease. Both had the type of fibrocavitary pulmonary histoplasmosis for which, in the opinion of the author, resective surgery is indicated. Neither could tolerate surgery. These two patients have been continued on 3 g of sulfadiazine daily since their last retreatment with amphotericin B. There has been no evidence of recurrence, but it is not known how much importance should be attached to this hopefully suppressive sulfonamide therapy.

Ignoring the death due to treatment, which will be discussed, the actual failure rate in the treatment of histoplasmosis among the patients studied by the author is at present 13.3 per cent.

The case of the patient who died from treatment has little relevance to the problem of histoplasmosis management other than for its precautionary value. She had received corticosteroid therapy for rheumatoid arthritis for several years before developing fever, severe anemia, thrombocytopenia, hepatosplenomegaly, and a diffuse pulmonary infiltrate. The bone marrow was positive for *H. capsulatum*. She was given a portion of a 5 mg infusion of amphotericin B before she developed hyperpyrexia and pulmonary edema. Despite rapid digitalization and diuretics, diffuse erythema and irreversible shock occurred. It is believed that death was precipitated by a Herxheimer type of reaction. Autopsy revealed the most extensive Histoplasma involvement ever seen by the author.

In the present studies, experience with cryptococcosis has shown it to be a most responsive systemic mycosis. The series reported by the author is unusual, perhaps, in containing so few patients with lymphomas and other neoplasms. Of the 30 patients described in Table 1, 23 had meningitis as the presenting finding. One patient relapsed 18 months after treatment but responded promptly to intrathecal and intravenous therapy. This patient has remained well for more than seven years since retreatment.

Failures in treatment for cryptococcosis should be explained in order that they may be considered in the proper perspective. Such cases are reported briefly in Table 2. In the author's opinion, none of the failures can be attributed honestly to the inadequacy of amphotericin B. The patient who died after four days of treatment with the insoluble suspension of amphotericin B was moribund on admission and was the first patient to be treated under the program. The second failure received only six days of low-dosage therapy before succumbing to septicemia due to *Escherichia coli* and *Staphylococcus aureus*. The third failure is illustrative of that type of person who in the United States is often called the "born loser." After having been treated quite successfully for lupus erythematosus disseminatus for several years with corticosteroids, she was admitted with a deep abscess due to *Nocardia asteroides*. She responded well to treatment, and her infection healed. How-

Table 2

Cryptococcal failures

(Number of days of treatment)

Primary:

- 4 days Rx (insoluble)
- 6 days Rx (Staph. & *E. coli* sepsis)
- 19 days Rx (*E. coli* sepsis, disseminated lupus)
- 14 days Rx (insoluble—death 6 months later)

Other causes:

- 96 days Rx (Ommaya-staph., meningitis)
- 110 days Rx (Hodgkin's & Salmonella sepsis)
- 103 days Rx (Epidermoid Ca of lung, I yr. $\overline{p}$ Rx)

ever, she later became febrile, disoriented, and semicomatose. Cryptococcal meningitis and pneumonitis were present. During the 19 days of amphotericin B therapy, she suffered and recovered from two episodes of severe *Escherichia coli* sepsis due to a blocked urethral catheter. The third episode resulted in death. The fourth "primary" failure was one of the first patients in the series to be treated with the insoluble suspension of amphotericin B. Therapy was interrupted on several occasions because of angina pectoris precipitated by too rapid infusions. Treatment was refused after 14 infusions over a period of 21 days. Death from disseminated cryptococcosis occurred six months later.

Cryptococcal "failures" listed under "other causes" had no evidence of residual cryptococcosis at the time of death, although the patient with the Ommaya reservoir did have a few microscopically observable degenerate forms in the ventricular fluid. These were not culturable after two months of incubation.

It would be undesirable to state a failure rate for the cryptococcosis patients in this program because it would be quite out of line with the series of others. If death is a sole criterion, 23 per cent of the patients have died. This is not a valid basis for statistical analysis, however. If failure of effectiveness of amphotericin B is at issue, then one patient relapsed (a 3 per cent failure rate), with successful retreatment.

Among the 30 patients with cryptococcosis, seven had no discernible evidence of meningeal involvement. It would be reasonable to expect the latter group to respond to therapy better than those with central nervous system disease. Limiting the discussion to those whose only known disease was within the lung, one can legitimately ask if the patients would not have recovered spontaneously. The author feels that some of them would and others would not. Figure 1 is a good example of a young female patient whose future was certainly in jeopardy. Pulmonary cryptococcosis is undoubtedly quite common in many geographic areas. It may be

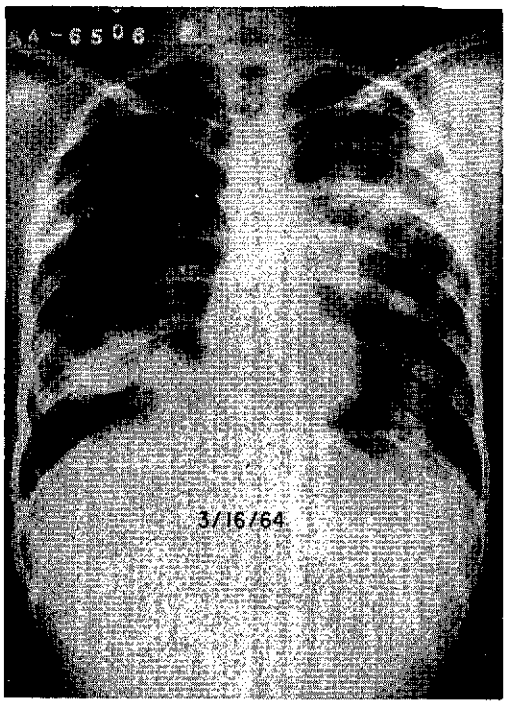

Figure 1. Multiglobular, bilateral pulmonary infiltrates in an adolescent female who liked to feed horses, chickens, and pigeons. Low-grade fever and left pleuritic pain were present. Sputum was positive for *C. neoformans*. Spinal fluid was negative.

suspected that most of the persons infected recover spontaneously, as do those with histoplasmosis, coccidioidomycosis, and tuberculosis. Which patient will be the one to suffer progression or late relapse? What are the criteria by which to judge? Certainly no one would deny chemotherapy to the patient with primary pulmonary tuberculosis, but there are those who question whether all patients with cryptococcosis should receive amphotericin B (*3, 4*). They are really asking themselves whether they know enough about this antibiotic to administer it safely.

Most of the patients with cryptococcal meningitis in the author's series have received intrathecal therapy. Whether this is important or not is unclear. Perhaps the same comment applies to daily versus alternate or every third day therapy intravenously. No randomized study of statistical significance seems to have been done,

and it is unlikely that it will be done unless the frequency of cryptococcal meningitis increases greatly. The problem of intrathecal, intracisternal, and intraventricular administration of amphotericin B will be discussed in connection with coccidioidal meningitis.

The desirable frequency of administration of intravenous amphotericin B is even more in limbo. It will remain so until it is known what happens to the antibiotic after administration. If it is quickly affixed to sterol components of fungal cell walls, assays may be meaningless. If it is stored and released below measurable levels, but still in an antifungal range, assays are also meaningless. Despite our early work in antibiotic assay (*7*), the author is at present very uncertain as to its importance in therapy. Rapid and permanent healing of observable lesions has been seen in patients receiving intravenous doses below the level of assayability. This same phenomenon apparently occurred in some of the early patients treated with oral amphotericin B by others as well.

The problem of dosage in the treatment of histoplasmosis and cryptococcosis is certainly not solved. Duration of therapy for chronic disseminated or meningeal disease due to histoplasmosis or cryptococcosis under the program in question has not been altered significantly since 1958. Two patients with cryptococcal meningitis received their major course of treatment intrathecally with excellent results. The author is of the belief—although there is no supporting proof—that all fungal meningitides to be treated with amphotericin B can be managed as advocated by Winn for coccidioidal meningitis; that is, with prolonged intrathecal, intracisternal, or intraventricular administration of the drug, intravenous dosage being limited to what is needed to treat the detectable disease outside of the nervous system. However, there is no proof that the injection of amphotericin B into the cerebrospinal fluid is essential for cure of either cryptococcal or histoplasmal meningitis, or, indeed, that such injections actually do improve prognosis. The experience of Winn with the much more difficult coccidioidal meningitis, however, strongly supports such instillations. The problem of treatment of fungal meningitis will be discussed in more detail in connection with coccidioidal meningitis.

The individual dose, whether administered daily or on alternate days, has been reduced since 1964 and is still being reduced. It can be seen from Table 3 that the dosages used in the present series are lower than those generally used by many physicians, and lower since 1964 than previously. Reduction of the dosage did not come about initially by experimental design. It took place simply because of increased toxicity, primarily renal, which became evident in 1964. For a brief period in 1962 it was possible to reevaluate the original insoluble suspension of amphotericin B against the colloidal preparation. Although closer nursing supervision was necessary, the insoluble suspension was effective and well accepted in several patients who had tolerated the colloidal suspension poorly or not at all. Reduction in the individual infusion dosage has not altered the therapeutic results, but it has reduced the difficulties connected with treating the patients. Caution should be observed in evaluating these data and the case report by Boone and Allison (*2*). Although experience has been limited in volume, it has been quite solid in post-treatment evaluation.

Table 3

Dosage with amphotericin B

Diagnosis		Dosage (mg)
Cryptococcosis		
	Median dose/kg/day	0.49
	Average dose/kg/day	0.526
	Average dose/kg/day	
	1956–63	0.59
	1964–68	0.45
	Average total dose	3.137
Histoplasmosis		
	Median dose/kg/day	0.55
	Average dose/kg/day	0.496
	Average dose/kg/day	
	1956–63	0.54
	1964–68	0.32

One should remember the early successes reported after oral administration of amphotericin B. Observations such as these are provocative, but they prove nothing until a carefully designed study is carried out based on the premise that currently recommended daily doses of amphotericin B are excessive.

The indications for treatment of coccidioidomycosis, slightly modified from Winn, are listed in Table 4. The table need not be modified basically for either histoplasmosis or cryptococcosis except to reduce the recommended dosage by half. True, pulmonary cryptococcosis generally behaves in a much different manner, and cavitation is unusual. The author believes that the indications suggested by Winn for coccidioidomycosis hold for histoplasmosis as well, although little surgery has been involved in the limited experience of the program. Of 15 patients with a clinical diagnosis of pulmonary histoplasmosis only, in no case could surgery have been helpful; either the character of the disease did not warrant resection, or the underlying chronic pulmonary disease made surgery impossible or undesirable. There has been no evidence that racial origin alters the severity of either histoplasmosis or cryptococcosis.

Physicians with experience in coccidioidal meningitis will undoubtedly agree to the principles outlined by Winn in Table 5. Winn emphasizes the importance of early treatment. The presence of complement-fixing antibody in the cerebrospinal fluid is a positive indication, but 25 per cent of his patients had negative serology. Clinical findings alone are sufficient justification for treatment. The author used the intrathecal route in most of the patients with fungal meningitis, combining amphotericin B with hydrocortisone sodium succinate without a phenol preservative in the injected medication (*7*). Arachnoiditis has not been a problem. Winn prefers the intracisternal route.

Every patient with coccidioidal meningitis should have cultures made of the intraventricular fluid. If the fluid is culturally positive, amphotericin B should be instilled intraventricularly by means of the Ommaya reservoir. In addition, if a noncommunicating hydrocephalus is present, both intracisternal and intraventricular instillations are indicated, in addition to a ventriculo-atrial shunt.

The frequency of instillation of amphotericin B into the cerebrospinal fluid is debatable. Winn advocates a three- to seven-day schedule until complement-fixing antibodies have been absent for three months. The same requirement would certainly be present for those without complement-fixing antibody who have either detectable fungi or persistent symptomatology. Bindschadler and Bennett (*1*) believe that amphotericin B must be instilled in amounts of 0.2 to 0.3 mg daily or on alternate days in order to maintain inhibitory concentrations against *Cryp-*

Table 4

Coccidioidomycosis: treatment with amphotericin B

1. All disseminated: 5 to 10 g or more
2. Pulmonary
   a) Coverage before and after surgery (2–4 weeks)
      1) extending or exacerbating chronic disease
      2) enlarging peripheral cavities
      3) repeated, severe hemoptysis
      4) abscessing nodules
   b) Multiple pulmonary foci with rising C.F.
   c) Severe symptomatic primary, short course
   d) Progressive primary—? short course
      1) dark skin
      2) rising CF
   e) All symptomatic primaries with diabetes, pregnancy, corticosteroid Rx or dark-skin origin

Table 5

Coccidioidal meningitis
(Modified from Winn)

1. Short-term IV amphotericin B or as indicated by extra-CNS involvement
2. Long-term "suppressive" intrathecal or intracisternal Rx if detected early
3. If response poor, trephine and sample intraventricular fluid
   a) If +, Ommaya reservoir and intraventricular amphotericin B
4. Ventriculo-atrial shunt only if hydrocephalus
5. Secondary infection requires removal of a shunt or Ommaya reservoir

tococcus neoformans, an organism which is more sensitive to amphotericin B than Coccidioides. Their assays of spinal fluid levels following intravenous administration of amphotericin B do not agree with those of the author (7). Methods of assay have been different, and it is difficult to know who is correct. The fact remains that Winn's schedule has been successful and probably should be a standard for reference.

Our experience at Louisiana State University with ventricular shunts and Ommaya reservoirs has not been happy. Bacterial or fungal superinfection has been a problem. This has occurred both as a consequence of the surgery involved and as a late result. When infection occurs, the shunt or reservoir must be removed and replaced before one can hope to control the complication.

Coccidioidal granuloma, except for the rarely encountered inoculation chancroidal lesion, is a manifestation of dissemination. Intravenous amphotericin B is indicated, but the amount should be guided by the evidence for nonlocalized disease and the titer of complement-fixing antibody. Local therapy by application, instillation, or irrigation is indicated. Adjunctive use of lidocaine or corticosteroid may be necessary because of irritative effects. If many bones are involved, as in osteomyelitis of the foot, amputation is desirable. Long bones can be treated by intramedullary irrigation and sump drainage of 12 liters per day of 0.5 mg/ml solution of amphotericin B.

Although few would disagree seriously with Winn's management of disseminated and meningeal coccidioidomycosis, there are those who reject his recommendations for the treatment of pulmonary coccidioidomycosis. Hyde (5) is a prominent disbeliever in surgery for this form of the disease. His conclusions are based primarily on review of the experiences at a number of hospitals. Individual studies would appear to be of greater significance than reviews.

If surgery is to be done for pulmonary coccidioidomycosis, the experience of Winn and

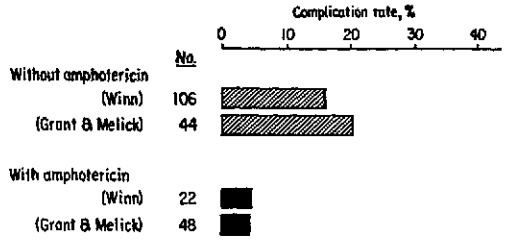

Figure 2. Experiences of Winn and Grant and Melick in the use of amphotericin B for surgery of pulmonary coccidioidomycosis.

that of Grant and Melick, depicted in Figure 2, fully justifies the pre- and postoperative use of amphotericin B. The complication rate depicted for Winn's cases without amphotericin B includes only major complications.

In the United States, surgery has become a minor modality of treatment for pulmonary tuberculosis. This is no reason to conclude, however, that the pulmonary mycoses should be considered similarly. The circumstances, and in some instances the pathogenesis and the pathology, are different. A multiplicity of antimicrobials are available for tuberculosis, most of which can be given for many months, but there is only one agent for coccidioidomycosis, histoplasmosis, and cryptococcosis. This antimicrobial can be given usually for not more than one year, and administration is expensive. The therapeutic indices for the principal tuberculostats seem to exceed the index of amphotericin B for the major mycoses. As a consequence of the latter consideration, the persistence of viable fungi in cavitary lesions is threatening if no amphotericin B is given, or if it is discontinued without surgery. The incidence of dissemination from such lesions is apparently low. The incidence of local extension is not yet fully documented. Hemoptysis, superinfection, new cavity formation, employability, and insurability are all strong factors in favor of resectional surgery under coverage by amphotericin B for the lesions listed in Table 4 (Section 2.a) and for similar lesions due to histoplasmosis when surgery is feasible.

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PARACOCCIDIOIDOMYCOSIS: SOME CLINICAL, PATHOLOGICAL, AND THERAPEUTIC CONSIDERATIONS

Mario Robledo

Paracoccidioidomycosis is the most common deep mycosis in Colombia and a number of other South American countries. In a series of 162 cases of deep mycotic infections studied in a general hospital in Bogotá, Peña (*25*) found 72 cases of paracoccidioidomycosis.

This mycosis—also known as South American blastomycosis, paracoccidioidal granuloma, Brazilian blastomycosis, Lutz-Splendore disease, and Almeida's disease—is endemic in Latin America. Cases have been described in most of the countries extending from Mexico to Argentina. So far, no cases have been reported from Chile. The highest incidence is found in rural areas with tropical climates in Brazil, Venezuela, Argentina, and Colombia.

It is caused by *Paracoccidioides brasiliensis*, a dimorphic or biphasic fungus whose habitat has not been as yet determined.

The portal of entry of the parasite is still a matter of discussion, and opinions concerning the pathogenesis are divided. Some authors (*2, 13*) sustain the theory that traumatic lesions, mainly in the oral mucosa, facilitate the entrance of the agents into the human organism. The observation of dental paracoccidioidal granulomas (*3, 8, 26*) lends support to this site as a possible portal of entry.

The lung as portal of entry has been assumed by González Ochoa (*10*), Londero (*14*), Mackinnon (*20, 21*), and Salfelder (*31*). Mackinnon (*21*), on the basis of experimental work, believes that paracoccidioidal mucocutaneus lesions in man are secondary to inflammation of the underlying striated muscle, this myositis being also secondary to hematogenous dissemination of primary lung lesions. In an attempt to prove the coexistence of mucocutaneous lesions and myositis in patients suffering from paracoccidioidomycosis, the author studied 24 deeply taken lip and tongue biopsies, including some muscle fibres, and severe paracoccidioidal myositis was found in all of them (Figure 1).

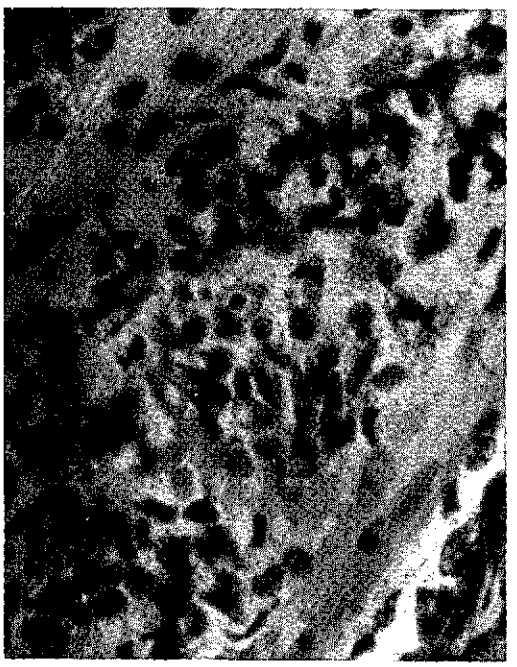

Figure 1. Tongue biopsy showing paracoccidiodal myositis.

Other sites of inoculation that have been reported are the tonsils (*6*) and the conjunctival (*2*), nasal (*2*), and anal mucosae (*22*). A few cases of infection presumably originating in the gastrointestinal tract have been reported (*10, 32*). Primary inoculation into the skin is apparently rare, and convincing evidence is difficult to obtain (*9*).

There are no reports of natural animal infection. The fungus has been isolated from the intestines of bats (*11*).

Since the pathogenesis of this disease is not established and the portal of entry of the parasite is not known, preventive measures against it have not yet been developed.

There is agreement on sex and age distribution in all the reports: males in the fourth and fifth decades are most frequently attacked. In a series of 70 cases (*28*), the present author found a lower age limit of 11 years and an upper limit of 62, with 37 patients in the fourth and fifth decades. Only five were females.

Symptomatology

Paracoccidioidomycosis has a wide variety of symptom complexes, and different clinical forms have been described depending on the most affected area: mucocutaneous, lymphangitic, visceral, and mixed forms. Actually, a clear-cut example of any of the pure types is rare; usually they are combined in various ways.

The mucous lesions are found most frequently on the surface of the lips, cheeks, palate, gums, tongue, and nose, and with somewhat less frequency on the eyelids. In the beginning, these lesions are erythematous and ulcerated, with characteristic tiny yellowish spots, interspersed with reddish dots, giving an over-all granular appearance. This picture was characterized by de Aguiar-Pupo (*7*) as a "mulberry-like erosive stomatitis" (Figure 2). The ulcers may become deeper, with destruction of tissues including even muscles and cartilages, and eventually produce deformities.

Cutaneous lesions may occur in any area of the body, but they are more frequent on the face,

Figure 2. Paracoccidioidomycosis. "Mulberry-like erosive stomatitis" on the upper lip.

around the mouth or nose, in continuity with mucous lesions. The skin lesions are usually ulcerovegetative, but there can also be papular, papulopustular, papillomatous (Figure 3), or crusted ulcers. They may occur in large numbers, and they usually appear on the face, al-

Figure 3. Paracoccidioidomycosis. Cutaneous and lymph node involvement.

though they may also be found on the extremities and trunk.

Fistulization of diseased lymph nodes, particularly in the cervical region, may give rise to a clinical picture very similar to scrofuloderma.

Keloid blastomycosis, or Lobo's disease, is now considered a different entity, with its own clinical, histological, and mycological characteristics (*29*).

Involvement of lymph nodes sooner or later in the course of the disease is a common feature of this mycosis (Figure 2). Sometimes the lymphadenopathy is the first and only clinical manifestation, and it can mimic tuberculosis or lymphoma.

The respiratory system is also frequently involved, and dysphonia, due to vocal cord involvement, is sometimes the first clinical symptom. In this location the gross lesions are very similar to those on the oral mucosa.

Pulmonary involvement is common in paracoccidioidomycosis; in some series, 85 to 100 per cent of the cases studied have lung involvement (*4, 31*), although in early reports of the disease lung lesions were thought to be infrequent (*1, 7*).

The roentgen findings are variable, usually located toward the bases of the lungs or toward the central field. The most common lesions are micronodular infiltrates, nodular condensations, and fibrotic areas. Cavities are seen less frequently, and calcifications are not usually seen at all (*30*).

Pathology

At autopsy, the pulmonary lesions resemble those of tuberculosis. They may be fibrotic, fibrocaseous, or cavitary. It is very common to find associated emphysema located mainly in the peripheral portion of the lungs. Pleural thickenings and adhesions are almost always present, but pleural fluids are not commonly found.

The gastrointestinal tract is not usually involved, except for the oral and pharyngeal mucosae.

Chronic cor pulmonale is a complication of pulmonary involvement very commonly found in autopsy material (*4, 31*). The present author and his co-workers found right cardiac hypertrophy in four out of five autopsies.

Involvement of many other organs has been observed, resulting in highly variable pictures. Adrenals, for example, are frequently affected, with or without Addison's syndrome, depending on the amount of destruction.

Arteritis is an important complication of paracoccidioidomycosis. Although there are few reports on this matter, it does not seem to be infrequent. Brass (*4*) in Venezuela found three cases in 36 autopsies. Angulo in Caracas has several cases (personal communication). The author had an opportunity to study two cases of paracoccidioidial arteritis, one of them with gangrene of the legs and Leriche's syndrome diagnosed while the patient was alive by microscopic study of the arteries of an amputated leg, and the other with mesenteric artery thrombosis microscopically diagnosed by studying the mesenteric arteries of the surgical specimen.

Histopathology

In the skin or mucous membrances there is a pseudoepitheliomatous hyperplasia (Figure 4) with intraepithelial microabscesses in which the parasites can be seen. The corium shows a suppurated-granulomatous inflammation with giant cells and occasional eosinophils. In compromised visceral tissue, the reaction is mixed, with caseous foci, scar tissue, abscesses, and tuberculoid granulomas. In the adrenals, the mycotic lesions are predominantly caseous with less tissue reaction than in other organs and with great numbers of yeast cells (Figure 5). The spores are usually easily seen even in the H-E stained sections (Figure 5). They lie free in the giant cells, and they are generally abundant. To detect the characteristic multiple budding of *P. brasiliensis,* it is necessary to search carefully, and in some cases special staining methods have to be used, the best of which is the silver impregnation of Grocott. With this stain, typical steering-wheel forms, dwarf yeast cells,

Figure 4. Lip biopsy showing pseudoepitheliomatous hyperplasia of the epithelium, and intraepithelial microabscesses.

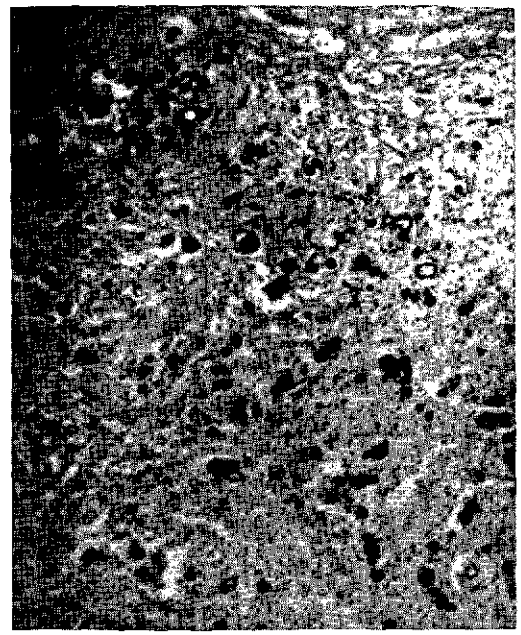

Figure 6. Lymph node biopsy showing abundant yeast cells of *P. Brasiliensis*. Grocott methenamine-silver stain. Magnification: × 100.

Figure 5. Adrenal gland with Caseous necrosis and abundant yeast cells. H-E. Magnification: × 400.

and regressive dust-like detritus are shown very well (Figures 6 and 7). Hyphae or pseudohyphae formation is exceptional, as is the presence of bacilliform buds. The size of the yeast cells is highly variable, from 2 or 3 to 40 or 50 miera (Figure 7).

Treatment

In 1940, Oliveira Ribeiro (*24*) in São Paulo, Brazil, began to use sulfas in the treatment of paracoccidioidomycosis. That step was a fundamental one, since no effective therapy had been available before. Many drugs, including iodides, gold compounds, dyes, and antimony salts, had been tried in the past without success. Almeida (*1*) reported good results with "specific" vaccines. A technique of electrocoagulation and surgical resection of local lesions was claimed to be of value.

Before the use of sulfonamides, the disease was usually fatal. Now clinical cures have even been reported and the prognosis has changed.

Sulfadiazine is the preparation most exten-

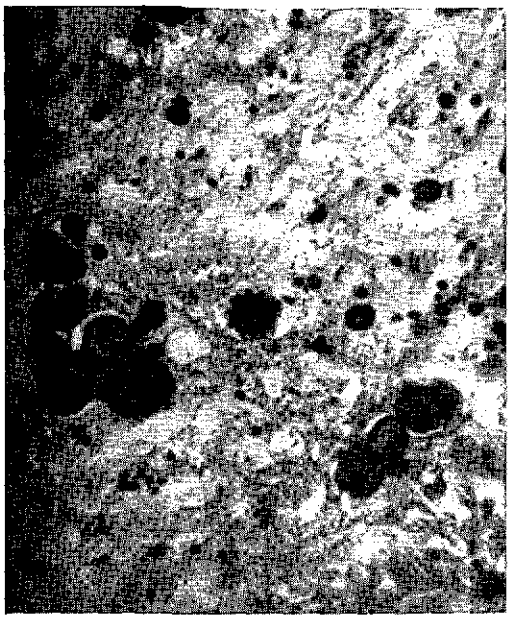

Figure 7. Giant and dwarf cells of *P. Brasiliensis*. Steering-wheel form in center of the field. Grocott methenamine-silver stain. Magnification: × 400.

sively used, doses being from 3 to 5 g a day, fractionally administered. Clinical improvement is usually noted a few weeks after the beginning of treatment. Relapses are frequent when the drug is discontinued. Sulfa resistance has occasionally been noted (*15*). Some pyrimidine derivatives (compound Ro 5–6846), can potentiate the antimicrobial effects of sulfonamides in sulfa-resistant cases. Good results have been obtained (*15*) with the association of both drugs. Slow-elimination sulfas are shown to be very effective. Sulfamethoxypyridazine in doses of 1 g the first day followed daily by doses of 500 mg has given satisfactory results. The new sulfa synthetized by Hoffman-La Roche Laboratories, known by the code Ro 4–4393, whose chemical formula is related to sulfadimethoxine and to the line of sulfadiazine, has been tried by several authors (*16, 17*). They have obtained very good therapeutic results without toxic manifestations and with the advantage of small doses of 1 or 2 g administered weekly. In 1957 Mackinnon *et al.* (*20*) obtained good results with diamidinodiphenylamine both *in vitro* and *in vivo* (one patient).

Treatment with sulfas must be started early in the course of the infection, the doses should be adequate, and it must be continued until clinical cure is obtained and serological reactions are negative.

The criteria of cure in a patient suffering from paracoccidioidomycosis, according to Prado-Sampaio (*27*), are clinical cure lasting at least two years, normal histology at the lesion area, normal sedimentation rate, and a negative serological reaction.

Amphotericin B (Fungizone, Squibb), has been shown to have marked fungistic action *in vitro* against a number of nonpathogenic and pathogenic fungi. In paracoccidioidomycosis it has been used by many authors (*5, 19, 23, 27*) with generally good results. This drug is poorly absorbed through the gastrointestinal tract, and the results with oral therapy have been disappointing. The blood levels obtained after oral administration are very low (*18*), whereas effective fungistatic blood levels are readily achieved by intravenous administration. The drug should be given intravenously every day or on alternate days. In general, the response to this agent is prompt. Common signs of intolerance are chills, fever, vein irritation, nausea, anorexia, aching, and malaise. All these symptoms are greatly reduced by the administration of steroids. In addition, steroids appear to have an effect in decreasing renal toxicity. The recommended average dosage of amphotericin B in different series of treated cases has been variable. In general, it ranges from 0.25 to 1 mg/kg of body weight per day of treatment, administered in 500 cc to 1,000 cc of 5 per cent glucose in water intravenously. A course of treatment is usually initiated with daily doses of from 15 to 20 mg given over a period of six or eight hours and increased by 5 mg daily until a desired level is reached.

The optimal duration of therapy for para-

coccidioidomycosis has not been established. It varies according to the circumstances of each patient. Negroni (*23*) recommends amphotericin B given in alternation with sulfas or androgens. Amphotericin B can be administrated in courses of one or two months each, to be repeated eight or nine times, for a total dose of 8 to 9 g. Androgens seem to have both a fungistatic and an anabolic effect. Sulfanilamides still have their place in the treatment of paracoccidioidomycosis, since amphotericin B cannot be administered to outpatients, it is an expensive drug, and it seems to have only a transitory fungistatic effect. Negroni (*23*) recommends the use of sulfas as a supplement to amphotericin B therapy or as a continuation of treatment for outpatients who have already attained cure with amphotericin B.

The general principles of management found to be of value in pulmonary tuberculosis and in other chronic infectious diseases are useful in complementing the therapy of paracoccidioidomycosis.

At the University of Antioquia Hospital, 38 patients suffering from paracoccidioidomycosis were treated from 1964 to 1968 (*30*): of 16 who received sulfas alone, six relapsed; of 22 who received both sulfas and amphotericin B, four relapsed. Toxic reactions occurred in 15 patients treated with amphotericin B, but treatment was discontinued in only three cases. Twenty of the 38 patients were followed for periods varying from six months to four years; two died as a consequence of the disease, while the remaining 18 improved and are now back at work.

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SOME IMMUNE RESPONSES TO *COCCIDIOIDES IMMITIS*[1]

H. B. Levine, G. M. Scalarone, and J. W. Fresh

Many field and laboratory studies of the last 20 years have indicated that immune responses play an important, if not dominant, role in the clinical history that follows exposure to *Coccidioides immitis*. Quite naturally, therefore, speculation has arisen concerning the prophylactic advantages of a vaccination program. There is immunologic, epidemiologic, and even ecologic support for this idea. But such a program also poses difficulties, both practical and academic, and these largely pertain to the immune responses in coccidioidomycosis.

Surveys reported by Smith, Pappagianis, and Saito (*21*) showed that Coccidioides infection, however mild, confers strong resistance to symptomatic reinfection in a very high proportion of subjects. This pattern of induced immunity pointed to the possibility that the fungus has potent immunogens. Pappagianis and co-workers (*16*), Converse *et al.* (*1, 2*), and others demonstrated this to be true in infected animals, and more recent studies showed that immunogenicity is a striking attribute even of killed coccidioidal particles, particularly spherules (*9, 10, 13, 14*). Subsequently, Pappagianis, Levine, and Smith found that such spherule vaccines were not unduly toxic in man in doses of less than 5 mg (*15*).

Other features pertaining to Coccidioides are compatible with the concept of control by vaccination. In the first place, the endemic predilection of the fungus for certain regions of North, Central, and South America (*3*) places certain populations at risk. In other words, populations that could benefit from an efficacious vaccine are geographically known. Secondly, within these geographical areas there are subpopulations, most notably Filipinos and Negroes, that are prone to suffer very severe consequences from infection (*3*). For these groups there is a clear-cut need for programs to help prevent occurrence of the disease, or, at very least, to impede its progress.

Thus a vaccination program is justified on the basis of need, urgency, identified populations at risk, and existence of an immunizing preparation of known worth in animals and low toxicity in man. Unfortunately, however, there are points against it as well. Pappagianis *et al.* (*15*) found that only about one third of the human volunteers acquired delayed dermal sensitivity to coccidioidin after vaccination. This could mean that at well-tolerated doses the threshold for successful immunization in the majority of subjects had not been reached. Much still remains to be learned about the relationship between delayed sensitivity and protective immunity. Spherule vaccination induced strong immunity in monkeys although very few of them acquired sensitivity to coccidioidin (*14*). The fact that serology or sensitivity is not altered in a uniform manner would pose problems if a trial in humans were being contemplated. There would not be

[1] Investigation supported by the Office of Naval Research and the Bureau of Medicine and Surgery, U.S. Navy, under a contract between the Office of Naval Research and the Regents of the University of California.

a consistent immunologic tag or marker among the vaccinees, and a diagnostic advantage would be lost in those that did convert.

In addition, a human vaccination trial would pose difficulties from the standpoint of logistics and interpretation of the results. Because of severe limitations in present therapy (*22*), we could not consider challenging volunteers in order to assess immunity; we would have to depend on natural exposure. In this situation, only about 40 per cent (*21*) of nonvaccinated volunteers residing in an endemic region might be expected to produce symptoms of infection with *C. immitis*, and in many the illness might not be sufficiently severe for them to consult a physician. For this reason, and also because mobility is a prominent aspect in our society, the number of volunteers would have to be large in order to obtain statistically meaningful results. This is an especially costly aspect, since the time of exposure cannot be predicted and the subjects would have to be followed for many years. The latter consideration would create logistic problems in organizing physicians and facilities for long-term clinical, roentgenographic, serologic, and sensitivity studies.

The erratic acquisition of delayed sensitivity following vaccination is one of the immune responses currently being studied in animals. Spherule-vaccinated mice readily survived intranasal challenge with 1,000 *C. immitis* arthrospores and showed an LD_{50} value of approximately 5,000 (*4*). In contrast, nonvaccinated mice showed an LD_{50} value of only about 50 arthrospores intranasally. As in monkeys, many of the immune animals—approximately 30 per cent—failed to demonstrate delayed dermal sensitivity to coccidioidin prepared commercially from the mycelial phase of growth. However, virtually all the spherule-vaccinated mice gave positive skin reactions when tested with a new coccidioidin prepared from the spherule growth phase (*8*). Pertinent data are summarized in Table 1, which shows also that formalin-killed mycelium is a poor sensitizing preparation and a relatively poor immunizing agent, as has been demonstrated earlier (*9, 10*).

In view of the past pattern of erratic sensitivity reactions with mycelial coccidioidin in spherule-vaccinated humans, there was great interest in testing the new more sensitive spherule reagent. Accordingly, Dr. Pappagianis conducted a small trial last year. The preliminary findings were disappointing. Although spherule coccidioidin in doses one tenth as great as those of mycelial coccidioidin was capable of eliciting comparable reactions, the percentage of reactors was not changed. It is still not known whether spherule coccidioidin will show a higher ratio of reactors at an equal dose.

The factors essential to a consistent response in man have yet to be identified. In animals, there are three considerations that seem to particularly influence the magnitude of the immune response: the phase and age of the coccidioidal culture from which the vaccine is prepared, the dose of vaccine injected, and the route by which it is delivered.

In regard to the first, numerous trials in mice (*4, 9, 10*) have confirmed that the spherule phase of growth is immunogenically superior to the arthrospore and mycelial phases. There is still additional evidence that immunogenic properties may reflect qualitative as well as quantitative differences among the several phases of coccidioidal growth (*8*). Endospores are also im-

Table 1

Delayed sensitivity reactions in mice tested with spherule or mycelial coccidioidins

Sensitizing preparations [a]	No. positive reactors/total	
	Spherule coccidioidin [b]	Mycelial coccidioidin [b]
Strain Silveira spherule vaccine	34/35	25/33
Strain Silveira killed mycelium	8/15	2/15

[a] 2.1 mg total in 3 equal doses at weekly intervals; animals tested 26 to 30 days after last dose; 35 nonvaccinated (control mice) were uniformly negative to the above doses of both coccidioidins.

[b] 0.008–0.009 mg of nondialyzable material per dose.

munogenic, but probably somewhat less so than spherules (*10*). The immunogens appear to reside almost exclusively in the spherule walls (*6*), and spherules grown for approximately 72 hours (*13*) are usually strongly immunogenic.

As to the second consideration, the minimum optimal vaccinating dosage for the mouse appears to be in the vicinity of 1.2 mg. However, dosages up to 2.4 mg are well tolerated. We generally use 2.1 mg, and this is administered in three doses of 0.7 mg at weekly intervals. Immunity becomes very strong after 36 days (*13*) and persists for at least 5½ months with little diminution.

Perhaps the most important determinant of the immune response in mice is the route by which the vaccine is delivered. In a dramatic example, intramuscularly vaccinated mice showed an LD_{50} value approximately 200 times that of control mice, whereas the LD_{50} in intravenously vaccinated mice was only about five times that of the controls (*12*). Not only was the intravenous route less effective for immunization than the intramuscular, but also the presence of intravenously administered vaccine impaired the animal's capacity to respond immunologically. Thus, when as little as 120 μg of spherule vaccine was administered intravenously into mice that had been vaccinated intramuscularly, strong immunity failed to develop (*12*). The intravenous treatment did not produce this effect by altering the expression of immunity; it prevented the development of immunity because 20 to 200 μg of vaccine could be given to already immunized mice without impairing their response (*12*).

Scalarone and Levine (*17*) have shown that the intravenous route of vaccination was also less efficacious than the intramuscular in inducing other immune responses. It did not induce delayed hypersensitivity well, it did not produce in mice a strong capacity to restrict fungal proliferation after infection, and it did not confer strong leukocytolytic properties on leukocytes in serum admixed with coccidioidin. There is also some suggestion that intravenously treated mice responded to an intraperitioneal endospore challenge with fewer inflammatory cells than did intramuscularly vaccinated mice.

Scalarone (unpublished) also tried to induce strong immunity intravenously by repeated small doses of vaccine. Even as many as 10 doses at 70 μg each were unsuccessful. Although it was speculated that intravenously delivered vaccine was eliminated by the host more rapidly than the intramuscularly injected spherules, the urine of animals vaccinated by either route with radio-tagged spherules showed comparable radioactivity over a seven-day period of monitoring.

There is evidence now that the inadequacy of the intravenous route of vaccination may be associated with the fact that this route tends to deposit the spherule particles primarily in pulmonary tissue. Table 2 shows that when live spherules were injected intravenously virtually all of them were found in the lung half an hour after vaccination. Intramuscularly injected spherules, on the other hand, remained primarily around the site of injection at the end of the same period.

By the 24th hour, the intravenously injected spherules had multiplied and were recovered in high numbers from the liver and spleen, although the lung was still the major repository. Likewise, when killed tritium-labeled spherules were substituted for live spherules, the intravenous route again deposited them primarily in the lung. After three hours, the liver showed strong radioactivity in mice injected either intramuscularly or intravenously, but this was to be expected because the spherules had been grown in totally labeled glucose and soluble components that would tend to accumulate in the liver. The insoluble moiety of the spherules, which included the immunogen-containing chitinous walls, tended to resist hydrolysis and remain at the site of injection (*11*). We infer, therefore, that the radioactivity in the lungs implied that much of the slowly solubilized immunogenic material was in the lungs. This point may be important, because in other studies it has been seen that

Table 2

Distribution of living (10^5 organisms, 1.0 mg) and nonliving tritium-labelled (7500 CPM, 0.7 mg) *Coccidioides immitis* spherules injected intravenously or intramuscularly into mice

Determination	Organ or site	Route	Hours after injection						
			0.5	1.5	3	24	72	168	336
H^3 [a]	Lung	I V	1422	–	1377	984	465	396	271
		I M	28	–	193	173	190	244	262
	Liver	I V	812	–	1014	1354	1349	1261	962
		I M	392	–	1109	2733	1813	1698	1642
	Spleen	I V	26	–	120	173	107	209	90
		I M	15	–	93	183	195	303	279
	Inguinal node	I V	22	–	40	97	80	84	58
		I M	32	–	37	161	124	172	115
	Kidney	I V	83	–	184	271	199	276	203
		I M	53	–	227	299	249	323	353
	Intramuscular inject. site	I V	–	–	–	–	–	–	–
		I M	3508	–	3425	3062	2968	2441	1146
Fungal numbers [a]	Lung	I V	$>10^3$	$>10^3$	–	$>10^3$	–	–	–
		I M	0	0	–	175	–	100	–
	Liver	I V	560	650	–	$>10^3$	–	–	–
		I M	0	15	–	40	–	10	–
	Spleen	I V	5	30	–	1125	–	–	–
		I M	0	0	–	0	–	800	–
	Inguinal node	I V	0	0	–	0	–	–	–
		I M	0	0	–	0	–	35	–
	Kidney	I V	45	120	–	675	–	–	–
		I M	0	0	–	0	–	500	–
	Intramuscular inject. site	I V	–	–	–	–	–	–	–
		I M	$>10^3$	$>10^3$	–	$>10^3$	–	$>10^3$	–

[a] Values correspond to recovered radioactive counts per minute or colony-forming fungal units per organ or site.

neither spherules administered intranasally into pulmonary tissue (*9*) nor finely ground spherule components given by the intranasal route (*18*) induced strong immunity.

The reason why killed spherules do not induce as great an immune response in pulmonary tissue as they do when injected into an intramuscular site is unknown to us. In the intramuscular site we observed that at three hours after injection there was the beginning of an inflammatory reaction with a few lymphocytes, but mostly polymorphonuclear cells, adjacent to the spherules. By six hours the response was predominantly neutrophilic, and only a few lymphocytes were present. And by 24 hours there was a rich polymorphonuclear neutrophilic response at the periphery of the spherules with lesser numbers of lymphocytes and macrophages. These responses were in substantial accord with observations in pulmonary sections of mice vaccinated intravenously or transcostally. It was not possible to associate differences in the immune responses with differences between cell responses in pulmonary and in muscular tissues. In some trials, however, it did appear as though the lymphocytic response was more pronounced with vaccine administered intravenously or transcostally into the lung than with vaccine injected intramuscularly.

Lymphocytes seemed to be the first line of defense when live spherules were introduced into pulmonary tissue by either the transcostal or intravenous routes. It is not known yet whether there is any relationship between this finding and the inadequacy of the intravenous vaccination route. Nevertheless, in concluding

this presentation, it is of interest to describe the lymphocytic response to spherules in pulmonary tissue. The reaction bears a very strong resemblance to the histiocyte ring or rosette response to Cryptococcus described by Schneerson-Porat, Shahar, and Aronson (*19*), and Shahar, Kletter, and Aronson (*20*). It appears to be one of the host's means to contain Coccidioides.

In our study, live spherules or live endospores were introduced transcostally, and histiologic changes in the lung were followed for 30 days. Endospores were soon phagocytosed by macrophages, and within 24 hours a mixed response was provoked which consisted of an infiltrate of phagocytes, polymorphonuclear neutrophils, and, occasionally, eosinophils.

The cellular response to injected spherules, on the other hand, was profoundly different from that above to endospores. Within six hours the spherules were generally surrounded by lymphocytes four to six cells thick. Occasionally the encirclement was even more extensive (Figure 1). Infrequently neutrophils and lymphocytes were seen inside the spherule. There were some plasma cells and macrophages and often a few neutrophils in the intervening cellular spaces, but the response in the vicinity of the spherule was predominantly and dramatically lymphocytic. As shown in Figure 1, the lymphocytic response took on the appearance of a rosette, and rosette formation was frequent at 24 hours after infection. The spherules were well encircled, and some macrophages were present. Lymphocytes and polymorphonuclear neutrophils were occasionally found within spherules at 24 hours (Figure 2). At 72 and 96 hours, lymphocyte encirclement of the spherules remained pronounced, and in animals surviving nine days the pattern was fundamentally unchanged, with spherules appearing to be contained primarily by rosettes of lymphocytes.

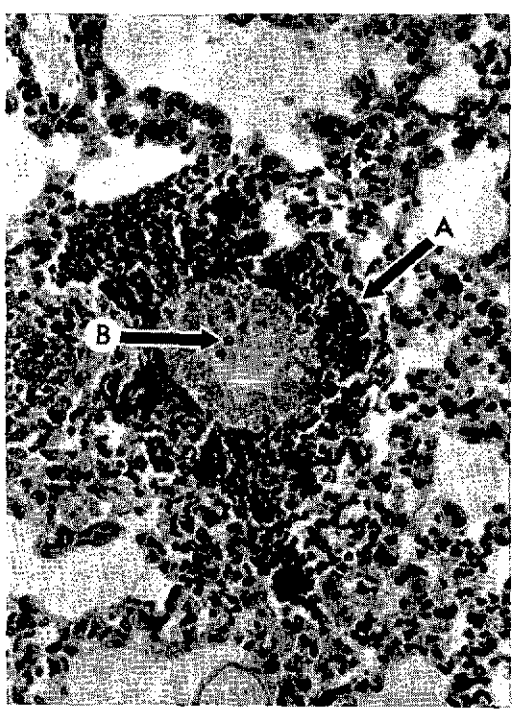

Figure 1. Spherule, 6 hours after injection transcostally into pulmonary tissue, surrounded by lymphocytes (A) with neutrophils and lymphocyte inside (B).

Figure 2. Spherule, 24 hours after injection transcostally into pulmonary tissue, surrounded by lymphocytes, some macrophages, with lymphocytes and polymorphonuclear neutrophils inside (A). Lymphocyte rings are forming around spherule loci at center bottom.

Mononuclear cells, polymorphonuclear neutrophils, and macrophages phagocytosed the endospores.

In our opinion, these data show that there are several categories of protective response to Coccidioides. Perhaps all are active to a greater or lesser extent in vaccinated and nonvaccinated animals. In the second category, lymphocytes offer an early means for containing spherules. Indeed, where lymphocytes were not present, spherules were usually seen proliferating extensively. Endospores, on the other hand, were readily phagocytosed, and macrophages and polymorphonuclear neutrophils appear to play an important role. With both structures, fibroblastic activity augments containment in older lesions. In vaccinated animals, the cellular responses associated with inflammation and containment were increased (*5, 7*). As discussed earlier, the phenomenon is influenced by the quality of the immunizing structure, its dose, the regimen of administration, and the route by which it is given.

In conclusion, it is fair to say that resistance, intrinsic or induced, is known in coccidioidomycosis but is poorly understood. Hopefully, with further understanding the difficulties cited in regard to a vaccination program may become quite academic.

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EPIDEMIOLOGY AND CONTROL OF RINGWORM OF THE SCALP

E. I. Grin

Ringworm of the scalp caused by anthropophilic dermatophytes is distributed throughout all the continents of the world, and in some regions there are endemic foci that constitute a public health problem of considerable importance. The disease is cause for concern not only because of the associated discomfort and bacterial complications but also because of the lasting psychological insults it can cause to the affected child, particularly in the case of favus.

Rational and successful control of tinea capitis depends primarily on epidemiological rather than on clinical approaches. The present paper deals with superficial ringworm of the scalp caused by anthropophilic dermatophytes and is based for the most part on observations and experiences gained in mass control campaigns in Yugoslavia (*5*) and several Mediterranean countries where there are large endemic foci of tinea capitis and where the author had the opportunity, as a consultant for the World Health Organization, to assist in the planning of effective measures for controlling the disease (*6, 7, 8, 9*).

Anthropophilic dermatophyte infections—those from *Trichophyton violaceum, T. tonsurans, T. schoenleinii, Microsporum audouinii,* etc.—are known to be cosmopolitan, but the geographical species distribution has its regional characteristics. For example, *Trichophyton violaceum* and *T. schoenleinii* infections are most prevalent in the eastern Mediterranean region, *T. soudanense* is found most frequently in the Sudan and other African countries, *T. tonsurans* (crateriforme) is characteristic of certain regions of Latin America and the southwestern part of the United States, *T. ferrugineum* is known particularly in parts of the Balkans, and so forth.

There is evidence that some of the dermatophytes—for example, *M. gypseum, Keratinomyces ajelloi, T. mentagrophytes*—exist in a saprophytic reservoir in the soil. In our own experimental investigations (*10*), however, we were able to demonstrate quite conclusively that the soil as a reservoir of infection for pathogenic dermatophytes already adapted to the parasitic life in man or animal has no epidemiological significance in the control of ringworm of the scalp. Specifically, it was possible to show (*11*) that pathogenic dermatophytes (*T. schoenleinii, T. violaceum, M. audouinii, T. ferrugineum,* etc.) in the debris of infected hairs or scales deposited in moist soil under laboratory or natural conditions lose their vitality after a few days because of the antagonistic action of microorganisms inhabiting the soil. This phenomenon does not occur, however, with dermatophytes that live permanently as saprophytes in the soil and sometimes appear facultatively as parasites for man (e.g. *M. gypseum*) or with pathogenic dermatophytes that have at last partly retained the ability to survive in the soil (e.g. *T. mentagrophytes*).

Ectoparasites, particularly *Pediculus capitis,* may be of some importance as passive transmitters of the disease, and our experiments with favus infection have indicated that such a

possibility does exist, although their actual epidemiological significance in spreading the infection appears to be very small.

Generally speaking, ringworm of the scalp has an uneven geographical distribution and, despite apparently identical or similar living conditions, a widely variable morbidity rate. Thus, a systematic survey of the entire region is necessary in order to properly measure the magnitude of the problem.

As we know, the disease is acquired almost exclusively in childhood before the age of puberty. It has been established that in general approximately 30 per cent of the infections occur in children under five years of age and, in all, about 96 per cent in those who have not yet reached the age of ten. Transmission from infected children to adults occurs very rarely, if ever.

The high morbidity rate of tinea capitis in an endemic area indicates that the remaining uninfected population is more or less not susceptible to the infection. Under natural conditions, even with very close and intensive exposure to the infection, usually only some of the exposed children will acquire the disease while the others, for reasons still unknown, appear to be not susceptible. Kligman (*19*), for instance, was able to demonstrate this phenomenon in experiments with *M. audouinii* infection. It is to be logically expected, therefore, that after a successful mass treatment campaign with a subsequent reduction in transmission of the disease new infections will be restricted, under proper surveillance, to sporadic cases only. The reservoir of susceptible population may again increase, of course, with the new generation.

Ringworm of the scalp caused by anthropophilic dermatophytes has all the characteristics of a familial disease, particularly in endemic areas. Once the infection has entered the family, it can be transmitted easily from one child to another, often producing simultaneous infections in the household (Figures 1, 2, and 3). The distribution in a family generally follows certain patterns, although many variations are encountered (Table 1). From the epidemiological standpoint, it is important to recognize that the infected family, and not the school, is the main source of infection for children. The prevalence of tinea capitis among schoolchildren may provide a useful index for measuring the magnitude of the problem in the general population, but schoolchildren should not be thought of as the exclusive group of infected people in a community.

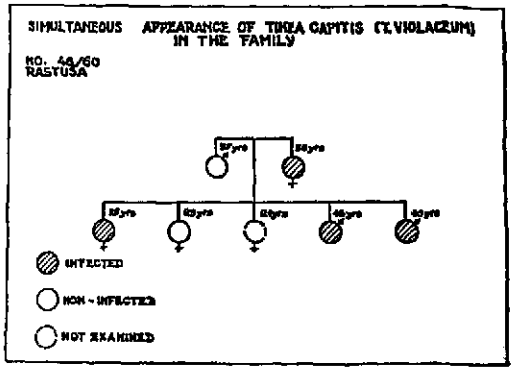

Figure 1. Simultaneous appearance of tinea capitis (*T. violaceum*) in the family.

Although there is no question that the prevalence of tinea capitis is greatest among children, extensive investigations could prove that infections are regularly observed among adults as well. In adults, tinea capitis, particularly when caused by *T. violaceum, T. schoenleinii,* or *T. tonsurans,* is of considerable

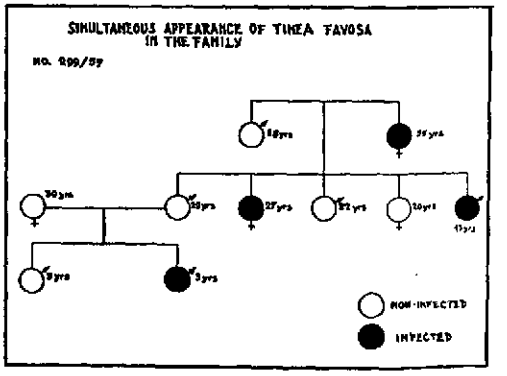

Figure 2. Simultaneous appearance of tinea favosa in the family.

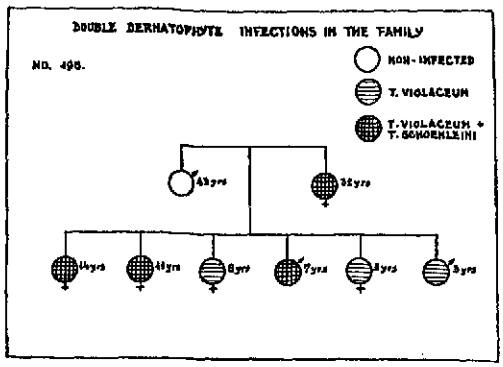

Figure 3. Double dermatophyte infections in the family (case 196).

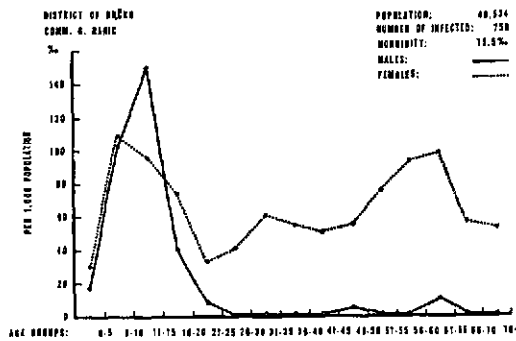

Figure 4. Morbidity rate of tinea capitis caused by *T. violaceum*, according to age and sex (high morbidity rate).

epidemiological importance and should not be regarded as an exceptional phenomenon. Thus, any control of tinea capitis confined to children alone is incomplete, and a considerable sector of the population continues to harbor a reservoir of infection. In our systematic surveys, around 30 per cent of the infected population was found to be over 20 years of age, and the predominant percentage of these affected adults were women, in whom the disease acquired in childhood continued to persist after puberty. The epidemiological importance of these findings is clear, since the mother may often be the source of infection or reinfection in the family (Figures 1, 2, and 3). No age limit for the occurrence of tinea capitis in adult life was found. Its symptoms are generally much less pronounced than in children, and hence the infection may pass unnoticed and unrecognized.

This statement on the sex and age distribution of ringworm of the scalp and on its epidemiological significance in adults, especially in the case of infections caused by *Trichophyton violaceum*, *T. tonsurans*, and *T. schoenleinii*, is based on investigations conducted in various parts of the world. Particular reference has been made to the work of Grin (*12*) in Yugoslavia, Maschkilleison (*22*) in the Soviet Union, Pipkin (*23*) in the United States, Berlin and Meyrovitz (*1*) in Israel, and Khan and Anwar (*17*) in Pakistan. Similar conditions may be presumed to exist in other countries as well, but the complete data have not yet been accumulated. The most comprehensive studies so far have been carried out in Yugoslavia. There, precise surveys of the endemic zones, conducted with the assistance of WHO, have revealed an almost uniform pattern of tinea capitis (*T. violaceum* and *T. schoenleinii*) morbidity in males and females (Figures 4 and 5).

The epidemiologic facts now in hand indicate that the control of tinea capitis caused by

Table 1

Relationship of family members infected with favus in 543 families

Infected persons	Number	Percentage
Father and child	1	0.2
Mother and child	75	13.8
Children only	332	61.1
Father only	3	0.5
Mother only	132	24.4

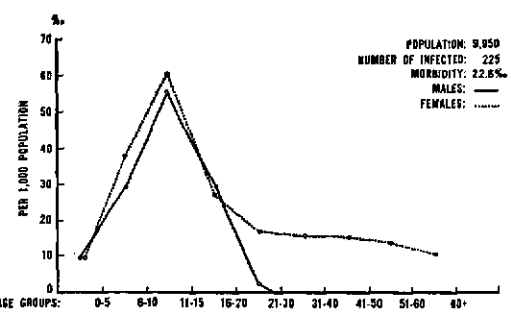

Figure 5. Morbidity of tinea capitis due to Trichophyton infection, according to age and sex, in 49 villages in the commune Bosanska Krupa (low morbidity rate).

anthropophilic species, especially where the disease is a public health problem, should be based on the following measures:

- Initial mass surveys covering a high percentage of the total population in all the respective regions;
- Suitable mass treatment policies for all infected households based on the use of griseofulvin;
- Case-finding through surveys at appropriate intervals; and
- Continuing surveillance toward the ultimate goal of eradication of the disease.

Although it is obvious that 100 per cent examination cannot be accomplished, every effort should be made to improve case-finding techniques so as to secure the gains resulting from mass treatment. If the initial systematic screening is based on voluntary attendance at clinics only, probably less than 50 per cent of the population will be reached, even when the best kind of propaganda is used. However, if special units are placed at strategic points, a larger group (70 per cent or more) can be encompassed, and if a house-to-house survey is done, an even higher proportion (about 90 per cent) of the people can be expected to be covered.

Diagnosis should be based on clinical findings confirmed by microscopic examination, plus the use of Wood's light in the case of fluorescent ringworm infections.

According to our experience, diagnostic errors in a mass survey can be as low as 2 or 3 per cent under good conditions. In most cases, these errors have occurred as a result of overdiagnosis. Excessive diagnosis can be of benefit, but it complicates the task of making evaluations under such conditions.

The brush-sampling technique suggested by Mackenzie (*21*) and Klokke *et al.* (*20*) for routine screening in the control of tinea capitis appears to be laborious and impractical in mass campaigns, and it does not seem to be adequate for precise clinical and microscopic examination by well-trained medical personnel.

A fully equipped mycological laboratory, directed by experienced mycologists, should be an integral part of any control program, and especially of pilot studies for the identification of the strains involved in a given area. Such a laboratory is essential not only for performing cultures from pilot studies but also for training the personnel engaged in a control program.

Following the initial mass survey, which if possible should be carried out on the basis of a family census, adequate treatment should be provided for all cases diagnosed. For this purpose, it is advisable to constitute a record of all the infected families, with an individual card for each infected member, containing complete data on surveys, treatment, resurveys, and all other relevant aspects. Such information is essential for the statistical analysis and evaluation of the control program.

In itself, the treatment of tinea capitis is one of the most important steps toward controlling the disease, since the cured patient ceases to form part of the chain of further transmission.

For many years, ringworm of the scalp was a therapeutic problem, but with the introduction of griseofulvin by Gentles (*3*) in 1958 this situation has changed dramatically. Griseofulvin is distinctly fungistatic. Taken orally, it is incorporated in the new keratinous structures that are formed during treatment, while leaving the fungi vital in the previously formed and infected distal part of the hair. Evidently, the infected portion of the hair, if not removed by some kind of local treatment, can cause recurrence of the disease or be a source of infection in the environment by spreading the infectious debris of the hairs. Thus, elimination of this potential source of infection, in addition to the oral use of griseofulvin, appears to be essential for the control of tinea capitis.

The first reports on the use of oral griseofulvin were published in 1958 by Williams and associates (*25*) in London and in the following year by Riehl (*24*) in Vienna, Blank and Roth (*2*) and Kirk and Ajello (*18*) in the United States, and Grin (*13*) in Yugoslavia. Since then, its efficacy in curing ringworm of the scalp

caused by dermatophytes has been demonstrated by numerous publications throughout the world. There are still significant variations, however, in the treatment schedules employed.

In general, the conventional daily dosage of 20 to 25 mg/kg body weight (1 to 2 g in adults and correspondingly less in children) for four to five weeks appears to be effective in producing a high rate of cure, although occasionally the therapy has to be continued for a longer time. A great number of other recommended treatment schedules are in use as well, and apparently they also give the same good results.

The effect of griseofulvin is not uniform in all patients treated by similar or even identical methods. Differences in drug response, which may be caused by the rate of hair growth, rate of absorption and local deposit of the drug, sensitivity of the particular strain, or other factors, may also account for individual failures. In our investigations (*14*), the therapeutic effect of griseofulvin was generally proportional to the concentration and duration of blood griseofulvin levels. Residual blood levels of over 1 μg/ml and peak blood levels of about 2 μg/ml were sufficient to effect a cure in most cases, whereas with further increases in the dose no proportional increase either in the blood concentration or in the therapeutic effect was observed.

While the infected person is being treated in a hospital or an outpatient clinic under medical supervision, the dosage of griseofulvin and duration of treatment can be suitably altered according to the response of the individual case. However, in mass programs in the developing countries, further simplification will have to be introduced.

On the basis of a controlled trial carried out in Yugoslavia, we are recommending for mass treatment a dose of 25 mg/kg body weight twice weekly for four to five weeks. There does not appear to be any advantage in increasing the dosage. Only in ringworm of the scalp caused by *T. schoenleinii,* because of the frequent local complications (folliculitis, reduced blood circulation, etc.), is a longer period of treatment (five to six weeks) at the same daily dosage recommended.

It would certainly facilitate the administration of griseofulvin in mass campaigns if one single treatment schedule were to be used. If the drug is available in sufficient quantities, this could be accomplished by using the schedule recommended for favus infections. Unfortunately, experiments with a single high-dose treatment, which would obviously be of great advantage for mass use, have not been successful. Our investigations have shown that a single high dose (100 mg/kg) brings cure in only about 20 per cent of the infected hairs in a *T. schoenleinii* infection.

Local treatment in addition to griseofulvin should be offered routinely, since it significantly enhances the recovery rate. Such therapy should consist in removal of the hair first at the beginning and once again after 18 or 20 days, supplemented by application of fungicides (for example, 10 per cent salicylic acid) and washing of the scalp. In our trials with *T. violaceum* infections of the scalp, the difference between the recovery rates in patients with and without local treatment was 9.9 per cent at six months after therapy.

For a mass campaign, it is essential to have complete evidence that griseofulvin can be taken safely at the full dosage according to the treatment schedule used. Such information is hard to obtain in the rural areas of developing countries unless the drug is administered under supervision. This step is necessary, however, in order to be able to evaluate the results.

Onychomycoses caused by dermatophytes are not rare among patients with tinea capitis of long duration. They should always be kept in mind in systematic surveys so that adequately prolonged treatment can be provided for.

In very low prevalence areas, control may consist merely in assuring that free and adequate supplies of griseofulvin are available, rather than carrying out mass surveys.

Prophylactic griseofulvin treatment of con-

tacts in the infected households was not shown to be of significant preventive value in our control trials. González Ochoa (*4*), working with tinea glabrosa in Mexico, also noted the lack of prophylactic action of griseofulvin. Similarly, cases of kerion capitis due to infection with zoophilic strains tend to heal spontaneously, and griseofulvin is of no great advantage in achieving cure. However, with superficial infections the drug is helpful in preventing inflammation and deep-seated suppuration that may otherwise develop.

In the course of our investigations (*15*), we were able to demonstrate clearly that there is no epidemiological connection between endemic foci of ringworm of the scalp and zoophilic infections transmitted by animals, which have an epidemiological course of their own and require control measures involving veterinary services almost exclusively. The transmission of zoophilic infection among human beings is apparently limited to few passages only and does not give rise to continuous spread of the disease from man to man. We were able to demonstrate this fact quite convincingly in *T. verrucosum* infections (*15*), and it may be assumed that the same or similar phenomena occur with other zoophilic infections as well.

Although griseofulvin sensitivity has been seen to vary considerably in cultures of different strains of dermatophytes (*16*), in general no correlation is observed between the degree of *in vitro* sensitivity to griseofulvin and the clinical response to therapy with this drug. To date there is practically no evidence of any significant decrease in the response of dermatophyte infections to griseofulvin treatment, although *in vitro* this tendency has been observed in some dermatophytes both after exposure to increased concentrations of griseofulvin and also after successive transfers of the cultures to plain media without exposure to griseofulvin.

It is well recognized that no single systematic survey followed by mass treatment is sufficient to achieve full control of the disease. However, with repeated campaigns it is possible to reduce the incidence of tinea capitis to the point where permanent medical facilities will be able to control residual cases, particularly if active case-finding measures are not prematurely discontinued. A few months after an intensive case-finding and mass treatment effort, it can be expected that the prevalence of the disease will be reduced 90 per cent or even more, but if no further action is taken the infection may gradually redevelop at a rate and to an extent inversely related to the percentage of population covered and proportional to the prevalence of the infection at the time of the initial treatment survey. Resurveys should therefore not be postponed beyond six months after any given survey. How many systematic resurveys are required will depend on the circumstances, but one or two should generally be sufficient to reach the surveillance stage of the campaign. New cases, relapses, and reinfections detected in the surveys should receive treatment without delay.

Obviously, the practical application of recommended control measures will have to be subject to great flexibility in order to allow for the specific epidemiological and other local conditions prevailing in each region. For instance, it would not be economical to continue periodic resurveys if only a few sporadic cases are found and if the prevalence of the disease is below the level at which it is a public health problem. In such instances, measures carried out as part of the regular health services to ensure that any increase in prevalence will be immediately recognized can still be very useful. At the same time, simpler procedures can be used that do not include resurveys of the entire population. For example, the declaring of tinea capitis a reportable disease may be sufficient to permit detection of sporadic cases and prevent local outbreaks of the infection.

When a state of low prevalence is reached, the control program may pass from the initial stage of the campaign to that of surveillance. This latter activity should then be integrated into the public health service if adequate facilities are available in the community.

The surveillance phase should include the following aspects:

- Regular surveys of schoolchildren and occasional surveys of the population in communities where endemic foci of superficial ringworm of the scalp existed formerly;
- Adequate and rapid action in case of increased prevalence; and
- Health education.

Maintenance of surveillance as part of proper health services is of great importance in the later stages of an eradication campaign. It should be concentrated on children and young persons up to 15 years of age and on female adults in infected households. The schools may be used to advantage during the surveillance phase, since they afford the best available population sector suitable for surveys, but the aim of mass examinations considerably limits their role.

It is rare in an endemic area, even when tinea capitis is under control, that all factors favoring transmission of the infection are completely removed. New infections may still occur sporadically. Thus, continued surveillance—or, if necessary, periodic resurveys to consolidate the results already achieved—are required.

From the very beginning of a campaign, full-scale efforts should be undertaken to improve hygienic conditions and living standards in the various communities. Any recommendations in this respect should be simple and inexpensive and should be designed as much as possible to take into account the capabilities of the communities concerned. They should be presented in such a form as to be understood and wanted by the population.

The prevalence of tinea capitis as an endemic disease can also decrease greatly without any specific campaign. This probably happens as a result of improved living standards, a phenomenon that is occurring nearly everywhere in the developing countries. This factor is slow-acting, however, and it may take generations before the point of eradication is achieved. In the meantime, the effect of improved socioeconomic standards is very important, especially when the prevalence of ringworm of the scalp has already been greatly reduced by the mass treatment campaign.

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DISCUSSION

Chairman Drouhet: The discussion of Session III is now open.

Dr. Pollak: I believe that with paracoccidioidomycosis one of the greatest questions is its pathogenesis. We are not all in agreement on this point. My own opinion is that the portal of entry for the fungus is the respiratory tract. In other words, the primary lesion would always be found in the lungs. It is true that we know of cases in which mucocutaneous ulcers are found, especially in the mouth, and we also know of x-rays in which pulmonary lesions are not observed. Contrariwise, there are cases with pulmonary lesions and no oral lesions. I believe that the mucocutaneous lesions are signs of dissemination. Most of the patients are peasants with poor oral hygiene, and in these cases the mouth is probably a *locus minoris resistentiae* where lesions are produced more easily. It is true that in many cases of dental granuloma *P. brasiliensis* is found in the culture of the apical granuloma when a tooth is extracted—a method we use for diagnosis rather frequently.

I insist again on the existence of subclinical forms. This is an important point about which very little is known. In our institute we find 30 per cent of the cases of paracoccidioidomycosis associated with tuberculosis. We have observed quite often that in certain complications of tuberculosis the lesions improve rapidly when corticosteroids are applied. However, in other cases new, more serious pulmonary lesions suddenly appear. In these instances the sputum culture shows *P. brasiliensis,* indicating that there was a subclinical form of paracoccidioidomycosis.

One final observation. We have noted that paracoccidioidomycosis and histoplasmosis in their very grave forms appear most frequently among European immigrants rather than the native population, and we believe this is an immunological phenomenon caused by the lack of previous contact with these fungi.

Dr. Negroni: With regard to the pathogenicity of paracoccidioidomycosis—or South American blastomycosis, as we call it—our experience in Argentina bears out what Dr. Pollak has said. I first drew attention to this disease in Uruguay back in 1937, so I have been able to observe it for some time. I have seen subjects who contracted the infection in endemic areas and then went to nonendemic areas where they have lived as long as 40 years before the lesions appeared. In other words, the parasite can remain dormant within the organism for many, many years without producing lesions.

In 1928, when I was working in the state of São Paulo, we removed a ganglion from a case of South American blastomycosis. It was submerged in formaldehyde for over five years, and yet we still obtained positive cultures from it. I bring this up to show that although the clinical and serological signs may indicate a cure, the parasite can remain alive for years.

Some of the Brazilian authors have had similar cases of asymptomatic patients with small ganglia that were found on examination to contain parasites. All this leads us to believe that patients who have had South American blastomycosis should be followed for the rest of their lives, even though they have been cured. If there is no recurrence, all the better. If the manifestations do come back, then they should be treated again.

In the case of a subject with disseminated lesions, we usually resort to amphotericin B therapy whenever well-equipped hospital facilities are available. The sulfas can give good results, too, but sometimes these drugs are not well tolerated, and sometimes the lesions are resistant to the treatment. Also, there are more recurrences with the sulfas than with the other drugs.

Consideration should be given to the efforts of combined treatment when tuberculosis is associated with this disease. We have seen that the use of isoniazid in the treatment of TB aggravates the blastomycosis lesions. Also, potassium

iodide should never be used when blastomycosis is present.

In the intervals between treatments, sulfamide should be continued, and if this is not possible, then very good results can be obtained with a vitamin D shock, as recommended by Charpy. We have also used testosterone intramuscularly, but I am less enthusiastic about this drug because of the potential danger of creating blastemic cancer in adults.

Dr. Baldó: I suppose you are all familiar with what health administrators call regionalization and "districtization," if I may coin a word. By region we mean a given country, department, province, or state. By district we mean any subdivision that constitutes a health district.

In Latin America, if I remember correctly, there are some 240 million people. Among these, there are some 90 million without medical care. In Venezuela, a country that I know well, we have a population of 10 million and a well-established health organization, but despite this, there are about 3 million rural inhabitants for whom medical care is not available.

Generally speaking, we find that in any given region with a capital there are hospitals with all the necessary resources. The problem begins with people living outside the capital. In a health district you have a health center with its facilities, and you also have additional hospitals, where preventive and curative medicine is practiced and personnel are trained to know everything possible about a given problem—in this particular case, paracoccidioidomycosis. Then there are the health subcenters. In these small units there is usually one general practitioner on duty, and there is the enormous problem of attending to the population, which is scattered and made up of small groups of, say, 500, 300, or 200 persons.

There have been great advances in mycology, but they are to little avail if we need a hospital for the administration of a toxic drug such as amphotericin B. I say, what good will these advances serve if we can only deal with a small number of patients?

We have had considerable experience in Venezuela administering sulfa drugs on a ambulatory basis, as with tuberculosis patients, at district health subcenters staffed with paramedical personnel. The physician believes he is the sole protagonist in medicine, but today this is no longer true. Auxiliary medical personnel have become important. The countries that have achieved medical progress are the ones in which each physician has from 10 to 12 auxiliaries available to help him. We cannot make progress as physicians unless we recognize the need to act in concert with our assistants.

In Venezuela we have succeeded in treating rural patients with paracoccidioidomycosis on an ambulatory basis in some seven or nine different regions. Since most cases are found in rural areas, that is where the medical workers have to be sent. We cannot disregard the socioeconomic structure of the country: hospitals cannot be counted on to care for these people. The patients have to go back to the fields to work, but they must also continue to have supervised medical treatment. We have data on cases in rural areas that have been followed for as long as three years. We know that paracoccidioidomycosis is not cured in three or four months. And this kind of ambulatory treatment is possible only with auxiliary, nonprofessional paramedical personnel.

All this is to say that I want you scientists to know there are administrators behind you ready to apply your findings at the practical level.

Dr. Shadomy: I would like to report to this meeting results that have been obtained in clinical and laboratory studies with two antifungal agents: 5-fluorocytosine (5-FC) and hamycin. Both these agents appear to have considerable potential in the treatment of systemic mycotic disease, and both have the distinct advantage of being administered orally.

The first, 5-FC, is a fluoropyrimidine active both *in vitro* and *in vivo* against yeastlike fungi, including *Cryptococcus neoformans* and *Candida*

albicans, and also against *Aspergillus fumigatus*. Initially, *in vitro* studies with *C. neoformans* were hampered by the fact that this agent acts as a competitive analogue for cytosine. Presence of free cytosine in culture media containing cell or tissue extracts resulted in a nearly total inhibition of antifungal activity. When tested in a synthetic medium, 5-FC was inhibitory for more than 95 per cent of 77 strains of *C. neoformans* at a concentration of 7.8 μg/ml, and fungicidal for 50 per cent at 15.6 μg/ml. These concentrations were subsequently found to be within the range of clinically achievable serum levels. In way of comparison, amphotericin B was fungicidal for 97 per cent of the same strains at 0.78 μg/ml, while hamycin was fungicidal for 97 to 100 per cent at 0.20 μg/ml.

Our clinical experience with 5-FC in the treatment of cryptococcosis now totals 21 cases (Table 1). These include both the cases described earlier by Utz and associates and six cases treated subsequently at the Medical College of Virginia in Richmond. Among these are 14 cases of strictly meningeal disease, four cases of meningeal disease with involvement of other tissues (renal, pulmonary, pleural, bone marrow, and subcutaneous), and three cases of purely pulmonary disease.

Of these cases, 11, or 52.3 per cent, were cured with 5-FC. The breakdown was as follows: meningeal only, six cases, including one that was negative on culture but the patient died of other causes; meningeal disease with involvement of other tissues, two cases, including one that was negative on culture but the patient died of staphylococcal septicemia; and pulmonary only, three cases. Seven cases—including five meningeal infections and two cases with both meningeal disease and involvement of other tissues—improved initially but ultimately relapsed with cultures positive for strains of *C. neoformans* that were totally resistant to 5-FC. No improvement was seen in the remaining three cases of meningeal disease.

These data show that the best results with 5-FC were obtained in the treatment of purely pulmonary infections, where there was a cure rate of 100 per cent and no relapses. The poorest results were obtained in the 14 meningeal infections, where there were six cures (42.8 per cent), five initial responses followed by relapse (35.8 per cent), and three cases in which no improvement at all was noted (21.4 per cent).

As indicated before, most previously unexposed strains of *C. neoformans* are inhibited by 7.8 μg/ml of 5-FC, while about half are killed by 15.6 μg/ml. These concentrations approximate the limits of the average range of values reported from bioassays of sera and cerebrospinal fluids in patients treated daily with 100 mg/kg of this

Table 1

5-fluorocytosine in the treatment of human cryptococcosis: form of disease and clinical outcome [a]

Disease	Apparent cure	Initial response, relapsed	No response
Meningeal only (14)	6 (1 DOC) [b] (42.8%)	5 (35.8%)	3 (21.4%)
Meningeal, other tissues (4)	2(1 DOC) (50%)	2 (50%)	—
Pulmonary only (3)	3 (100%)	—	—
Totals	11(2 DOC) (52.3%)	7(33.3%)	3(14.4%)

[a] *Antimicrob Agents Chemother, 1968*, 344 (1969), and 6 cases subsequently seen at Medical College of Virginia, Virginia Commonwealth University, Richmond.

[b] DOC = death due to other causes

agent. Failure to achieve levels of 5-FC in biological fluids well in excess of inhibitory and fungicidal concentrations may explain the high frequency with which resistant strains have emerged clinically. More recently, bioassay data have been obtained for sera from several patients treated daily with 150 mg/kg of 5-FC. In one, a maximum level of 39 μg/ml was detected, with a mean for nine separate specimens of 31.1 μg/ml. In a second, the maximum measured level was 78 μg/ml with a mean for five specimens of 61.6 μg/ml. The effect of increasing the maximum daily dosage of 5-FC from 100 to 150 mg/kg can be seen when these values are compared with those obtained from a similar patient who received the lower dosage. The highest 5-FC serum level measured in this latter patient was 33 μg/ml, with a mean for seven specimens of 21.3 μg/ml.

Only a limited number of cryptococcosis patients treated at the Medical College of Virginia have received a dosage of 150 mg of 5-FC per kilo of body weight (Table 2). Thus, the ultimate clinical effect of the increased dosage cannot be determined at this time. However, a possibly significant trend is developing. Four of the 14 cases seen in Richmond have been treated with the higher dosage. Only one of these patients relapsed with emergence of a 5-FC-resistant strain of *C. neoformans*, whereas resistant strains emerged in 5 of 10 patients treated at the level of 100 mg/kg. It is also of interest that the pretreatment isolate obtained from this particular patient was of borderline susceptibility, with minimal inhibitory and fungicidal concentrations for 5-FC of 7.8 and 500 μg/ml, respectively.

The results of *in vitro* susceptibility studies with *C. neoformans* and 5-FC are summarized in Table 3. At the time of our first report on the *in vitro* activity of 5-FC, it appeared that only 2 to 3 per cent of the *C. neofomans* strains were resistant to this agent. Subsequent experience, however, suggests a much higher figure. Recently, 52 human isolates of *C. neoformans* were studied. Only one of the isolates recovered from patients prior to treatment was resistant, and a second was susceptible only at concentrations above 7.8 μg/ml. In contrast, isolates from 7 of 14 patients, or 50 per cent, obtained after

Table 2

5-fluorocytosine in the treatment of human cryptococcosis: dosage and clinical outcome [a] (14 courses of treatment)

Outcome	Dosage	
	100 mg/kg [b]	150 mg/kg [c]
Apparent cures (7)	4(40%)	3(75%)
Relapses and/or failures (7)	6(60%)	1(25%)
C. neoformans S → R (6)	5(50%)	1(25%)
Levels, μg/ml		
Serum	6.8–33	23.0–39
	9.8–19	34.0–86
CSF	3.8–15	7–24.0

[a] Data through Jan 1970 from Medical College of Virginia, VCU.
[b] 10 courses [c] 4 courses
S → R = emergence of resistant strains

Table 3

5-fluorocytosine *in vitro* susceptibility studies with *C. neoformans* clinical isolates [a]

Total number of isolates: 52	
Susceptible	40(77%)
Resistant	12(23%)
Occurrence of resistant strains	
Courses of treatment: 25	
Resistant strains: 12(48%)	
Prior to treatment	1(8.3%)
Demonstrated S → R	9(75%)
No pretreat. data	2(16.7%)
S → R, high MFC	5(41.7%, MFC ≥ 32 μg/ml)
Documented courses of treatment: 24	
Cures: 11(45.8%)—all susceptible strains	
Failures: 10(41.6%)—all resistant strains	
Unknown: 3	

[a] Includes data for 14 cases from Medical College of Virginia, VCU, 10 from NIH, and 1 case treated elsewhere with strain sent for study.
S → R = emergence of resistant strains

treatment with 5-FC were totally resistant to this agent.

Experience with 5-FC and Candida species is less extensive than that with *C. neoformans.* Fourteen clinical isolates of *C. albicans* or *Candida sp.* have been tested against 5-FC in our laboratories (Table 4). Five, or 35.7 per cent, were susceptible to 5-FC at concentrations of less than 12.5 μg/ml; two, or 14.3 per cent, were susceptible to concentrations beyond achievable serum levels; and the remaining seven, or 50 per cent, were totally resistant. Unlike the situation with *C. neoformans,* there does not appear to be any relationship between the susceptibility of a Candida isolate and prior exposure to the drug. Nor has the phenomenon of emerging resistant strains seen with *C. neoformans* been observed with the Candida species. Thus, while resistance will be a major drawback to the treatment of systemic Candida infections with 5-FC, it should be detectable prior to election of therapy. Speciation does not appear to be a factor in resistance, since one of three and two of four isolates identified as *Candida sp.,* not *C. albicans,* and as *C. albicans* were resistant.

Eight cases of systemic candidiasis have been

Table 4

5-fluorocytosine *in vitro* susceptibility studies with *C. albicans* or *Candida sp.* clinical isolates

Total number of isolates: 15	
Susceptible	5(33.3%)
Moderate resistance	2(13.3%, MIC ≥ 30 μg/ml)
Resistant	8(53.3%)
Occurrence of resistant strains	
Courses of treatment: 9	
Resistant strains: 5	
Pretreatment resistance: 4	
Courses of treatment	
Cures: 4 (including 2 DOC)—all susceptible	
Failures or no response: 5—4 resistant strains, 1 susceptible	
Speciation and susceptibility:	
Candida sp.	*C. albicans*
S-3, M-1, R-4	S-2, M-1, R-4

DOC = death due to other causes

Table 5

5-fluorocytosine clinical results with Candida infections [a]

Diagnosis	Apparent recovery	Improved only with relapse	No response or failure	Culture data
Pyelonephritis (3)	2(1 DOC)	–	1	R-*C. albicans* & *Candida sp.* F-*Candida sp.*
Peritonitis (1)	–	–	1	*C. albicans*
Endocarditis (1)	–	–	1	*C. albicans*
Fungemia (agammaglobulinemia) (1)	1(DOC)	–	–	*C. albicans* & *Candida sp.*
Granuloma (1)	1	–	–	*C. albicans*
Bladder diverticulitis (1)	–	1	–	*Candida sp.*
Totals	4(50%)	1(12.5%)	3(37.5%)	

Candida sp., 4; 5-FC resistant, 4; *C. albicans,* 5; 5-FC susceptible, 4.

[a] Data through Jan 1970 from Medical College of Virginia, VCU; all treated with 100 mg/kg.

DOC = death due to other causes

R = recovery

F = failure

treated with 5-FC at the Medical College of Virginia (Table 5). All received 100 mg/kg, and total dosages ranged from 10 to 164 g. *C. albicans* was isolated from four cases and *Candida sp.*, not *C. albicans*, from three; one infection was mixed, with both *C. albicans* and *Candida sp.* being recovered. There were three cases of pyelonephritis and one case each of bladder diverticulitis, granuloma, and endocarditis, as well as one case of intestinal candidiasis with fungemia in a patient with Swiss-type agammaglobulinemia.

One patient with pyelonephritis and the patient with granuloma were cured of their infections. Isolates from both were susceptible to 5-FC, although the strain of *Candida sp.* recovered from the patient with pyelonephritis was inhibited only by 3.13 μg/ml. Infections were also cleared in the patient with agammaglobulinemia and in a second case of pyelonephritis, but these patients subsequently died. Postmortem cultures were negative for Candida species in both. The isolate from the case of agammaglobulinemia was inhibited by 1.8 μg/ml of 5-FC; susceptibility data were not obtained for the isolate from the second patient. Initially, the infection in the patient with bladder diverticulitis cleared, but it later relapsed and required surgical treatment. In this case, isolates obtained both before and after treatment with 5-FC were susceptible to 0.23 μg/ml or less of 5-FC. The two patients with peritonitis and endocarditis failed on 5-FC; surgery was required in both, and treatment with amphotericin B was needed to clear the infection in the latter. In addition, a third patient with pyelonephritis died. In all three instances, isolates of *Candida sp.* or *C. albicans* were found to be resistant to 5-FC.

The second agent I should like to discuss is hamycin. This is a polyene antibiotic closely related to candicidin and amphotericin B but different from these compounds in solubility properties and absorption spectra. The original reports on this drug stimulated a considerable degree of interest, as they indicated that it possessed a high level of antifungal activity and was also capable of producing clinically effective antifungal concentrations in serum on oral administration with a minimum of side effects.

In vitro, hamycin was found to be highly active against most of the deep-seated fungal pathogens (Table 6). Using a routine procedure in which the drug was diluted serially in blood agar, hamycin was found to be inhibitory for *Blastomyces dermatitidis* at concentrations as low as 0.008 μg/ml. Inhibition was obtained for strains of *Histoplasma capsulatum* at concentrations ranging from 0.10 to 0.78 μg/ml. Species of *Aspergillus fumigatus* were inhibited at 0.10 to 6.25 μg/ml. Inhibition of *Sporothrix schenckii* was obtained only at concentrations of 1.56 to 3.13 μg/ml or more. Using a procedure in which the drug was serially diluted in broth, hamycin was inhibitory for a majority of 77 strains of *C. neoformans* at 0.05 μg/ml, and fungicidal at 0.10 μg/ml. Under identical test conditions, amphotericin B was some ten times less active.

Hamycin has been tried in 21 patients for treatment of systemic fungal disease at the Medical College of Virginia (Table 7). Of these, 14 had blastomycosis, four had histoplasmosis, and one each had cryptococcosis, aspergillosis, and chromoblastomycosis. Repeat courses of therapy were required in one patient each with blastomycosis and histoplasmosis, giving a total of 23 courses. Apparent recovery was observed in 10 of the patients with blastomycosis (71

Table 6

Hamycin *in vitro* susceptibility studies

Species		MIC [a]	MFC [a]
B. dermatitidis	(7)	.008–.016	–
H. capsulatum	(5)	.10–.78	–
A. fumigatus	(6)	.10–6.25	–
S. schenckii	(4)	1.56–3.13	–
C. neoformans	(77)	.05–.39	.10–.78

[a] Minimal inhibitory and fungicidal concentrations, μg/ml. First 4 species tested in mycelial form on blood agar with incorporated drug; *C. neoformans* tested in yeast-beef broth.

Table 7

Hamycin clinical results [a]
(21 patients; 23 courses of treatment) [b]

Infection	Patients	Apparent recovery	Improved only or relapsed	No response
Blastomycosis	14 (1 rpt)	10	4	1 [c]
Histoplasmosis	4 (1 rpt)	–	4	1 [c]
Cryptococcosis	1	–	1	–
Aspergillosis	1	–	–	1
Chromoblasto-mycosis	1	–	–	1 [c]
Totals	21[23]	10(44%)	9(39%)	4(17%)

[a] Data as of Jan 1970 from Medical College of Virginia, VCU, Richmond.
[b] Dosages: 10 mg/kg tableted hamycin; 20 mg/kg micronized hamycin.
[c] Inadequate course of therapy.
rpt = repeat course of treatment

per cent), while four (35 per cent) improved but subsequently relapsed. None of the patients with histoplasmosis demonstrated complete recoveries; relapses were seen in all four cases. While the one patient with cryptococcosis responded to hamycin, he relapsed and treatment with amphotericin B was required. No response was seen in either the chromoblastomycosis or the aspergillosis cases.

Clinical studies with hamycin have employed several different preparations and dosages. Initially, the drug was available only as a hard, pressed tablet and was administered at a maximum daily dosage of 10 mg/kg. More recently, it has been prepared in the form of an encapsulated, micronized powder with the maximum daily dosage increased to 20 mg/kg. These changes have produced apparently better clinical results (Table 8). Such was the case in one study in which clinical improvement with nega-

Table 8

Hamycin clinical results with varying dosages

Infection	Patients	Apparent recovery	Improved only or relapsed	No response
20 mg/kg, micronized material [a]				
Blastomycosis	5	4	1	–
Histoplasmosis	2	1	1	–
Totals	7	5(71%)	2(29%)	–
2–50 mg/kg, tableted material [b]				
Blastomycosis	7	2	1	4
Histoplasmosis	2	–	–	2
Cryptococcosis	1	–	–	1
Totals	10	2(20%)	1(10%)	7(70%)

Serum levels: 2–50 mg/kg tableted: .01–.10 μg/ml
10–20 mg/kg micronized: .05 > .30 μg/ml

[a] *Antimicrob Agents Chemother, 1967,* 113 (1968).
[b] *Amer Rev Resp Dis* 95: 506 (1967).

tive cultures was reported in four of five cases of blastomycosis and one of two cases of histoplasmosis. Neither the change in physical form nor the increased dosage resulted in serious gastrointestinal disturbances or in consistent abnormalities in the organ function tests.

Increased hamycin dosages also have resulted in serum levels well in excess of 0.012 μg/ml, which is the average minimal inhibitory concentration of the drug for *B. dermatitidis*. In patients receiving 10 mg/kg of the tableted material, hamycin serum levels rarely exceeded 0.04 μg/ml and usually were in the range of 0.01 to 0.02 μg/ml. In contrast, hamycin serum levels were in the range of 0.04 to 0.05 μg/ml and often exceeded 0.10 μg/ml in patients receiving 20 mg/kg of the micronized material. In more recent studies, average serum levels as high as 0.33 μg/ml, with individual high values in excess of 1.0 μg/ml have been measured in patients receiving 20 mg/kg. Levels as high as 3.5 μg/ml have also been measured in a limited number of individuals receiving 40 mg/kg of micronized hamycin.

An absolute association between hamycin serum levels and clinical results cannot be made. However, certain relationships have become apparent. These may be seen, for example, in the results of the study in which the micronized and tableted preparations were compared. In one patient with blastomycosis who had shown initial improvement with negative cultures followed by relapse and positive cultures, hamycin serum levels never exceeded 0.03 μg/ml. In a second patient with histoplasmosis, hamycin serum levels as high as 0.096 μg/ml were measured; these levels, however, were less than the *in vitro* minimal inhibitory concentration of hamycin, 0.78 μg/ml, measured against the patient's isolate of *H. capsulatum*. This patient subsequently relapsed and has been treated, apparently successfully, with amphotericin B.

The above association between hamycin serum levels and clinical outcome has not been seen in all patients. For example, one patient described in the earlier study as an apparent cure after treatment with 20 mg/kg of hamycin has recently yielded sputa that are positive for *B. dermatitidis*. Serum levels in this patient were as high as 0.06 μg/ml. In contrast, in a second patient also described in the same study, hamycin serum levels were in the range of only 0.014 to 0.016 μg/ml. This patient was apparently cured of blastomycosis and has remained culturally negative for over three years.

The results I have discussed represent only a limited experience with these two new agents. However, I feel that the data should be of interest to this group because of the obvious advantages that oral preparations offer in the management of fungal diseases among outpatient populations and among large groups—both of which factors will sharply limit, I am sure, any consideration of therapeutic agents that might be useful in treatment of the medical problems being discussed here.

Chairman Drouhet: I think 5-fluorocytosine is the first antifungal agent that has given resistant strains during treatment, so we should be very careful not to have any resistant strains of *Candida albicans* or *Cryptococcus neoformans* in circulation.

Dr. Mayorga: I would like to comment on our experience in managing chromoblastomycosis with the antimetabolite 5-fluorocytosine. We treated five patients on a schedule of 100 to 150 mg per kilogram of body weight for different periods of time with the following results:

One patient presented a severe leukopenia after one week of treatment, and administration of the drug therefore had to be stopped.

Another patient with a small, localized lesion in the hand seemed to be cured after we had administered approximately 800 g of the drug orally. Some activity was still observed, however, especially on the border of the lesion.

Two more patients with extensive lesions showed a dramatic improvement in the first few weeks of treatment, but after having received approximately 300 and 800 g, respectively, the fungus was still observable by direct examination.

It is my personal opinion that the therapeutic value of this agent for chromoblastomycosis deserves to be studied on a larger scale.

Finally, I would like to ask Dr. Borelli to comment on his experience with the use of thiabendazole in chromoblastomycosis, because I think he has observed similar results with that drug.

Dr. Borelli: I have tested thiabendazole in mice and in man. In one experiment, 48 mice, half of them males and half females, were inoculated peritoneally with *Fonsecaea pedrosoi*. Both groups developed an equal number of progressive visceral and/or localized lesions. Of those who became ill, half had received the thiabendazole and half had not. The two patients treated with full doses of 2.2 g daily for six months appeared to be completely cured. Every 15 days a biopsy was taken from the scars, and we were able to verify by histology and by culture the continued presence of the parasite. Subsequently, there was a clinical relapse and we had to interrupt treatment because of lack of tolerance.

As to amphotericin B, my experience has led me to the following conclusions: (1) Sulfa-resistant paracoccidioidomycosis is an indication for its use. (2) The dosages for candidiasis, paracoccidioidomycosis, coccidiodomycosis, cryptococcosis, and histoplasmosis depend on the response in each case. Most cases will be cured with maximum foreseeable rapidity with a dose of 0.1 to 0.25 mg per kilogram of body weight four times a week, applied in a glucose solution of 1 mg/10 ml at the rate of 33 drops per minute, or approximately 100 ml per hour. The treatment routine should not be interrupted.

With regard to paracoccidioidomycosis, in my opinion it does not make sense to expect inhalation to be the agent's usual means of penetration. Sulfa drugs are the treatment of choice for paracoccidioidomycosis. I have had experience with sulfadiazine, sulfamethoxypyridazine, and sulfadoxine (Ro 4-4393). This last was the most active. It reaches suppressive levels with doses of 0.5 g given every 48 hours. Treatment of paracoccidioidomycosis should be prolonged without interruption for three years. This has been my own practice for the past 15 years. There are no firm criteria for cure. All virgin cases will be cured with sulfa therapy properly applied. To achieve effective results, these patients should receive psychological support, and sometimes even financial assistance. I suggest that they be given a permanent card that they can present at any public clinic in order to receive their periodic doses of sulfa free of charge.

Amphotericin B in low dosages, as indicated above, and diaminodiphenylacine hydrochloride may be considered, when administered over longer periods of time, as substitutes for the sulfas, just as a parachute is a substitute for a good engine.

Dr. Ajello: What are the economics of large-scale tinea capitis control programs? How much is needed in the way of money, people, and drugs?

Dr. Grin: This is very difficult to say. It depends on the extent of the campaign.

I am very glad that Dr. Baldó gave some recognition to the mycoses as a public health problem. Certainly ringworm of the scalp could be dealt with very easily in the developing countries, since it does not require highly qualified personnel to carry out the field work. Paramedical personnel are used in Yugoslavia. Every year we examine about 200,000 persons and treat approximately 2,000. I think it is very important to start such campaigns in the developing countries, because the effort is very useful in raising the standards of hygiene. As an illustration, in Yugoslavia the consumption of soap increased 15 to 20 per cent after our campaign. These programs are a first step toward practical health education for people living in primitive conditions. Because of this effect, I would say that any amount of money spent on antimycotic control programs is worthwhile. However, I would recommend that such public health efforts in the developing countries be concentrated first on diseases that are easily controlled.

Dr. Mackinnon: With regard to South

American blastomycosis, the involvement of muscles in experimental laboratory animals kept at rather low room temperatures is such a striking and important feature that it would not be surprising if some degree of muscle involvement were also recorded in man. The distal parts of the limbs appear to be the areas most likely to be affected. The cutaneous lesions seen in the face and neck suggest that a myositis of the cutaneous muscles may be the underlying cause of these epidermic lesions.

I was very interested in Dr. Robledo's references to myositis in South American blastomycosis, and I would like to ask him which groups of muscles were most often affected, and how frequently the adrenal glands were involved.

Dr. Robledo: We became interested in the problem of myositis in paracoccidioidomycosis because of the reports of Dr. Mackinnon. In postmortem examinations, we have studied muscles of the extremities, muscles of the face, and deep muscles that have high temperatures, such as the psoas and the diaphragm. We have not studied enough cases to have significant data yet, but it has been our impression that involvement is greater in the muscles of the legs than in the deep ones.

I wish to add that the last word on paracoccidioidomycosis has not been spoken from any viewpoint. I feel we have a lot to learn and investigate.

Dr. Conti-Díaz: When Dr. González Ochoa referred to the treatment of sporotrichosis in his paper, he did not make any mention of the local heat treatment we have used with success in Uruguay. We believe it is a useful approach, particularly in cases of intolerance to iodides.

Dr. González Ochoa: I wish to mention that it is far easier to give the patient a few spoonfuls of potassium iodine than to expose him to steam for several hours each day.

Chairman Drouhet: I wish we had time to discuss some of the important problems of public health that we have not yet touched on. I regret particularly that Professor Seabury did not have an opportunity to talk about the treatment of North American blastomycosis. He has treated many cases and has had excellent results. I am also sorry we were not able to mention the fungal complications of modern therapy—complications after the use of antibiotics, after immunosuppresive therapy, after surgical intervention in the heart and other organs, and after the use of contraceptive pills producing Candida vaginitis.

From our experience at the hospital of the Pasteur Institute and other hospitals in France we agreed on a moderate dosage of amphotericin B by perfusion—0.5 mg/kg, or half the recommended dosage—for treatment of the deep fungal infections. In some cases where the usual intravenous dose of amphotericin B would be toxic, such as renal candidiasis associated with artificial kidneys, we have obtained very good results with oral amphotericin B at 100 mg/kg combined with 5 and 10 mg amphotericin B by perfusion in adults. Even with this small dosage, the cases were cured in a short time. One case of peritonitis—*Candida parapsilosis* infection following peritoneal dialysis—was cured after a week's treatment by the introduction of 10 mg every day.

Session IV

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ECOLOGY AND EPIDEMIOLOGY

Chairman

Demosthenes Pappagianis

Rapporteur

H. G. Muchmore

ECOLOGY AND EPIDEMIOLOGY OF SPOROTRICHOSIS

Juan E. Mackinnon

The present paper will deal with the infection produced by *Sporothrix schenckii* Hektoen and Perkins, 1900. A better understanding of clinical, epidemiological, and therapeutic features will be helped by a discussion of ecological aspects of the causal organism.

Sources of the infection

There is almost complete agreement that plant debris, wood, straw, and similar materials constitute good substrates for the growth of *S. schenckii* in nature. However, the various research workers reporting isolation of the fungus from nature have described quite different characteristics in the cultures. Our own recently published results (*65*) differ from those of Howard and Orr (*47*) and in turn from those of Mariat (*66*): three papers and three different results.

We isolated seven strains (*65*), followed by two others, from rotten fallen palm-tree trunks, from dry grass accumulated by armadillos and wild rodents inside their holes or nests, and from a sandy soil covered by mosses and partially protected from direct sunshine. Our cultures showed dark brown oval-shaped, sometimes biconvex, radulaspores forming sheaths or "sleeves" of conidia around the hyphae. After detachment of the conidia, the hyphae exhibited multiple spicules. All the nine strains grew at 37°C but not at 39°C. When injected intraperitoneally into male mice they produced the typical vaginalitis, epididymitis, and orchitis, and eight of them originated metastases in the bones of the tail and paws.

We also isolated strains that looked like pale strains of *S. schenckii* when growing on Sabouraud's glucose agar but produced a downy whitish growth on corn meal agar and on willow wood unlike the flat growth of *S. schenckii*. Some were identified as *Ceratocystis sp.*, while others produced the Graphium form and still others did not produce synnemas. They did not produce true "sleeves" of radulaspores. In some of the inoculated mice there were small hard nodules in the omentum, and a number of parasitic forms similar to the cigar-shaped or yeast-like bodies of *S. schenckii* were seen. These lesions were nonevolutive and never gave rise to metastases. Nevertheless, the fungus was frequently recovered three to four weeks after inoculation.

Mariat (*66*) isolated colonies from a decorative plant with thorny leaves that was suspected of being related to a case of sporotrichosis. The fungus was also isolated from the soil around this plant as well as from other similar plants and soil in the greenhouse. The isolated strains were pale, had little or no pathogenicity, and failed to convert, or only partially converted, to the yeast phase of growth at 37°C. These strains were different from our strains isolated from nature.

Mariat, Escudié, and Gaxotte (*68*) isolated pale nonpathogenic strains of *Ceratocystis sp.* from the scalp of Africans and developed an interesting hypothesis involving the possibility

that *S. schenckii* might be the conidial stage of a heterothallic species of the genus Ceratocystis. Mariat (*67*) refers in a very recent paper to a strain of *Ceratocystis sp.* showing some pathogenicity for the hamster, and Mariat, Destombes, Diez, and Nazimoff also report some pathogenicity for the mouse.[1]

Howard and Orr (*47*) isolated nine strains regarded as *S. schenckii* or nonpathogenic varieties from rat dung, wood, and soil. Most of them did not grow at 37°C. We studied five of these strains received from Dr. Howard. Four of them did not grow at 34.5°C and were nonpathogenic for the mouse. They produced dark brown conidia but appeared spherical when fully developed. They did not detach easily from the hyphae, which failed to show the spicules seen in *S. schenckii*. The fifth strain scarcely grew at 37°C, it did not produce fuliginous spores, and it was nonpathogenic.

Many other authors contend that they have isolated *S. schenckii* from various substrates. Du Toit (*28*) believed that he had isolated two strains from the air and from timber at the Venterpost mine in Transvaal, although he admitted that these strains showed little virulence. Emmons (quoted in *12*) later identified Du Toit's strains as Graphium.

In the light of such results, we are not inclined to rely on reports of *S. schenckii* isolation from nature without further details about the isolated strains. There are references in the literature to isolations from horsehide and horsehair (*70*), from straw used for packing pottery (*34*), from shrimp (*73*), from materials gathered on tide-washed coastal areas (*23*), from moss (*21, 24, 38*), from straw and from soil in Brazil (*90, 100*), from the intestinal contents of one of 1,270 bats in Colombia (*45*), and so on, but no mycological details are provided, and the experimental pathogenicity is either not studied at all, or else affirmed without giving any further details. In other papers some differences between human and wild strains are quoted (*24*). The diagnosis of sporotrichosis is easy because the patient is affected by only one species among many other nonpathogenic contaminants and the clinical picture is frequently characteristic. The isolation of various species from nature presents a different problem, however, even if inoculated animals are used; nonpathogenic species related to Ceratocystis, Graphium, and the like can survive for weeks and even appear together with *S. schenckii* in the cultures (*65*). On routine diagnosis media such as Sabouraud's glucose agar, young cultures of the above-mentioned fungi show similarities with *S. schenckii*.

In view of these difficulties, it is important that the experimental pathogenicity of the isolated strains be assayed and that the evolutive vaginalitis, epididymitis, orchitis, and metastases in the bones of the paws and tail be obtained. Proper attention should also be given to the purity of the inoculated cultures. The existence of nonpathogenic strains of *S. schenckii* may be regarded as a possibility.

Some papers leave no doubt about the pathogenicity of strains of *S. schenckii* isolated from nature. Simson, Helm, Bowen, and Brandt (*102*) inoculated volunteers and observed that the strains considered undistinguishable from *S. schenckii* by Brown, Weintroub, and Simpson (*12*) did, in fact, produce typical sporotrichosis lesions.

De Beurmann and Gougerot (*27*) isolated the species from a fern, from a beech tree, and from oat grains. They reported that the strain isolated from oat grains showed a slight degree of virulence, which increased following passage through animals. They published a drawing in which the fungus looks like *S. schenckii*. The experience of these authors with experimental disease in rats deserves consideration, and the present writer is prepared to concede that the strain isolated from oat grains was similar to those isolated from patients. We have noted

[1] Mariat, F., P. Destombes, E. Diez, and O. Nazimoff. Pouvoir pathogène expérimental chez le hamster et la souris d'une souche de *Ceratocystis*. Manuscript of paper read at the meeting of the Société Française de Mycologie Médicale, December 1969. Received from the authors.

that the virulence of strains recently isolated from nature is similar to that of strains isolated from man. The variations in pathogenicity that De Beurmann and Gougerot claim to have observed may well be due to variations in temperature in the animal room. It has been our experience that a rapid course and over-all spread are favored by low environmental temperatures (*62, 64*).

An important contribution to the study of the ecology of *S. schenckii* may be the direct recognition of its growth on natural substrates. Brown, Weintroub, and Simpson (*12*) noted a black discoloration in the wood, while the identity of the fungus was based on the many "triangular" or biconvex conidia. De Beurmann and Gougerot also described black discoloration of oat grains invaded by *S. schenckii*. We failed to observe the "triangular" conidia in our materials, probably because they were scarce or because not all the strains were capable of producing them. Mariat, Lavalle, and Destombes (*69*) found these conidia in Mexican strains, and they were also produced by a Uruguayan strain (*65*).

Benham and Kesten (*10*) inoculated living carnation and rose buds with *S. schenckii*. This species and others caused rot in the carnation similar to that attributed to *Sporotrichum poae*. The latter was nonpathogenic in the rat and monkey. There is no basis for believing that plants can act as intermediate hosts in sporotrichosis.

There are some few cases of human transmission. When several cases occur in a family or group, as reported by Silva and Guimarães (*100*), they can be traced to the same extrahuman source.

At the beginning of the century, Lutz and Splendore (*59*) undertook the study of a disease in rats. It was observed as nodules in the tails of animals caught during a campaign against plague. The rats also showed localizations in the paws and viscera. The authors reported five cases in man, and one of them was attributed to a rat bite. Following the growth of the fungus, the disease was found to be identical to sporotrichosis, which was then being studied in France. Lutz and Splendore thought that the fungus lived in the mouth of rats and was transmitted by biting.

In 1908, Gougerot and Caraven (*43*) discovered sporotrichosis in a dog. Shortly thereafter, Carougeau (*17*) found it in a mule and recorded its transmission to a veterinarian who was wounded while cutting an abscess. In 1915, Meyer (*70*) stated that 10 to 30 cases per year were recorded in horses in Pennsylvania. He ascribed no importance to the transmission of sporotrichosis from one horse to another or from horses to man. The infection was most frequently contracted on land recently cleared and put to agricultural use. In these areas, dry tree trunks, branches, splinters of wood, and the like serve both as the cause of the injuries and as good material for growth of the pathogen. Meyer discussed the infection of man by animal bites, suggesting that the fungus was transmitted passively and reached the animals through their food.

It is quite probable that sporotrichosis occurs commonly in some domestic animals in Latin America. Albornoz (*1*) recorded a case in a mule in Colombia. Piratininga (*81*) and Saliba, Sorensen, and Marcondes-Veiga (*92*) saw similar cases in Brazil. Londero, Castro, and Fischman (*56*) quoted further cases seen by Leão and by Mello and also reported two cases in dogs. Freitas, Moreno, Bottino, Mos, and Saliba (*35*) reported 12 cases in dogs and eight in cats. The animals were not considered to be possible reservoirs.

Thermal and hygrometric requirements of the agent of sporotrichosis

Brown, Weintroub, and Simpson (*12*) undertook experiments on dry, sound, and seasoned timber and on oven-dried maltose agar. Good growth was recorded at 100 per cent relative humidity, fair at 97.5, feeble at 95, very slight at 92.5, and nil at 90 per cent. It is concluded that well-seasoned timbers will sustain the

growth of the pathogen as long as the relative humidity is between 95 and 100 per cent. The temperature at the Venterpost mine was between 26° and 27°C. *S. schenckii* grows very well at temperatures between 20° and 32°C. At 37°C it grows in the yeast phase. We have determined the maximal temperature for growth —or thermotolerance—of 12 strains, and we observed that *S. schenckii* does not grow at all at 39°C and only very poorly at 38°C. Seven strains had been isolated from nature and five from cases of cutaneous sporotrichosis.

Our observations in Uruguay suggest the influence of temperature and relative humidity in the epidemiology of sporotrichosis on the earth's surface as well. It is more frequently contracted during the months of autumn and early winter (March to July) (*60, 61, 62*). Periods of several days with repeated rainfalls, relative humidity values close to saturation, and temperatures between 16° and 20°C are not unusual during the Uruguayan autumn. In 1944, nine cases were recorded, all the subjects having contracted the infection over the period May 21 through June 7. During the last 10 days of May that year the relative humidity was close to saturation. Following these observations, it was possible for us to forecast small outbreaks. Similar features were seen by Silva (*26*) in Rio Grande do Sul, Brazil. The growth of *S. schenckii* in nature was presumed to be the cause of the seasonal variations. Sporotrichosis is a rare disease in countries with scarce rainfalls, such as Central and North Chile; cold countries, such as Canada (*31*); and semiarid lands, high plateaus, and mountains. Nevertheless, microclimates favorable for the growth of *S. schenckii* in adequate substrates may be artificially created not only in mines but in nearly any place at all, as for instance in the three cases observed by Balabanoff, Koevu, and Stoynovski (*6*) in a paper factory.

Modes of infection

It is agreed that the fungus is usually introduced into the tissues as a result of an injury in the skin. The experiments published by Baker (*5*) demonstrate that the injection of the fungus into the paws of mice produces an evolutive infection in most of the animals.

Numerous sources of injury have been reported: farming implements, thorns of plants such as the barberry shrub (*32*) or the rosebush (*49*), splinters of wood, sphagnum moss in tree nurseries (*21, 24*), straw for packing earthenware (*41*), and other articles (*100*). In Uruguay, almost half the patients related their infection to armadillo hunting. *Dasypus septemcinctus* digs its hole in the earth, and the hunter can seldom draw it out without breaking the ground and thrusting an arm inside the burrow. Skin traumas frequently occur during these maneuvers, and the patients ascribe these injuries to being clawed by the animal, being scratched with improvised wire hooks, or rubbing against rocks, pricks of thorny shrubs, and other sharp objects.

Singer and Muncie (*103*) presented six patients who contracted the infection within a limited area of Long Island from grass mulch used on bulb farms. In Mexico, Barba Rubio (*7*) attached importance to animal bites. Even the peck of a hen and the sting of an arthropod have been noted as the cause of sporotrichosis (*75*).

A pulmonary portal of entry is possible. Forbus (*33*) reviewed the cases considered to be primary pulmonary sporotrichosis and concluded that the first to be proved was that of Warfield (*107*), reported in 1922. He presented a second of his own. Forbus' criticism was justified, but neither Warfield's nor Forbus' case is demonstrative. The mycological study of these cases is not probatory. Forbus insisted that the fungus be isolated from tissues, and modern surgical treatment has permitted this.

Scott, Peasley, and Crimes (*95*) presented two cases with positive cultures from sputum and from tissue. The complement fixation test was negative with Histoplasma, Coccidioides, and Blastomyces, but positive with *S. schenckii*.

Ridgeway, Whitcomb, Erickson, and Law (*89*) reported two cavitary cases with the isolation of *S. schenckii* from tissue. The mycologi-

cal study was not complete, but the diagnosis appears to be quite reliable. In the second case, the complement fixation test was positive with *S. schenckii* and negative with Histoplasma, Coccidioides, and Blastomyces.

Siegrist and Ferrington (*99*) reported a case in which *S. schenckii* was isolated from sputum, bronchial washings, and surgical material. The microscopic appearance of the culture was typical, and the inoculation of mice proved positive. Moreover, the skin test was strongly positive and the precipitin test (double diffusion agar) was positive, too.

Beland, Mankiewicz, and MacIntosh (*9*) isolated *S. schenckii* from a surgical specimen and obtained classical orchitis in inoculated male mice.

Experiments in mice also support the possibility of a pulmonary portal of entry. Sethi, Kneipp, and Schwarz (*97*) placed a drop of suspension fluid from a yeast-phase culture in the nares of 21 previously anesthetized mice. The presence of the fungus was demonstrated in the lungs of 17 mice and in the livers of nine. In four of the mice from the latter group the fungus was also recovered from the spleen. Generalization of the infection occurred around the 26th day after the inoculation.

Conti-Díaz and Civila (*19*) conducted an experiment under conditions similar to those found in nature. They used Piggott and Emmons' device (*83*) with calcium chloride to avoid the problem of humidity due to the animals' breathing. Chips of wood with growths of the fungus were suspended inside the device. The movements provoked in the air caused brownish-colored conidia xerospores to become detached, and these could be easily recognized in the lungs and in tissue sections because of their color. The fungus could be recovered from the lungs of two animals out of 20 and from the liver and spleen of one.

Brown, Weintroub, and Simpson (*12*) demonstrated that air currents with a velocity of 200 feet per minute (60 meters) were sufficient to detach the spores.

Some authors have contended that the rat can be infected by the intestinal route. Sethi (*96*) fed 14 hamsters with the livers of heavily infected mice. Five animals gave positive cultures from the lungs, liver, and spleen. Small lesions were seen in the lungs and liver and in two spleens, but there were none in the intestinal tract. The author assumed that the infection was acquired through the lungs.

Effects of the infection: the spectrum of infection

The range of possibilities is broad, from sporotrichosis infection through self-limited cases to severe ones.

Sporotrichosis infection

In 1953, Mackinnon, Artagaveytia-Allende, and Arroyo (*63*) recorded strongly positive skin tests with 1:1,000 sporotrichin in two out of 50 persons without a previous history of sporotrichosis. These two positive reactors were not sensitive to histoplasmin, coccidioidin, paracoccidioidin, or trichophytin. Consequently, they were considered to be demonstrative of the existence of sporotrichosis infection.

In 1960, Castro (*18*) reported rates of sensitivity varying from 9 to 16 per cent in São Paulo, thus also suggesting the existence of sporotrichosis infection.

Pereira, Padilha-Gonçalves, Lacaz, Fava Netto, and Castro (*81*) recorded 10 positive reactors among 92 children between two months and 12 years of age in a group living under poor hygienic conditions in São Paulo. It was not possible to ascertain whether any of them had actually suffered from sporotrichosis. The same authors also observed six positive reactors among 100 children two to 13 years of age living in an asylum where sporotrichosis had never been recorded. Together with Silva and Neves (*25*), these authors used the same antigen in Portugal and found only one positive reactor among 78 persons. In Germany, working with Wernsdörfer (*108*), they had negative results from a group of 55 subjects. A cellular sporotrichin was used in these surveys.

Schneidau, Lamar, and Hairston (*94*) found a rather high rate of sensitivity in a Louisiana study: 11.2 per cent among 349 prison inmates and hospital patients, and 32.3 per cent among 34 tree nursery employees. Looking for some correlation between these reactions and those to other antigens, they concluded that there is a strong possibility that some positive reactions to histoplasmin cross-react with persons sensitive to *S. schenckii,* and that the reverse is unlikely.

Ingrish and Schneidau (*48*) found 15 positive reactors to sporotrichin among 203 persons in Arizona. Cross reactions in persons sensitive to histoplasmin or coccidioidin were discarded, and the experiments in guinea pigs sensitive to Sporotrichum and Histoplasma showed too low a rate of cross reactions. This result is rather intriguing, since only a few cases of sporotrichosis have been recorded in southwestern United States and only eight of the 15 positive reactors had migrated to Arizona from other areas.

Self-limited sporotrichosis

The agent of sporotrichosis elicits remarkably strong immunological effects in man. Clinical cases usually respond easily to treatment, and cases of spontaneous cure, or self-limited sporotrichosis, have been reported by Errecart (*29*), Padilha-Gonçalves (*78*), and others. Helm and Berman (*46*) contend that spontaneous cure only occurs in a few cases, and most often in those showing the flat plaque-type of lesion.

Self-limitation may be due to an enhancement of the immunological defenses, to the effects of high environmental temperatures, or to other causes yet to be identified. Miranda, Cunha, and Schweidson (*71*) and Padilha-Gonçalves (*78*) cured several cases by intradermal injections of sporotrichin. The present author and co-workers, on the other hand, have cured many patients with thermotherapy (*36, 64*). We shall discuss this effect later.

Evolutive sporotrichosis

Most of these cases are cutaneous or subcutaneous. The lymphatic form appears as a primary complex or chancriform syndrome. Disseminated cutaneous sporotrichosis is considered to be an effect of hematogenous dissemination from a visceral primary lesion; however, a cutaneous form may also lead to a disseminated case.

Disseminated sporotrichosis is not frequent. Almeida, Sampaio, Lacaz, and Castro-Fernandes (*3*) recorded two cases in a series of 344 patients. Fernández, Perdomo de Fernández, and Dávila (*30*) recorded one case in Uruguay. Bone lesions are not rare in these patients, and one such case was reported by Curban, Lacaz, Belfort, Dillon, and Auada (*22*). As in the inoculated rats, the affected bones are those of the distal parts of the limbs, especially the carpus and the metacarpus. The case of Moore and Kile (*74*) was considered of pulmonary origin.

Among the thousands of cutaneous cases from the gold mines in the Transvaal, Lurie (*58*) reported five instances of dissemination: one gummatous lesion in a hand; lesions of the muscles; lesions of the bones in two cases; and skin, subcutaneous, muscular, visceral, and bone lesions in a man with sarcoid reactions.

Even cases of meningitis have been recorded by Schoemaker, Bennett, Fields, Whitcomb, and Halpert (*98*) and by Klein, Sue-Ivens, Seabury, and Dascomb (*50*). The cases of primary pulmonary sporotrichosis have already been discussed.

The incidence of sporotrichosis

Incidence of the disease varies widely from one region to another. Singer and Muncie (*103*) collected 275 cases in the medical literature in the United States up to 1952, while, according to Schneidau, Lamar, and Hairston (*94*), 75 additional ones had been recorded up to 1964. Foerster (*32*) states that it is more prevalent in the midwestern United States and in the Mississippi river basin. However, since not all the cases are published, it is impossible to know the true incidence of the disease.

The number of published cases from the United States is relatively low compared to reports from some of the other countries of the

Americas. In São Paulo, Almeida, Sampaio, Lacaz, and Castro-Fernandes (*3*) reported 344 cases up to 1955, and Sampaio, Lacaz, and Almeida (*93*) stated that 0.5 per cent of the patients entering the Dermatology Clinic were cases of sporotrichosis. In another São Paulo clinic, Rotberg, Defina, and Pereira (*91*) found 148 cases among 10,534 patients. Sporotrichosis is also frequent in Rio de Janeiro (*80*). In the state of Rio Grande do Sul, Londero, Fischman, and Ramos (*57*) collected 57 cases in the city of Santa Maria over a five-year period, and Silva (*26*) observed 86 cases in Pôrto Alegre over eight years. Campos (*15*) stated that 0.22 and 0.38 per cent of the patients who attended two dermatological clinics in Pôrto Alegre were sporotrichosis cases. It is most interesting to compare the percentages of patients with sporotrichosis at the dermatological clinics in Brazil with the situation in a country where sporotrichosis is rare. Gay Prieto (*39*) reported that the percentage in Madrid, Spain, is 0.003. Thus, according to these data, the disease is 1,000 to 4,000 times more frequent in southern Brazil than it is in Madrid.

On the other hand, Morães and Oliveira (*76*) report that sporotrichosis is rare in Manaus. Surveys to establish the rate of positive reactors to sporotrichin should be carried out in order to know whether self-limited and subclinical forms of the infection are common in torrid zones.

In Uruguay, 164 cases of sporotrichosis are on record. The percentage of cases at the Dermatology Clinic of the Hospital de Clínicas is 0.14 per cent.

Sporadic cases are seen in Argentina, and about 40 have been reported so far. Freire (*34*) observed eight cases in Chaco and states that the disease is not rare in this province. According to Niño (*77*), most of the Argentine cases are from Chaco, Santa Fe, and Buenos Aires. Grinspan and Madeo (*44*) recorded a small outbreak of three cases in Buenos Aires. The disease is believed to exist in the northern provinces bordering Paraguay, where it has been reported by González and Rivarola (*40*) and Canese and Añasco (*16*).

In Colombia, Restrepo, Calle, Sánchez, and Correa (*88*) have found 47 cases, which make for a total of 124 for the entire country up to 1962.

In Venezuela, Campins (*14*) found 14 cases reported in the literature up to 1958. Convit, Borelli, Albornoz, Rodríguez, and Hómez (*20*) recorded 44 additional cases, almost all of them from elevations of over 500 meters where rainfall was heavy.

Only five cases have been published in Ecuador up to 1964, according to León (*54*). Veintemillas (*106*) quoted the only known case in Bolivia; he states that the disease is not rare in this country. No references are available from Chile. In Peru, Miranda, Fernández, Golden, and Suárez (*72*) reviewed the literature and quoted 20 cases up to 1967. The disease is known in French Guiana, where Silverie and Ravisse diagnosed two cases (*101*). It has been recorded in Panama by Calero and Tapia (*13*), and in Costa Rica by Bolaños and Trejos (*11*). Trejos and Ramírez (*104*) have surveyed 114 cases in the latter country and state that the disease is known in El Salvador. It also occurs in Honduras and Guatemala (*54*).

In Cuba, Alfonso-Armenteros (*2*) has surveyed 20 cases. The disease was recently diagnosed in Guadeloupe by Audebaud, Escudié, and Courmes (*4*).

In Mexico City, Ramírez (*85*) recorded 27 cases in a period of only 18 months. According to González Ochoa (*42*), patients stem from practically every state of Mexico. Garrett and Robbins (*37*) quoted data provided by Dr. O. Germes, who claims that 0.3 per cent of the patients in a dermatologic clinic in Juárez, Chihuahua, are cases of sporotrichosis. This rate is similar to those recorded in southern Brazil.

Sporotrichosis is of very rare occurrence in Canada, according to Fischer and Markkanen (*31*).

Localization and prevalence by sex and age

In all the countries, localization in the upper limbs is predominant, and this is presumed to be related to occupational causes. When the disease is acquired while playing, particularly in the case of children, localization in the face becomes rather frequent (*57*).

In Uruguay, 90 per cent of the cases occurred in males and only 10 per cent in females. In Medellín, Colombia, however, according to Restrepo, Calle, Sánchez, and Correa (*88*), 25 per cent of the cases were females. The rate of female infection for Caracas, as reported by Convit, Borelli, Albornoz, Rodríguez, and Hómez (*20*), was 32 per cent, and for São Paulo, according to Almeida, Sampaio, Lacaz, and Castro-Fernandes (*3*), 47 per cent. In Rio Janeiro, 38 out of the 68 patients of Padilha-Gonçalves and Peryassú (*80*) were females.

Only six children under 14 years of age are among our 164 patients in Uruguay. In Mexico, Ramírez (*85*) saw seven children under 14 years of age among 27 patients. Londero, Fischman, and Ramos (*57*) noted that 22.8 per cent of their patients were children between one and 10 years old, while in Pôrto Alegre only 6.6 per cent of the cases recorded were in this age group (*26*). This difference is explained by Londero and collaborators by the frequent opportunities that children have to come in contact with vegetation, particularly while playing in yards. In 1965, Londero confirmed his results (*55*). Almeida, Sampaio, Lacaz, and Castro-Fernandes (*3*) recorded 20 per cent of their cases among children between one and 10 years of age. León (*54*) observes that four of the five cases reported in Ecuador were in children.

It is the present author's opinion that there is no real evidence of special susceptibility on the part of males and adults; rather, their opportunities for infection are greater.

Effect of ambient temperature on infection

High temperature values and humidity rates favor the growth of *S. schenckii* in nature and increase the possibility of infection. In addition, environmental temperature can influence the course of an infection already established. An assay of five strains from human cases and seven strains isolated from nature showed that the fungus grows poorly at 38°C and does not grow at all at 39°C. These temperature values, which are critical for the growth of the fungus, are close to the inner temperature of man and of the animals used in the laboratory. If we recall that *S. schenckii* is a saprophyte and not a parasite, that the living tissues of man and animals constitute an abnormal substrate, that human serum has a fungistatic effect on the species (*8*), and that the fungus elicits a strong immunological response in man, we may assume that its thermotolerance in tissues is lower than that recorded in cultures. The fungus adopts the yeast phase at 36° to 37°C, and any proximity of the tissue temperature to the maximum tolerance rate is bound to endanger its survival. This harmful effect is demonstrated by experiments in rats and by thermotherapy in man.

Lesions in the bones of the paws and tail were recorded by Mackinnon and Conti-Díaz (*64*) in 13 of 15 rats inoculated intracardially and kept in a room at a temperature ranging from 5° to 15°C, but no lesions occurred in any of the 13 rats kept at a room temperature of 31°C.

The author and co-workers (*64*) successfully treated one human case of the lymphatic type of sporotrichosis in the hand and forearm by means of hot wet dressings applied two to three times daily for periods of 30 to 40 minutes over three months. Galiana and Conti-Díaz (*36*) treated two cases with local heat, another case by local application of a rubefacient, and five cases by both methods: hot wet dressings and the rubefacient, nicotinic acid tetrahydrofurfuryl ester, Trafuril (Ciba Laboratories). All nine patients were cured. They were not treated with potassium iodide or any other specific drug. The healing effect of heating was confirmed by Laca (*51*) and by Trejos and Ramírez (*104*).

Sporotrichosis is usually localized in zones of the body, limbs, and face whose temperatures are

influenced by the temperature of the environment. The ecological conditions of the tissues would be suitable for the growth of a fungus showing a low thermotolerance whenever they are cooled by the air, but they might become unsuitable if the temperature of the tissues increases to a value close to the inner body temperature. The local rise of the temperature would delay or stop the multiplication of the fungal elements by modifying the host-pathogen relationship in favor of the host. The usually strong immune response of man to *S. schenckii* and the nonspecific defenses would provoke a final sterilization of the lesions and consequently a cure. The local immune effects may also be favored by a rise in temperature.

The problem of pulmonary and disseminated sporotrichosis deserves some attention. We did not have an opportunity to study strains of *S. schenckii* isolated from such forms. Are these cases due to special strains, or are they due to immunological deficiencies? At the moment we can only speculate on this question.

Various aspects of sporotrichosis in different countries

In Uruguay, 88 per cent of our cases are lymphangitic forms. Even in some lesions of the face, the lymphatic vessels have been involved. In other countries, the relative frequency of this form is not so predominant, or else the nonlymphangitic forms predominate. Almeida, Sampaio, Lacaz, and Castro-Fernandes (*3*) observed lymphatic forms in 62 per cent of their patients in São Paulo. In Rio, Ramos e Silva and Padilha-Gonçalves (*86*) recorded 40 lymphatic forms among their 68 cases (58 per cent). In Medellín, Colombia, Restrepo, Calle, Sánchez, and Correa (*88*) recorded only 11 lymphatic forms among 47 patients (23.4 per cent), the rest being epidermal forms that were sometimes very atypical, simulating chromoblastomycosis, as shown in another publication by Restrepo, Calle, Robledo, and Rivera (*87*). In Venezuela, Convit, Borelli, Albornoz, Rodríguez, and Hómez (*20*) report lymphatic forms in only 21.6 per cent of the patients studied, and Vegas estimates a rate of 50 per cent (*105*). In Mexico, the lymphatic forms predominate: 67 per cent according to González Ochoa (*42*) and 82 per cent according to Latapí (*52*).

The above reports show a low relative frequency of the lymphatic forms in Medellín, Colombia, and in Caracas, Venezuela; high relative frequencies in Uruguay and Mexico; and an intermediate relative frequency in São Paulo, Brazil. Do these figures reflect actual facts? If so, the causes underlying these differences should be examined.

It is hard to determine differences accurately. The uneven thoroughness and experience of the reporting workers in the field may account for variations in the stated incidence of atypical sporotrichosis and in the relative frequency of so-called common forms. Vegas (*105*) ascribes the higher relative frequency of lymphatic forms to failure to recognize the atypical, fixed, and minimal forms. On the other hand, the higher frequency of nonlymphatic forms might also be due to the absence of early diagnosis in areas with inadequate medical facilities. Lavalle (*53*) remarked that the lymphatic forms have a tendency to become confined after some time. This is confirmed by our experience, and it is quite logical, since the new immunological status is not adequate for a permanent chancriform syndrome.

Padilha-Gonçalves and Mattos (*79*) reported two reinfections in the same patient without involvement of the lymphatic vessels. It is the present author's opinion that in countries where sporotrichosis is frequent the opportunities for reinfection must be likewise frequent in a partially immunized population, the chancriform syndrome being replaced by fixed forms in at least some of these cases.

Can these differences be accounted for by the effects of climate, and of temperature in particular? One is tempted to accept this theory after looking at the map, which shows that Medellín and Caracas are between latitudes 5° and 10° N, Uruguay between latitudes 30° and 35° S, and central Mexico around latitude 20° N but at a

high altitude. Nevertheless, on considering the possible influence of the differing immunological status of the populations, one is inclined to believe that both the immunological status and the temperature of the environment are operative and that they may have different effects. The immunological status of a part of the population may account for the absence of the chancriform syndrome, while high temperatures in the environment would diminish the intensity of the syndrome, limiting its size, speeding up its course to fixed and superficial forms, and turning some cases into self-limited ones.

Summary

Sporotrichosis is a two-factor infection caused by the fungus species *Sporothrix schenckii* Hektoen and Perkins, 1900.

Different workers have isolated a number of nonpathogenic species of fungi from nature that are regarded as varieties or related species, and consequently present knowledge on the ecology of *S. schenckii* may appear confusing. Despite the existence of typical mycological characteristics, the pathogenic effect of each strain isolated from nature must be studied. Typical pathogenic strains have been isolated from wood, plant debris, and soil.

High hygrometric and moderate temperature values are necessary for *S. schenckii* cultures in the laboratory, and the observation of epidemics in the mines of the Transvaal as well as the seasonal incidence of sporotrichosis in Uruguay warrant the assumption that a similar situation occurs in nature.

The disease is usually contracted through injuries of the skin. Experiments in animals have demonstrated the possibility of pulmonary infection by the inhalation of fuliginous conidia, or xerospores. A number of human cases recorded during the last decade demonstrate the existence of pulmonary sporotrichosis.

The sporotrichin skin test reveals a strong immunological response to *S. schenckii* in man, and also the existence of sporotrichosis infection without actual disease. Self-limited cases have been recorded.

The disease is relatively common in certain parts of southern Brazil, Venezuela, Colombia, Mexico and Uruguay. It is very uncommon in semiarid areas and cold countries. There is no evidence of susceptibility to a particular sex or age group, and the differences that have been recorded in some countries can undoubtedly be ascribed to occupational factors.

In inoculated rats and mice, the fungus develops in those tissues and organs that easily become cooled by the outside environment, such as the vertebrae of the tail, bones of the paws, and testicles. A high temperature in the environment, from about 31°C, inhibits the evolution of the disease immediately after inoculation. Since the thermotolerance of *S. schenckii* is between 38° and 39°C, it is thought that the temperature of the tissues becomes unsuitable for its multiplication. The healing effect of temperature can also be seen in man. This therapeutic action may be due to the direct effect of temperature, or also to immunological reactions that may be simultaneously enhanced by the rise of temperature.

Climate may also have an effect on the clinical manifestations of cutaneous sporotrichosis.

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ECOLOGY AND EPIDEMIOLOGY OF CHROMOMYCOSIS

F. Montero-Gei

Chromoblastomycosis, or verrucose dermatitis, is a chronic mycotic infection of the skin and subcutaneous tissues characterized by the formation of warty cutaneous nodules and by verrucose, ulcerated, and crusted lesions, which are sometimes pedicellate and irregular, presenting a cauliflower-like appearance. The disease is caused by several species of dematiaceous fungi, which become dark brown septate bodies in the tissue and multiply by septa. The study of the localization and mode of spread of this group of fungi is, of course, a very interesting problem for the epidemiologist.

The disease has been described in all continents, and its incidence is greatest in the tropical and subtropical zones lying between 30°N and 30°S. In the Americas, most of the cases have been reported from Brazil, Costa Rica, and Cuba, while in Africa, Madagascar occupies first place.

According to a study by Romero and Trejos (*11*) based on data published by Carrión and Silva in 1947 (*3*), Costa Rica, with one case per 24,275 inhabitants, has the highest incidence of all. A study by Montero-Gei in 1967 (*10*) revealed 18 new cases, and Solano (*12*) reported on 36 in 1966, thus increasing the incidence and identifying certain regions of the country in which the disease is endemic.

In Africa, this mycosis has become increasingly important in recent years, and, according to Brygoo and Segretain (*1*), its incidence is greatest in Madagascar. The region of Antandroy has a case rate of one per 6,819 population, which is unusually high for a mycotic disease.

At present, all the evidence points to the fact that chromomycosis is not a contagious disease in the sense of its being spread from person to person or from animals to humans. It must be regarded, rather, as a disease of nature that is transmitted to humans from some reservoir in the soil or in certain plants.

Profuse production of spores is one of the well-recognized characteristics of the fungi, and it is important to consider the different methods by which they are dispersed. Typically, the spores are resistant to abrupt changes in temperature and humidity, and they are particularly resistant to heat, drying, and other physical conditions. Moreover, they are viable for extremely long periods of time. It is well known that the spores of certain plant diseases can remain viable for several years in the ground or on leaves or plants, until such time as growth conditions are favorable. Another property of the fungi is that they can produce antibiotic substances that inhibit growth or are lethal to other fungi or bacteria. We also know that they are prevalent in the Tropics and rare in colder climates.

It has been demonstrated that the etiologic agents of chromoblastomycosis are excellent producers of spores, but it is not yet known whether other structures of these fungi, such as fragments of mycelium, are capable of causing infection, or whether these fungi produce antibiotics to compensate for their slow rate of growth.

Most cases of the disease are found in males. In Costa Rica, Romero and Trejos (*11*) reported that of 34 cases only one was in a woman. In the 18 cases of Montero-Gei (*10*), again, only

one was in a female, and of the 36 cases studied by Solano (*12*), three were in females.

All races are equally susceptible, although the majority of cases have been described in Caucasian individuals between 25 and 50 years of age. Of the Costa Rican cases reported, the youngest was in a boy 14 years old and the oldest in a man of 70 (*12*).

The lesions are most commonly located on the lower extremities, but they have also been reported on the hands, arms, shoulders, face, buttocks, and neck. Localization is determined by the site of a dermal injury, by means of which spores of the etiologic fungus are introduced into subcutaneous tissue.

The patient's occupation is considered one of the most important factors, since most cases occur in agricultural workers who lack the protection of shoes and who are easily exposed to traumatic accidents in which vegetable fragments and thorns are involved.

The first evidence that the etiologic agents of chromoblastomycosis grow in nature was presented by Conant in 1937 (*4, 5*), when he proved that the species known as *Cadophora americana* Nanfeld, 1927 was a strain of *Phialophora verrucosa*. *Cadophora americana* has been isolated from wood pulp by Kress and co-workers (cited in *6*).

In Costa Rica, Ruíz (personal communication), studying species of the genera *Aspergillus*, succeeded in isolating a black fungus that was later identified as *Fonsecaea pedrosoi* (*13*). It was found in a pasture located near San José. The technique used was to agitate the various herbs slowly at a height of 20 cm over a Petri dish containing coconut milk agar. All attempts to isolate the fungus again from the same location were negative.

Trejos (*13*) demonstrated by means of autoinoculation that a strain isolated from soil was pathogenic for man.

It may be concluded that the etiologic agents of chromoblastomycosis do not require a specific type of soil, as is the case with *Histoplasma capsulatum* (*7*), but rather that the endemic areas are located in the ecological life zones of moist and wet tropical forests and in lower montane wet and rain forest environments where the mean annual biotemperature ranges from 12° to 24°C and the mean annual rainfall is from 2,000 to 4,000 mm (*8*).

The theory has been advanced that these fungi are parasites of certain plants which grow in the ecological environments described above.

In our laboratory, the artificial inoculation of various plant specimens with strains isolated from our cases has led us to conclude that temperature and humidity are more important factors for growth than is the substrate itself. For example, fungi grew quite well on filter paper in a Petri dish kept at room temperature. Thus, we saw that the growth of these strains, like other fungi pathogenic to man (*7*), can be controlled by temperature and humidity. Indeed, the preference of fungi for moist humid conditions and warmer temperatures is well known in the Tropics.

There are relatively few references in the literature to cutaneous sensitivity in chromomycosis (*2, 9*). However, it may be concluded that the antigens prepared to date using the conventional techniques for histoplasmosis and coccidioidomycosis lack specificity and fail to produce appreciable cutaneous reactions in affected persons. For this reason, it has not been possible to carry out any epidiomiologic studies based on the skin test, and we have no data as yet on the existence of subclinical infections. Moreover, the status of animals as reservoirs of the disease has not been demonstrated.

We believe, finally, that the general condition of the host is a very important factor in the establishment of infection. In our endemic areas we have observed that although many farmers and laborers are injured with thorns, vegetable fragments, and dirt-contaminated tools, only a small percentage of them becomes infected. It is believed that certain conditions must exist in the host to make him susceptible to infection by this group of fungi, and that hence the personal habits, nutritional condition, and metabolic status

of these patients in terms of carbohydrates, proteins, vitamins, and enzymatic processes must be studied. It may well be that deficiencies in certain of these areas make the host more readily susceptible. Indeed, we have patients who have had chromoblastomycosis for many years who have also contracted other mycotic diseases whose incidence is quite low in our environment, and for this reason we consider it important to report two cases of double infection: chromomycosis-paracoccidioidal granuloma and chromomycosis-keloidal blastomycosis.

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EPIDEMIOLOGY AND ECOLOGY OF MYCETOMAS

Ernesto Macotela-Ruiz

Introduction

Mycetomas are chronic tumoral lesions located primarily in the subcutaneous tissue with fistulas that excrete pus generally containing granules made up of saprophytic colonies of Actinomycetes or true fungi. This definition excludes as mycetomic lesions those subcutaneous tumoral processes or abscesses produced by fungi or Actinomycetes in which the parasite does not have the tendency to form more or less structured colonies.

Six aerobic species of Actinomycetes have been isolated as pathogenic agents of mycetomas: *Nocardia brasiliensis, N. asteroides, Streptomyces pelletieri, S. madurae, S. somaliensis, and S. paraguayensis* (*16, 21, 24, 30, 40*). The species of true fungi that have most commonly been found to produce mycetomas are *Madurella mycetomi, M. grisea, Leptosphaeria senegalensis, Pyrenochaeta romeroi, Monosporium apiospermum, Cephalosporium falciforme,* and *C. recifei* (*15, 20, 23, 37, 38, 39*).

There are some subcutaneous forms of actinomycosis, for example the cervicofacial type, that should also be considered mycetomas. The pathogenic agent is anaerobic *Actinomyces israelii.*

The following terminology is recommended: actinomycotic mycetoma, when the pathogenic agents are aerobic Actinomycetes; maduromycotic mycetoma, when true fungi are present; and actinomycosis, when the disease is caused by *Actinomyces israelii,* including its mycetomic morphology.

Special note should be taken of the fact that actinomycotic mycetomas are only the tumoral lesions that have just been defined. Nocardiasis is the term used for the pulmonary, meningoencephalic, and subcutaneous infections in which Nocardia is present in its filament form but does not form granules (*12*).

Frequency and distribution

There are several reports on the frequency and distribution of mycetomas (*1, 4, 7, 19, 35*). However, we consider that the work published by Mariat in 1967 (*31*) is the most extensive and precise international survey on this subject to date. The geographical distribution and etiological types of the 854 cases studied by him are summarized in Table 1. It is noteworthy that 57 per cent of the total were actinomycotic mycetomas.

An interesting finding of the survey is that *Nocardia brasiliensis* is the most common agent of mycetomas in Mexico. This fact has been confirmed by other studies, such as that of González Ochoa (*13*), who found that 94 per cent of the mycetomas observed at the Institute of Tropical Diseases were produced by this aerobic Actinomycete. On the other hand, in Africa the most commonly observed mycetomas are of the maduromycotic type.

Although the geographical distribution of the species most frequently isolated (Table 2) is varied (*31, 39*), there is a certain general pattern.

Recently some new species have been de-

Table 1

Survey of mycetomas (Mariat, 1967)

Origin	Actinomycotic	Maduromycotic	Unclassified	Total
United States	9	8	5	22
West Indies	1	3	—	4
Mexico	202	4	—	206
South America	49	58	1	108
Africa	196	249	19	464
Asia	25	5	—	30
Australia and New Zealand	2	—	4	6
Europe	3	11	—	14

scribed: *Pyrenochaeta romeroi* (Borelli, 1959) (*8*), *Neotestudina rosatii* (Segretain and Destombes, 1961) (*38*), and *Leptosphaeria senegalensis* (Segretain, 1959) (*37*). Also, it is now accepted that *Nocardia asteroides* can produce mycetoma as well as nocardiasis.

The reported frequency of mycetomas depends to some extent on the location and type of mycological study centers. However, there does seem to be a tendency toward endemic zones within a particular region, as has been found in Morelos, Mexico, by Atala (*3*).

At the Service of Dermatology and Medical Mycology of the National Medical Center in Mexico City, we were able to confirm only 1,000 cases out of a total of 6,000 patients referred to the Center with suspected mycotic infections (Table 3). Of these 1,000 cases, 913 were superficial mycoses, and 87 were deep mycoses. Of the latter, 24 cases were aerobic mycetomas—22 caused by *Nocardia brasiliensis*, one by *Streptomyces madurae*, and one by *Monosporium apiospermum*. We also found three anaerobic mycetomas. Sporotrichosis (20 cases) and systemic candidiasis (15 cases) in opportunistic conditions (lupus erythematosus, hemolytic anemia, diabetes, leukemia, renal chronic failure, rheumatic cardiopathies) were the next most frequent deep mycoses.

Table 2

Mycetomas: geographical distribution of the most common species

Species	Geographical distribution
N. brasiliensis	Universal; predominates in Central America and Mexico
N. asteroides	Universal
S. madurae	Universal
S. somaliensis	Predominates in Africa
S. pelletieri	Predominates in Africa
S. paraguayensis	Predominates in South America
M. mycetomi	Universal
M. grisea	South America, Africa
L. senegalensis	Africa
P. romeroi	South America, Africa
C. lunata	Africa
M. apiospermum	Universal; predominates in the Americas
Cephalosporium	Universal
N. rosatii	Africa

Table 3

Distribution of mycological cases studied at the National Medical Center, Mexico City
(1,000 confirmed cases of 6,000 consultations studied)

Superficial mycoses		913
Deep mycoses		87
Actinomycotic mycetoma	23	
Sporotrichosis	20	
Coccidioidomycosis	10	
Candidiasis	15	
Mucormycosis	3	
Cryptococcosis	5	
Chromomycosis	2	
Maduromycotic mycetoma	1	
Actinomycosis	8	

The host

Mycetomas are more commonly found in males. Race does not seem to be a factor. The disease is most often present in young rural workers. However, there are some exceptions of affected persons who have lived exclusively in urban areas.

In our recent series of 24 aerobic mycetomas, 20 were observed in males, and the average age at onset was 19 years. A total of 21 patients came from rural areas, while three had always lived in Mexico City. Their professions were varied, but all who came from the rural areas were involved in some way with farming. Of the three urban-dwelling patients, one was an engineer, one was an auto repair specialist, and one was a mason. With one exception, the socioeconomic level was relatively low. It is interesting to note that three cases produced by *Nocardia brasiliensis* came from the endemic sugar cane region described by Atala (*3*). None of the patients showed any general disease that could favor opportunistic infection.

Figure 2. Actinomycotic mycetoma of the spine in an auto repair worker (*N. brasiliensis*).

Antecedents of multiple and frequent trauma were present in all the cases, and in 81 per cent of them the mycotic tumor was located at the site of a previous wound. Inadequate habits of dress—in particular, use of scant footwear or none at all—were very common among the patients. It is believed that these factors together account for the frequency distribution in the localization of mycetomas (*15*), which in descending order is (1) lower limbs, (2) trunk, (3) upper limbs, and (4) neck and face (Figures 1, 2, 3, 4, 5, and 6).

The characteristics of traumatism are variable, but in general it is a question of inadequately treated wounds, as pointed out by Mackinnon

Figure 1. Actinomycotic mycetoma of the foot in a rural worker (*N. brasiliensis*).

Figure 3. Actinomycotic mycetoma of the buttock in a rural worker (*N. brasiliensis*).

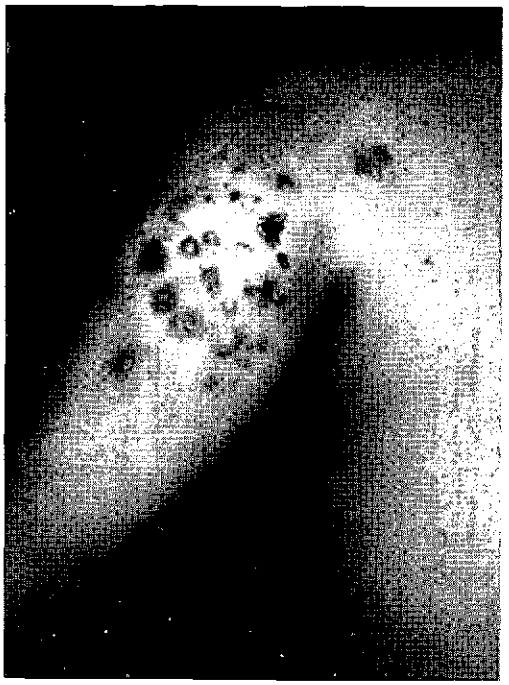

Figure 4. Actinomycotic mycetoma of the posterior aspect of the arm in a farmer (*N. brasiliensis*).

Figure 5. Maduromycotic mycetoma of the posterior aspect of the arm in a rural worker (*M. apiospermum*).

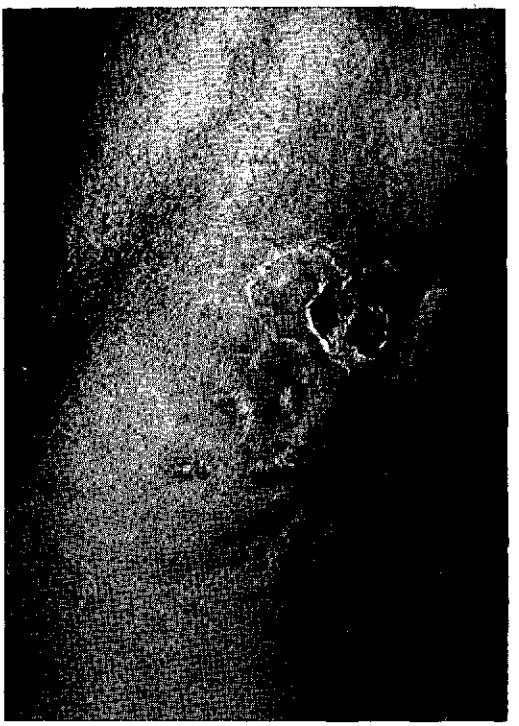

Figure 6. Actinomycotic mycetoma of the lower posterior aspect of the thigh (*N. brasiliensis*) in an engineer.

(*21*). A history of previous surgical procedures of multiple microtraumas in the gums has been seen in cervicofacial actinomycosis, while in some aerobic mycetomas it is possible to identify the exact traumatic agent, such as the thorn of *Machaonia ottonis* reported by Borelli (*8*).

In the case of mycetoma produced by *Nocardia brasiliensis,* Beirana (*6*) has pointed out the importance of previous homologous sensitization of the patient. On the other hand, González Ochoa and Baranda (*14*) described a cutaneous-specific test using polysaccharides from *Nocardia brasiliensis* for the diagnosis of mycetoma. Rodríguez (*36*) obtained positive results ranging from 9 to 47 per cent in a normal population using a protein fraction from *Nocardia brasiliensis.* In a recent study using the antigen obtained according to the technique of González Ochoa and Baranda (*14*), we failed to find any positive reactions in a group of 300 unaffected individuals from the endemic area of Morelos. The

Table 4

Mycetoma: characteristics of granule according to causative agent

Species	Color	Size	Clubs
A. israelii	Yellow	500 μ	+
N. brasiliensis	Yellow	100 μ	+
N. asteroides	Yellow	100 μ	+
S. madurae	White, pink	1 mm	+
S. somaliensis	Yellow	2 mm	—
S. pelletieri	Red	500 μ	—
S. paraguayensis [a]	White	250 μ	+
M. mycetomi	Black	1 mm	—
M. grisea	Black	1 mm	—
P. romeroi	Black	0.5–1 mm	—
M. apiospermum	White	500 μ	—
M. rosatii	White	500 μ	—
Cephalosporium	White	500 μ	—

[a] Experimental granules in a hamster (Strain 285, School of Medicine of the University of São Paulo, Brazil, Professor C. S. Lacaz).

same test was positive, however, in cases of mycetoma produced by *Nocardia brasiliensis*.

The parasite

As mentioned before, there are several species that can produce mycetomas (Table 2). Some aerobic Actinomycetes and true fungi have been isolated from soil (*2, 11, 17, 18, 29*) and from vegetable material, as reported by Baylet *et al.* (*5*) in Senegal. In the host, the parasites group together forming granules whose macro- and microscopic morphology is generally constant

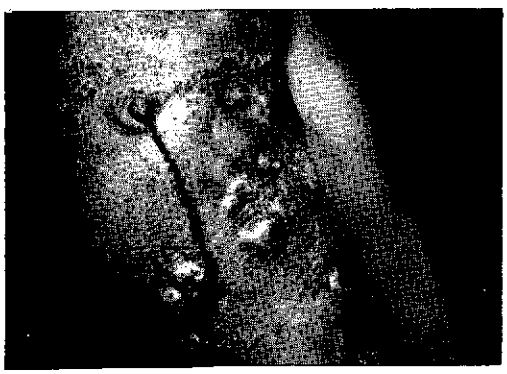

Figure 7. Actinomycotic mycetoma of the knee in a sugar cane worker (*N. brasiliensis*).

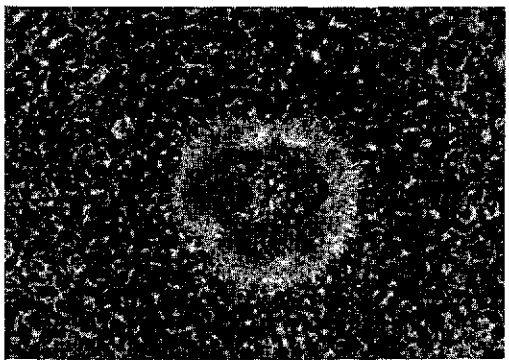

Figure 8. Granule of *N. brasiliensis* in purulent exudate (contrast phase) showing the peripheral clubs.

(*9*) (Table 4; Figures 7, 8, 9, 10, 11, 12, and 13).

As in some cases of superficial (*28*) and deep (*26*) mycoses, mycetomas can be produced by two species simultaneously. An example is the co-occurrence of *Madurella mycetomi* and *M. grisea* reported by Niño (*34*) in a black granule mycetoma of the foot.

Microscopically, it is easy to differentiate actinomycotic from maduromycotic granules. The former are composed of filaments approximately 1μ wide, and the latter of filaments measuring 5μ across, frequently with associated vesicles. The size, presence of clubs and of cement, and staining properties aid in the provisional diagnosis of the species, which can be confirmed only by culture. Several authors have studied experi-

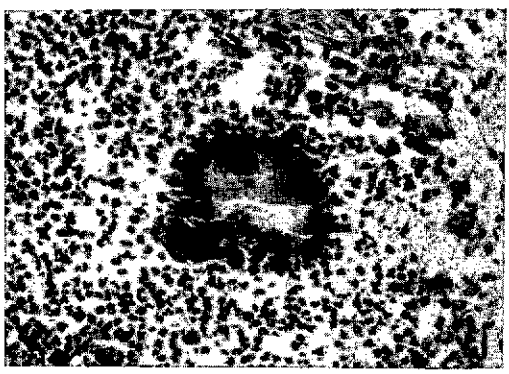

Figure 9. Granule of *N. asteroides* from a mycetoma of the trunk showing its filamentous center surrounded by clubs (Ziehl-Neelsen).

Figure 10. Granule of *Streptomyces* (*Nocardia*) *pelletieri* in tissue (H&E stain).

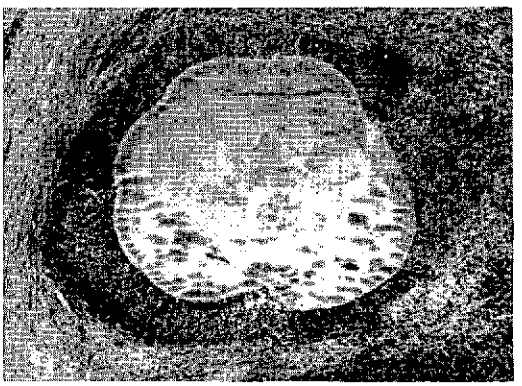

Figure 11. Granule of *Streptomyces somaliensis* in tissue (H&E stain).

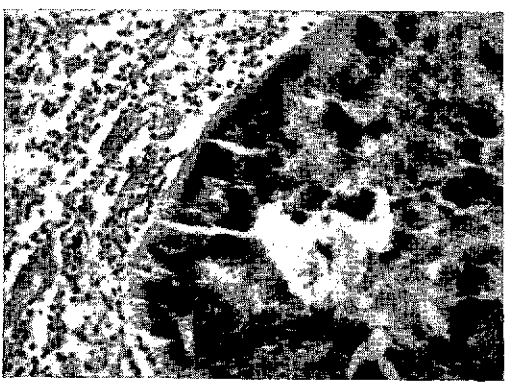

Figure 12. Granule of *Streptomyces* (*Nocardia*) *madurae* showing its typical clubs (H&E stain).

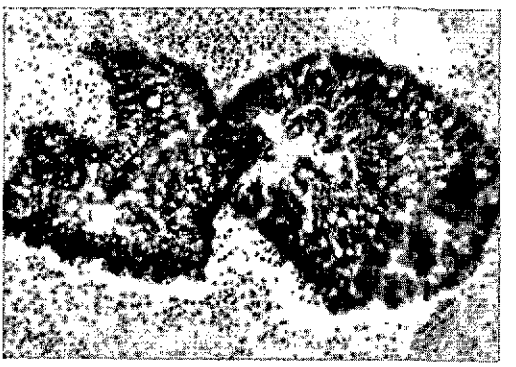

Figure 13. Granule from a mycetoma produced by *M. mycetoma*. Note the coarse filaments and vesicles (H&E stain).

mental pathogenicity in hamsters and mice, obtaining intraperitoneal (*16, 22, 33*) or testicular (*41*) granules.

The mechanism of infection

Most authors agree that the microorganisms are introduced by local trauma (*31*), although some have suggested that a previous pulmonary infection is related to the process (*38*). Emmons (*10*) found that repeated intramuscular inoculation of guinea pigs with Actinomyces increased the number and duration of abscesses. Murray *et al.* (*33*) obtained similar results using multiple peritoneal inoculations of mice with *Madurella mycetomi* and found an increased number of granules when the fungus was inoculated in association with dead cultures of *Mycobacterium tuberculosis*.

In a recent study, Macotela and Mariat (*27*), working with three different kinds of laboratory animals, succeeded in producing peritoneal granules or mycetomas by intraperitoneal or subcutaneous inoculations of *Nocardia brasiliensis* or *N. asteroides* (Table 5). Some of the animals were previously sensitized with dead cultures of homologous Nocardia. The proportion developing intraperitoneal granules or mycetomas varied (Tables 6 and 7).

The experimental subcutaneous lesions showed two kinds of histopathological patterns: abscesses and granulomas. Both of these contained

Table 5

Experiment for the production of mycetomas in mice, hamsters, and guinea pigs [a]

Group	Sensitization	Inoculation	Route
A	—	—	—
B	—	Vehicle	IP
C	Vehicle	*N. asteroides*	SC
D	Vehicle	*N. brasiliensis*	SC
E	*N. asteroides*	*N. asteroides*	SC
F	*N. brasiliensis*	*N. brasiliensis*	SC
G	Vehicle	*N. asteroides*	IP
H	Vehicle	*N. brasiliensis*	IP
I	*N. asteroides*	*N. asteroides*	IP
J	*N. brasiliensis*	*N. brasiliensis*	IP

[a] Each group in the first column includes 6 mice (3 males, 3 females), 4 hamsters (2 males, 2 females), and 2 guinea pigs (1 male, 1 female).

the typical granules (Figures 14, 15, and 16). The growth of the lesions did not seem to be influenced by the sex of the animal or by previous sensitization with dead homologous organisms. This finding was later confirmed by González Ochoa (*13*), who produced mycetomas on the footpads of mice without previous sensitization by inoculation with *Nocardia brasiliensis*.

It is interesting to note that when performing

Figure 14. Granule of *M. mycetomi* showing the coarse filaments (4 μ width) and interfilamentous cement (H&E stain).

a series of biopsies on these animals it is possible to follow the development of the typical granules.

The interior of the actinomycotic granules does not contain any other kind of bacteria, and they have two clearly defined areas that are easily distinguishable with an electron microscope (*25*) (Figures 17 and 18). Nevertheless, in cultures from human and experimental lesions it is possible to see various forms of bacterial flora in which *Staphylococcus aureus* is always present (*13*). We were able to isolate *Staphylococcus aureus* in 20 of the 23 recent human cases of

Table 6

Occurrence of intraperitoneal granules

Group	Sensitization	Inoculation	Mice	Hamsters	Guinea pig
G	Vehicle	*N. asteroides*	2/6	2/4	1/2
H	Vehicle	*N. brasiliensis*	2/6	1/4	0/2
I	*N. asteroides*	*N. asteroides*	4/6	3/4	0/2
J	*N. brasiliensis*	*N. brasiliensis*	4/6	4/4	2/2

Table 7

Occurrence of experimental mycetomas

Group	Sensitization	Inoculation	Mice	Hamsters	Guinea Pigs
C	Vehicle	*N. asteroides*	2/6	2/4	1/2
D	Vehicle	*N. brasiliensis*	4/6	4/4	1/2
E	*N. asteroides*	*N. asteroides*	3/6	2/4	0/2
F	*N. brasiliensis*	*N. brasiliensis*	4/6	4/4	0/2

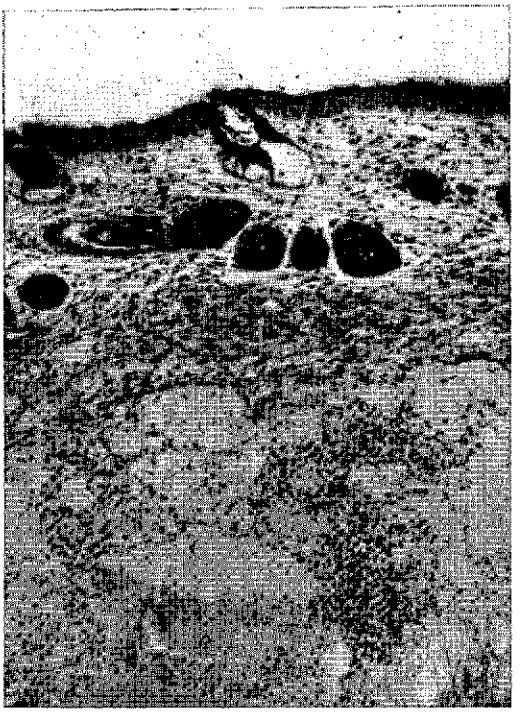

Figure 15. Experimental mycetoma by ***N. brasiliensis*** in a hamster. Abscessed histopathological pattern (hemalum stain).

mycetoma caused by *Nocardia brasiliensis*, as well as other common saprophytic skin bacteria.

In conclusion, the following points regarding the epidemiology and ecology of mycetomas are considered important:

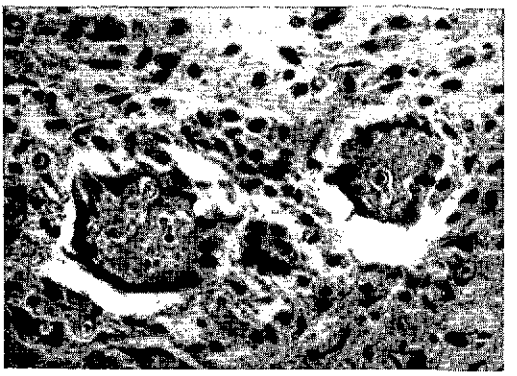

Figure 17. Experimental mycetoma by ***N. asteroides*** in a mouse. Granulomatous histopathological pattern showing foreign body cells (Giemsa's stain).

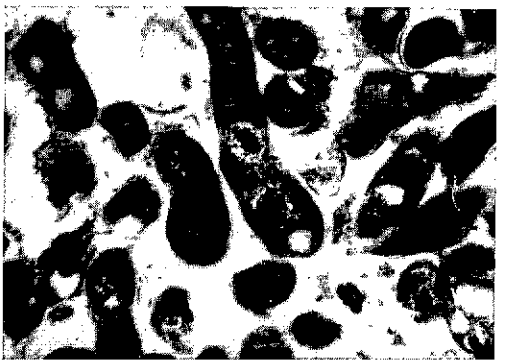

Figure 18. Ultrastructure of the central area of a granule from a human case of mycetoma produced by ***N. brasiliensis*** (X 38,000).

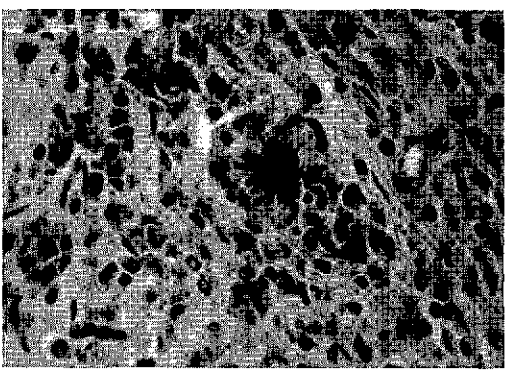

Figure 16. Experimental mycetoma by ***N. brasiliensis*** in a hamster. Granulomatous histopathological pattern (hemalum stain).

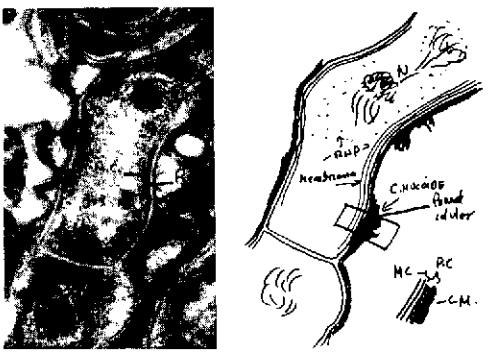

Figure 19. Ultrastructure of the filament of ***N. brasiliensis***. N = nucleus; PC = cellular wall (X 80,000). Courtesy of Dr. González-Angulo.

• Their distribution is universal, with the greatest frequencies occurring in tropical and subtropical developing regions.

• The pathogenic agents normally live in the soil.

• Although these organisms grow in the host and produce varied histopathological reactions unquestionably related to immunological and bacteriological conditions that have not yet been completely studied, it is currently believed that a previous sensitization is probably not necessary.

• Conditions related to the precipitating trauma are of great importance (associated bacterial flora, temperature of the anatomical region, etc.).

• The further evolution of mycetomas depends on the species of the parasite, its sensitivity to chemotherapeutic agents, and the availability of medical care.

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EPIDEMIOLOGY OF COCCIDIOIDOMYCOSIS[1]

Demosthenes Pappagianis

In the Americas, the coccidioidomycosis zone extends from approximately 39°N 120°W in northern California to 40°S 65°W in Argentina. The endemic zones in the United States have been well delineated, thanks to the efforts of Charles E. Smith and collaborators, Palmer and Edwards, Maddy, Emmons, and many others. In addition, there is increasing recognition of cases outside the known endemic areas (*16*). González Ochoa, Glusker, and others have contributed to knowledge of the endemic zones in Mexico, and through the efforts of Negroni in Argentina, Campins in Venezuela, and Mayorga, Castro, and Trejos in Central America we have some idea of the occurrence of clinical coccidioidomycosis in these other areas of the Hemisphere. It is to be hoped that the discussions in this Symposium will elicit for all of us students of the disease a clearer picture of its precise clinical magnitude in the Latin American countries.

It is of particular interest, as pointed out by Negroni (*36*), that following the discovery of the initial case of coccidioidomycosis by Posadas in Argentina another 35 years elapsed before a second case was observed in that country. Indeed, by 1965, Negroni (*36*) had recorded a total of only 27 diagnosed cases of the disease. Campins (*3*) accumulated a total of 35 cases from Venezuela. Mayorga (*35*) reported on six cases in man from Guatemala and Honduras, while Guatemala and Nicaragua yielded a total of eight subhuman animal cases. By 1968, Alberto Guzmán (*15*) had performed coccidioidin skin tests on 19,846 persons in Honduras, with a yield of only 0.2 per cent positive. Robledo (*41*) has recently related the diagnosis of two cases of coccidioidomycosis in Colombia, and his subsequent skin test surveys, showing 3.3 and 6.3 per cent coccidioidin reactors in the northern and north central regions, respectively, suggests that that country may well be a source of substantial numbers of infections, too. An interesting contrast, however, was the report by Peña (*37*), in which clinicopathologic studies on 162 systemic mycotic infections acquired in Colombia included no cases of coccidioidomycosis.

For those of us who, through serologic studies as well as general communications, have been used to recognizing several hundred clinical cases confirmed serologically and/or culturally each year in the United States, our curiosity is whetted to learn precisely the extent of overt clinical disease in the Latin American countries. Most strongly represented in the reports of Negroni and Campins are cases of disseminated coccidioidal disease. Perhaps the patient population from which those cases are drawn is less inclined to seek medical attention, so that the primary infections without dissemination are more likely to go unrecognized. Or perhaps routine chest x-rays are not as common among that population, and thus patients with coccidiodal pulmonary residual cavities or coccid-

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ioidomas, which are recognized with some frequency in the United States, are not brought forth. We know that this form of the disease has in fact been noted by Negroni and by Campins in their series.

Skin-test sensitivity is another useful measurement of the presence of coccidioidomycosis. In this connection, the data collected by Robledo *et al.* in Colombia, the earlier studies of Hómez in Paraguay, and the efforts of Guzmán in Honduras constitute first steps toward acquiring what is hoped will be definitive information on the type and location of the disease. We view with interest the results of skin tests accumulated by Manych (*34*) in Czechoslovakia and by Imperato (*22*) in Mali. The latter found some 6 to 7 per cent reactors to coccidioidin among the 11- to 20-year-old group. This, however, is somewhat of an enigma in view of the lack of clinically proven cases of coccidioidomycosis from these areas. While cases have been reported from various places in Europe, including one very recently from Poland (*25*), they are almost without a doubt infections acquired either in the Western Hemisphere or from products that reached Europe from the Western Hemisphere endemic zones. A native of Samoa was reported to have coccidioidal spherules with endospores in his sputum and presumably had acquired a coccidioidal infection from a consignment of used clothing sent from California (*7*). In the present author's view, the human *patient,* rather than the positive skin test, remains the sentinel of choice for detection of significant distribution of *C. immitis.*

In the meantime, we must point to the existence of excellent animal sentinels, as demonstrated by Emmons (*11*) in his important field studies in Arizona in the early 1940's. Rodents infected under natural conditions proved then and could prove in other circumstances to be important substitutes for the human detector of *C. immitis.* Thus, collaborative work with a mammologist to trap selected rodents could contribute definitive information on the distribution of *C. immitis.* Culturing of lungs, liver, spleen, and omentum on the available selective media could be of invaluable assistance in demonstrating the presence of *C. immitis* from a given geographic location. As will be mentioned below, cultivation of the organism from the soil could also be an important endeavor. In the absence of a known point source of exposure of humans, it may be that systematic, persevering studies on field animals will be the only means of ascertaining the presence of *C. immitis* in a given location. Such work, however, requires a great deal of patience. The marked natural variation among some strains of *C. immitis,* as observed by Friedman *et al.* (*12*) and Huppert *et al.* (*21*), makes it difficult to select organisms from infected animals for further confirming studies.

Maddy and Reed have made use of domestic animals as natural sentinels of coccidioidal endemicity in Arizona, and Converse and Reed (*4*) demonstrated the utility of monkeys and dogs placed out-of-doors as sampling devices for the presence of *C. immitis,* also in Arizona.

Several epidemic-like outbreaks of coccidioidal disease have made it possible to pinpoint specific endemic areas or "pockets" of *C. immitis* distribution. Following the development of several cases of coccidioidal granuloma among grape pickers of Philippine origin, Stewart and Meyer (*46*) were able to isolate *C. immitis* from the soil under the bunk house where the workers had lived on a ranch at Delano, California. Similarly, the epidemic among zoology students reported by Davis *et al.* (*5*) was followed by isolation of *C. immitis* from the precise animal burrow that had been dug by the students. In 1954, a class of anthropology students excavated an Indian camp site in the desert area near Inyokern, California. Four of these students developed clinically apparent primary pulmonary coccidioidomycosis. Recognition of the site of exposure led Plunkett and Swatek (*38*) to sample soil from the excavation site, and they were able to recover *C. immitis* over several successive months. Walch and co-workers (*49*), pursuing the clue of a specific area of exposure in three cases of coccidioidomycosis in San Diego

County, isolated the fungus from the soil at the site of exposure. In 1963, Winn *et al.* (*50*) reported on a localized epidemic of coccidioidal infection in the town of Woodville in an area already recognized as endemic in the San Joaquin Valley of California. This, incidentally, was followed by isolation of the organism from soil in areas where children were exposed to the fungus and led to the development by Levine and Winn (*30*) of intranasal inoculation of mice as a sensitive method for obtaining the organism from soil specimens.

An outbreak of coccidioidal infection involving some 26 individuals in Canoga Park in the western part of the San Fernando Valley in 1965 was followed by isolation of the organism from a trench in which 22 of the youngsters had been playing. In 1967, Roberts and Lisciandro (*40*) reported an epidemic of coccidioidomycosis involving 10 children at El Paso, Texas. Soil obtained from an area where they had been playing yielded *C. immitis*. Our own recent experience involved the development of coccidioidal infections in archaeology students digging in Northern California (*31*). These students were exposed during excavation of an old Indian burial site, and we have subsequently demonstrated the presence of *C. immitis* at this site utilizing the previously mentioned method of Levine and Winn (*30*). Another outbreak was reported from the area of Beeville, Texas, and was followed by isolation of *C. immitis* from a rodent burrow where the subjects had been exposed while digging in soil (*47*). Additional outbreaks have been reported elsewhere as well (*13, 18, 23, 27*), although soil isolations were not attempted in all of them. The significance of soil isolations is that they provide identification of the precise site of exposure.

Occurrences such as the outbreak involving several house physicians and nurses exposed to a contaminated plaster of Paris cast (*8*) and the many laboratory-acquired infections (*24*) have not really contributed to information on the epidemiology of coccidioidomycosis in nature, but they have certainly helped to reemphasize the high infectivity of *C. immitis*.

While specific epidemics or outbreaks with subsequent isolation of *C. immitis* from the soil permit a precise characterization of infectious areas, what of the large mass of clinically recognized as well as asymptomatic coccidioidal infections? We still remain ignorant of the infectious dose required for man, although we know that inhalation of 10 arthrospores can lead to severe infections in monkeys and rodents. In studies of the outbreaks cited above it has been concluded that relatively large doses of the fungus must be inhaled during a very dusty digging or playing activity. In the few investigations involving isolation of *C. immitis* from the air, the success has been meager. A study by Hoggan *et al.* (*17*) yielded one isolate from the air in the Camp Roberts area of California. Ajello *et al.* (*1*) isolated *C. immitis* from two air samples in Phoenix, Arizona, and gave some credit for their success to a windstorm and very dusty conditions that had developed. In the study by Hoggan *et al.* (*17*), it is of interest that the single isolate of *C. immitis* was recovered during the sampling of approximately 170,000 liters of air. On the basis of a 500 ml tidal volume, a normal adult would inhale this quantity of air over a period of approximately 15 days. Thus, the single *C. immitis* isolate represents a sampling of *one* adult human breathing for two weeks and would appear to indicate a scanty concentration of these spores in the air.

The ecology of *C. immitis* has been very nicely reviewed by Ajello (*1*). Maddy's (*32*) concept of conformity between the Lower Sonoran Life Zone and *C. immitis* endemicity has been the focal point of many ecologic considerations. While there have been some deviations from this (*31*), the guidelines of an arid or semiarid climate with a long hot and dry season, a moderate-to-low rainfall, winters generally without severe or prolonged freezing temperatures, and alkaline soils appear to describe an appropriate residence for *C. immitis* in the soil. The tropical or semitropical areas of Mexico and

Colombia should be included as exceptions (*14, 41*).

C. immitis has been recovered from the soil at depths of 10 to 15 cm, even during the hot dry season when the surface layers of soil reached 60.5°C and were free of viable *C. immitis*. It has been shown that rodent burrow soil may yield a much greater proportion of positive samples for *C. immitis* than soils obtained at the surface or in the depths at random (*9, 10*). Egeberg and his co-workers have presented an interesting proposal and supporting evidence to suggest that *C. immitis* may survive in the soil of the endemic areas owing to the selective effect of high concentrations of calcium, magnesium, and sodium chlorides and sulfates. These salts, plus an elevated temperature (40°C), suffice to inhibit two biological antagonists of *C. immitis*—namely, *Penicillium janthinellum* and *Bacillus subtilis*. Recently, Sorensen (*45*) has shown that the addition of fertile, unsterilized garden soil to a culture inoculated with *C. immitis* leads to the rapid spread of bacteria and fungi competitive with *C. immitis* and inhibitory to its growth. Soil obtained from a natural site from which *C. immitis* was recovered provided no such growth of competitive bacteria and fungi, and *C. immitis* appeared to grow uninhibited in the medium adjacent to this sample of soil. The direct observation by Maddy (*33*) of the growth of *C. immitis* in the soil of the Arizona desert and by Kaplan and Ajello (*1*) of arthrospores in Arizona soils adds support to the recognition of survival and presumption of growth of *C. immitis* in the soil.

While the endemic areas are known to generally have moderate-to-low rainfall, the presence or absence of a winter's rain and a prolonged dry season have marked effects on the incidence of the disease. The winter of 1968-1969 was one of relatively heavy rainfall in California, and the following year, 1969, was one of a prolonged dry season before the advent of rains. Possibly as a result of this, some 78 new cases of coccidioidomycosis were reported in California during the first six weeks of 1970. Interestingly, the 428 cases of coccidioidomycosis reported in California during 1968 represented a 40 per cent rise over the year before and a sizable increase over the usual number of cases recorded annually in that state. The smaller state of Arizona reported 555 cases in 1968 and generally has a higher annual number than California.

The age of occurrence of coccidioidomycosis deserves comment. We are particularly interested in the lack of precise information on coccidioidomycosis in children. Larwood (*28*) has reported on a coccidioidal infection acquired *in utero* that led to death 25 days after birth. Thus, children can encounter *C. immitis* even at this early age. In a recent report on some 37 pediatric patients, Richardson *et al.* (*39*) concluded that dissemination was apparently a rare occurrence in children. In five years of pediatric clinic experience they had witnessed only one case of dissemination. However, on the basis of the 37 patients that they had studied over an 18-month period, the total number of patients observed during the five-year span would have been estimated at 121. One dissemination in 121 patients is not greatly different from the figure of one dissemination per 100 clinically apparent infections in adult Caucasian males described earlier by Smith *et al.* (*44*).

We have now accumulated records on 158 cases in children under the age of 12 who have had clinically apparent and serologically proved coccidioidal infections (Table 1). Of the 158 cases, extrapulmonary dissemination occurred in 33, or 21 per cent of the total. Thirteen of the patients had osteomyelitis; 13, meningitis; six, involvement of soft tissue with cutaneous or lymph node abscesses; and one, peritonitis. Four of the 158 died. The youngest infected in this group was five weeks old, and the youngest who underwent dissemination was three months. There were eight Negro, 13 Mexican, three American Indian, and nine Caucasian patients with disseminated disease. Unfortunately, we have no baseline figure of *total* infections with which to compare these figures.

Of the 15 coccidioidomycosis patients de-

Table 1

Serologically proven cases of coccidioidomycosis in children 12 years of age and under

Total	158 cases
Extrapulmonary dissemination	33 (21% of total)
Osteomyelitis	13
Meningitis	13
Soft tissue–cutaneous abscess, lymph node abscess	6
Peritonitis	1
Deaths	4
Age of youngest infected	5 weeks
Age of youngest with dissemination	3 months
Disseminated group, racial derivation	
8 Negro	
13 Mexican	
3 American Indian	
9 Caucasian	

scribed by Verduzco *et al.* (*48*) in the State of Coahuila, Mexico, ten were eight years of age or younger. Campins (*3*) indicated that six of the 35 Venezuelan cases were under 12 years of age. The work of Dickson and Gifford (*6*) indicated that 3 per cent of the "Valley fever" cases seen by physicians in the San Joaquin Valley were in the preschool age group (presumably under six years of age). Nevertheless, a clearer picture of coccidioidal infection and disease among children is definitely needed. This may be particularly so in the Latin American countries, where a place of residence may be more stable than that of the usual mobile family in the United States. In a stable population, adults who survive should by and large have acquired their coccidioidal infection and become resistant to subsequent infection, while children should constitute the bulk of the susceptible group at risk.

Somewhat related to the occurrence of coccidioidal infection in youngsters is the recognition by several workers that coccidioidin sensitivity in an endemic area may diminish. Larwood (*29*) made such an observation in the schoolchildren of Kern County. Elementary schoolchildren in 1939 had shown a coccidioidin reactivity of 55 per cent, which dropped to 15 per cent in 1969 and to 8 per cent in 1964. High school students reacting at a rate of 68 per cent in 1939 and 40 per cent in 1959 reacted at only 24 per cent in 1964. Klotz and Biddle (*26*) noted that of 237 students tested over a five-year period, seven, or approximately 2 per cent of those who originally reacted to coccidioidin, became negative. Sievers (*43*) shows a declining reactivity to coccidioidin among Indians in the southwestern United States. In a group of approximately 1,500, he noted the peak reactivity to be approximately 55 per cent in persons between 15 and 29 years of age. This fell to about 12 per cent in the age group 60 and over. One shortcoming of these studies was the failure to use 1:10 coccidioidin to determine also whether reactivity had merely weakened or whether it had disappeared. This could have important implications with respect to lasting resistance to exogenous reinfection by *C. immitis*.

There are other epidemiologic problems in relation to coccidioidomycosis as well. For example, Salkin (*42*) has reported on the occasional case of what appears to be endogenous reinfection or exacerbation of existing disease in cases without dissemination. Such instances are infrequent, however. In addition, there is a tendency for many workers to state that "dark-skinned individuals" are more susceptible to dissemination. The greater tendency toward dissemination among Negroes and persons of Philippine descent is quite clear. However, the evidence is less clear with respect to individuals of Mexican or other Latin American background. Mexicans are well represented in the western and southwestern parts of the United States and contribute sizably to the cases of coccidioidomycosis. However, in the studies of Huntington *et al.* (*19, 20*) the incidence of disseminated and fatal coccidioidomycosis among Mexicans was not greatly different from that among Caucasians. Sievers (*43*) provided much needed information on the extent and characteristics of coccidioidomycosis among American Indians of the Southwest. Of a group of 2,112 patients admitted to the hospital, 12 cases of disseminated coccidioidomycosis were diagnosed.

Unfortunately, the total number of patients with diagnosed, clinically apparent coccidioidomycosis was not ascertained, and therefore it is not possible to compare this figure of disseminated cases with that recognized for other population groups. Campins (*3*) did give some racial distributions of the patients in his group of 35 cases. However, we need clarification from North, Central, and South America as to what position the American Indian holds in the stratification of susceptibility to coccidioidal dissemination. It is evident from our own studies (*31*), as well as those of others who have recovered *C. immitis* from ancient Indian burial grounds or campsites, that perhaps the original American was a victim of this disease and may even have provided a nidus from which infection is acquired by the not-so-original Americans today.

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ECOLOGY AND EPIDEMIOLOGY OF CRYPTOCOCCOSIS[1]

H. G. Muchmore, F. G. Felton, S. B. Salvin, and E. R. Rhoades

Introduction

An understanding of the occurrence and distribution of human mycoses depends first on the correct diagnosis of clinical cases and second on the accurate recognition of normal persons who have been infected with the fungus but who exhibit no evidence of illness. In the case of coccidioidomycosis and histoplasmosis, epidemiologic understanding came after the development and application of serologic tests and sensitins capable of demonstrating delayed dermal hypersensitivity. The results of such tests in man, coupled with similar studies in animals, have helped greatly to determine the geographical distribution of the etiologic agents in the environment and have also been instrumental in clarifying the frequency and severity of the two diseases. Thanks to these methods, they have come to be regarded as frequent infections producing little or no clinical illness, with only an infrequent fatal outcome, rather than the rare and usually fatal infections they were formerly thought to be.

It seems probable that human infection with cryptococcosis follows a pattern similar to that noted above for coccidioidomycosis and histoplasmosis. The present paper will examine the evidence now existing as it may bear on this hypothesis and speculate on the nature of the information that will be forthcoming when reliable serologic and dermal hypersensitivity tests become available and are utilized.

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World distribution

Cryptococcosis in man and animals has been reported from all regions of the world except the Arctic and Antarctic. Published scientific articles are a major source of epidemiologic information on this infection. However, interpretation of these reports must be made cautiously, since they may reflect factors other than case distribution or prevalence, such as economic conditions or the presence of mycologically oriented investigators.

Table 1 presents a compilation of the human cryptococcosis cases noted in the *Review of Medical and Veterinary Mycology*[2] for those nations reporting the largest number of patients and for those in Latin America with a total of more than five patients. The period covered is approximately 1942 through 1968. A very rough estimate, taking into account previous tabulations (*8*), indicates that about 1,000 reports of human cryptococcosis have appeared in the medical literature since the first case was described in 1896.

This low number of published cases contrasts with the number of deaths due to this fungal infection known to occur in the United States of America alone. Ajello (*2*) states that an annual average of 66 deaths due to cryptococcosis have been reported since 1952 and that a total of 788

[2] Published by the Commonwealth Agricultural Bureaux, Kew, Surrey, England.

deaths were recorded during the period 1952-1963. If this death rate is assumed to be constant, then it may be postulated that some few thousands or more have died of this disease in the United States alone. Further consideration of the data compiled for Table 1 reveals that cases are being reported with ever-increasing frequency, and from more and more places throughout the world. These changes undoubtedly reflect economic and consequent educational changes occurring rapidly in many countries. Within the United States there are reports identifying patients from each of the 50 states.

On the basis of data abstracted from the same periodical noted above, reports of the disease in animals are not nearly as frequent as for human cryptococcosis, but they are likewise increasing steadily in number. The countries from which these reports originate are the same as for human disease. Economic factors seem to play a role, since infections in cattle have been seen most frequently, followed by those in pets (cats and dogs) and zoo animals. Each of these groups are of economic importance and hence are likely to come to the attention of veterinary practitioners. Notable by their complete absence are reports of naturally occurring infections in lagomorphs or birds. This phenomenon apparently supports the remarkable resistance of these groups of animals to experimental infections in the laboratory. Lastly, it must be noted, somewhat sadly, that cryptococcosis seems to be an especial nemesis for the Australian koala. No studies of any possible relationship between the growth of *C. neoformans* and eucalyptus trees or their sap, or birds that frequent these trees, have appeared.

The occurrence of *C. neoformans* in soil, especially soil contaminated with pigeon droppings, has been demonstrated by several investigators (*1, 6, 7*), beginning with Emmons in 1951 (*3*). Staib (*12*) suggested that the predilection of *C. neoformans* for pigeon (and other bird) droppings might be explained by the fact that this yeast is able to utilize creatinine as a nitrogen source, while other members of the genus Cryptococcus are not. The literature indicates that the fungus occurs widely in the United States, and presumably in other areas of the world; thus there should be frequent opportunity for man to encounter it.

Table 1

Reported cases of human cryptococcosis, 1942–1968

United States	400+	Australia	55
Venezuela	50	England	33
Brazil	30	South Africa	20
Argentina	25	France	20
Canada	17	Czechoslovakia	19
Mexico	6	Kenya	18
Colombia	5	Germany	15
		India	15

Studies in Oklahoma

The occurrence of cryptococcal meningitis in three men within one year's time in a small community in Oklahoma stimulated the authors to make a special study. We demonstrated *C. neoformans* in the work environment of each of these three patients, but not at the houses in which they lived (*10*).

Between 1949 and 1969 a total of 45 cases of human cryptococcosis have been identified in Oklahoma. Some data related to these patients are given in Table 2. Most of the cases involved the central nervous system (CNS), while seven patients had lung involvement only. The number of total cases is too small to permit any con-

Table 2

Cryptococcosis in Oklahoma: distribution by race and sex 1944–1969

	Total	Lung	CNS	DISS	Other
Male	33	6	22	4	1[a]
Female	12	1	8	1	2[b]
White	39	7	28	3	1
Black	4	—	1	1	2
Red (Indian)	2	—	2	—	—
Totals	45	7	31	4	3

[a] Perinephric abscess
[b] Bone = 1; skin = 1

clusions about racial predilection, but even so, the proportions approximate the over-all distribution of races in Oklahoma. Males constitute 73 per cent of the group and females only 27 per cent. The annual breakdown of these cases is presented in Table 3.

Soil sampling has been carried out in each of the 77 counties of Oklahoma. *C. neoformans* has been recovered from soil samples from only 10 of these counties. Each of these 10 counties has also had one or more of the cryptococcosis patients noted above. Examination of the patient's work environment, especially areas open to bird contamination, provided the most rewarding samples.

Studies by Staib (*14*) in Germany and by Ishaq (*5*) and Farhi (*4*) in Oklahoma describe several of the conditions conducive to survival of *C. neoformans* in the environment. The conditions are mild alkalinity of soil, a cool and shaded situation, and moderate humidity. Addition of pigeon manure to the soil provides alkalinity and supplies nutriment, especially creatinine, favorable to the multiplication and to the more prolonged survival of the yeast. The yeast is better able to withstand heat if it is in a dry situation. Under these conditions its cells become progressively smaller, with very little capsule in evidence, often reaching a diameter of less than 3 microns.

Development of a suitable sensitin for the detection of delayed hypersensitivity was reported in 1961 (*11*). The present authors used this material to test a small group of subjects and published their findings in 1968 (*9*). The results from a group of 477 Oklahoma residents are shown in Table 4. A total of 89 test subjects, or 19 per cent, showed induration of 5 mm or more with cryptococcin, while 50 per cent of the subjects reacted to histoplasmin. There were some who reacted to only one or the other of the two sensitins.

Table 3

Cryptococcosis in Oklahoma: annual distribution 1944–1969

Year	No. of patients	Year	No. of patients
1944	1	1957	0
1945	0	1958	2
1946	0	1959	2
1947	1	1960	3
1948	0	1961	3
1949	0	1962	4
1950	0	1963	2
1951	1	1964	1
1952	1	1965	1
1953	0	1966	1
1954	1	1967	9
1955	2	1968	5
1956	1	1969	3

Comment

The number of published reports of cryptococcosis throughout the world is small. Deaths in the United States since 1940 due to this disease exceed the total figure for all cases published throughout the world. The paucity of informa-

Table 4

Cryptococcin and histoplasmin reactors in four groups of Oklahoma residents

Group	Cryptococcin			Histoplasmin		
	No. tested	Induration ≥ 5mm	% positive	No. tested	Induration ≥ 5mm	% positive
Kingfisher County	82	26	32	82	45	55
Medical students	90	11	12	97	35	36
Cleveland County	155	26	17	111	65	59
Seminole County	150	26	17	150	77	51
Totals	477	89	19	440	222	50

tion makes it difficult to conclude whether the relative frequency of cases as reported throughout the world is in fact a fair reflection of the incidence of this disease in each country. It is probable that other factors such as the location and activities of physicians and mycologists interested in this particular fungus play an important role in the distribution of these reports.

The wide distribution of *C. neoformans* throughout the temperate and tropic zones is well documented, but multiple reports have appeared from only a few areas, such as Europe and the United States of America. The frequent association of this yeast with pigeon roosts and other areas contaminated by pigeon droppings appears to be an unquestionable factor in the spread of this infection to man and animals. The conditions for the survival of the yeast in nature have been elucidated in part, and perhaps this knowledge could be used in devising control measures.

The large size of the *C. neoformans* cell with its capsule has made it difficult to understand how airborne infection can occur. However, the studies of Farhi (*4*) showing the progressive diminution of size of the cell and disappearance of the capsule over prolonged periods in soil offer an attractive explanation to the problem, since cells of the size she describes are more plausible as airborne infectious particles than the large cells seen in vigorously growing cultures.

The frequency of human infection is unknown at the present time, and this knowledge will come only with the development and application of skin-testing and serologic techniques that will permit the detection of subclinical infections among healthy individuals. Experience with coccidioidomycosis and histoplasmosis suggests that many such people probably exist. Efforts should be made to detect pulmonary cases, and these should be facilitated by use of the selective differential medium reported by Staib (*13*).

The skin-testing results reported here must be regarded as preliminary, and we do not believe that the data are sufficient yet to permit conclusions as to the efficacy of the cryptococcin to detect hypersensitive individuals, and hence subclinical infections. Testing of guinea pigs infected with *C. neoformans, Candida albicans,* and *Histoplasma capsulatum* does not reveal any evidence of cross-reactions induced by this cryptococcin. We do not know whether these observations regarding the absence of cross-reactions are valid for human subjects.

If we can assume for a moment that the observed reactions do in fact reflect subclinical infections with *C. neoformans,* then we may speculate as to the numbers of such inapparent infections among healthy Oklahoma residents. On the basis of the observed reactor rate of 19 per cent, we can hypothesize 437,000 such infections among the 2,300,000 residents of Oklahoma. The contrast between this large number and the 45 cryptococcosis patients actually diagnosed in Oklahoma recalls the iceberg that Dr. Ajello described earlier in this Symposium. Using the numbers hypothesized above for Oklahoma, we have constructed the iceberg diagrammed in Figure 1. The contrast of the tiny spike of known patients projecting through the surface of clinical recognition as against the enormous subsurface mass of inapparent infections (as-

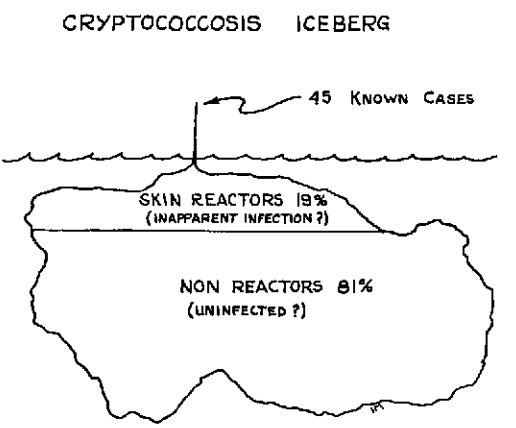

Figure 1. This hypothetical iceberg for cryptococcosis in Oklahoma is reconstructed from the number of known clinical cases, the number of skin reactors to cryptococcin, and the population of Oklahoma (1960 census of 2.5 million). Although this construction parallels that of histoplasmosis and coccidioidomycosis, it is emphasized this figure is extrapolated from a small amount of data.

sumed from positive skin tests) brings home forcefully that much work remains to be done before the accuracy of such extrapolations can be ascertained.

Summary

Cryptococcosis occurs throughout the world in the temperate and tropic zones. It is exclusively confined to mammals, and a wide variety of species are attacked, a notable exception being the Lagomorpha.

The causative yeast, *Cryptococcus neoformans,* is also widely distributed and is particularly frequent in pigeon habitats and in soil contaminated by their droppings. The yeast survives best in mildly alkaline soil in a shaded, cool location, neither excessively wet or excessively dry. Dryness favors survival in hot locations, and progressive dryness leads to decreasing size of the yeast cell.

The extent of inapparent human infection is not known. Preliminary data obtained by cryptoccocin skin testing of a small number of healthy subjects living in Oklahoma yielded a reactor rate of 19 per cent, which suggests that inapparent infections may be common, at least in that area. Problems of cross-reactions due to other infections remain to be elucidated.

Comparison with other fungal diseases indicates it should be possible to find patients with mild chronic pulmonary cryptococcosis and also acute pulmonary cryptococcosis, and efforts should be made to discover these cases.

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ECOLOGY AND EPIDEMIOLOGY OF HISTOPLASMOSIS

Howard W. Larsh

The ecology of *Histoplasma capsulatum* has been the subject of many investigations over the past three decades. From the studies, at least a partial understanding of the relationship between the fungus and its environment has evolved.

Histoplasmosis was discovered in 1906 by Darling (*4*), who considered the etiologic agent to be a member of the genus Leishmania. In 1912, da Rocha Lima (*5*) suggested that the causal agent was a yeast and not a Leishmania. Historically, from these observations and from the criteria available, the agent had to be considered an obligatory parasite. Up to 1934, all the cases on record had been diagnosed at necropsy and the fungus had not yet been isolated on artificial cultural medium. That year, however, De Monbruen (*6*), studying clinical material from Dodd and Tompkins' case (*7*), established that the fungus could grow as a saprophyte and that it was a dimorphic organism. This was the first case of histoplasmosis diagnosed ante mortem.

During the period from 1934 until 1948, when Emmons (*9*) successfully isolated *H. capsulatum* from soil, numerous studies were undertaken to ascertain the natural reservoir of the fungus. In addition, experimental procedures simulating natural environmental conditions were used in an effort to determine factors that may influence the growth of *H. capsulatum* in nature.

Subsequent laboratory studies revealed that the growth and development of *H. capsulatum* on natural products or on artificial cultural medium involves two physical factors: temperature and humidity (*24*). These two factors were also shown to be essential for maintenance of the species in nature. Under laboratory conditions, the fungus was destroyed below the minimal or above the maximal requirements for temperature and humidity.

Later it was learned that these two physical factors were not the sole requirements for establishment of *H. capsulatum* in the environment (*16*). It has proved difficult, if not impossible, to establish this fungus in natural soils under optimal environmental conditions, even when millions of viable units are inoculated. Thus, there must be additional, undetermined factors that permit successful competition between *H. capsulatum* and the normal flora and fauna of natural soils. These obstacles do not exist in sterilized natural soils, where the fungus will grow and produce an abundance of mycelia with luxuriant sporulation (*21*). However, experimental studies contribute very little toward determining the specific factors that influence the ecology of *H. capsulatum* in nature. Particularly, it does not solve the problem of understanding the colonization of the fungus in its natural environment under competitive circumstances.

All the available evidence suggests the importance of an environmental source of infectious particles. Thus, a comprehensive study of the specific associations of the fungus in nature is necessary in order to adequately understand its ecology. Since the original isolation of *H. capsulatum* from the soil by Emmons (*9*), this organism has been shown to occur in nature

with a wide geographical distribution. Isolated in at least thirty different countries, in temperate as well as tropical regions of the world, it is no longer associated with a specific endemic area. This widespread occurrence alone should be sufficient evidence to involve factors other than temperature and humidity in the ecology of *H. capsulatum*.

In the documentation of histoplasmosis epidemics, circumstances, activities, or associations of the patients with the environment often suggest the source of the infecting particles. Storm cellars, silos, caves, abandoned chicken houses, and other habitats have been incriminated as areas in which histoplasmosis has been contracted. It is obvious that the relationship of *H. capsulatum* to these environments determines the ecology of the fungus. The first positive isolations by Emmons were from a mound of soil at the entrance of a rat burrow next to a chicken house where he had previously trapped infected rats. Although it was not definitely stated, one could assume that the soil was contaminated with chicken excrement. Zeidberg *et al.* (*35*) were the first to clearly point out the association of chickens and the presence of the mycelial phase of *H. capsulatum* in soil. However, chickens have proved not to be the sole significant ecologic factor. Other birds have contaminated and enriched the soil with their excrement, and isolations from these environments have occurred without any relation to chickens (*1, 34*). Another association that has been well documented from many areas is the growth of the fungus from decayed bat guano samples (*12*). Thus we know that *H. capsulatum* has been isolated from a variety of specimens and locations, both with and without association with bird habitation, but with greatest frequency from soils enriched with organic material.

In 1955, Zeidberg and co-workers (*36*) studied the physical and chemical factors affecting *H. capsulatum* in soil. The most interesting observation was that soils from which *H. capsulatum* was isolated had an appreciably higher acidity than did negative soils. Most of the isolations were from chicken houses or from soils contaminated with chicken manure. Smith and Furcolow (*31*) have demonstrated growth-promoting substances for *H. capsulatum* and *Blastomyces dermatitidis* in infusion of starling manure. In these studies, the best growth and sporulation of *H. capsulatum* occurred on a medium of starling manure and loam soil intended to simulate the natural soil reservoirs associated with avian manure.

In an effort to determine the effect of chicken excrement on the growth of *H. capsulatum*, a water extract of this material was produced for laboratory studies. This extract, designated Chimanex, was added in varying amounts to sterilized soil, natural soil, and Sabouraud's dextrose agar medium. A definitive inoculum was added to each experimental medium and incubated at 25°C for 30 days. After incubation, quantitative counts were made from each soil and artificial cultural medium to determine the number of infective units per milliliter of sample. A stimulatory effect was observed at lower levels and a deleterious effect at higher levels. Luxuriant mycelial growth and sporulation occurred at the 2.5 and 5.0 per cent additive levels, whereas the fungus was destroyed at 25 per cent. No quantitative appraisal was obtained from the natural soil to which Chimanex was added. *H. capsulatum* was observed, but rapid overgrowth of the plates by other organisms prevented a realistic evaluation.

A preliminary chemical analysis of Chimanex performed by Dr. Aronson at the U.S. National Institutes of Health in Bethesda, Maryland, showed it to contain 1.20 mg/ml total nitrogen, by Kjedahl method, and 0.21 mg/ml nonprotein nitrogen. A more sophisticated and comprehensive chemical analysis might have revealed the specific factor or factors playing a major role in the establishment of *H. capsulatum* in given soils. Sufficient information was obtained to offer an explanation for why the fungus is found in places where the excrement has aged but not where it is fresh: The high concentration of certain chemicals in fresh excrement, especially

ammonium and ammonium compounds, destroys the fungus.

Stotzky and Post (*32*), in discussing the ecology of *H. capsulatum*, recently made the following statement: "Although the fungus (*H. capsulatum*) appears to be associated with animal droppings, primarily those of birds and bats, the essential unrestricted geographical distribution of animal droppings and the high saprophytic ability of the fungus suggest that the type and availability of energy sources are not primarily factors responsible for its ecology."

The precise role of animals and their ectoparasites in the ecology of *H. capsulatum* has not yet been determined. It has long been known that domestic and wild animals have been infected by this fungus, and in recent years it has been proved that certain species of bats harbor the fungus, but the ecological significance of these aspects of *H. capsulatum* needs further investigation.

An appreciation of the epidemiology of histoplasmosis requires knowledge of the incidence, geographical distribution, and sources of the infecting inoculum. During the past 25 years many investigators have presented viewpoints on these factors, but not to the complete satisfaction of all epidemiologists. Early in the Twentieth Century, histoplasmosis was considered to be a rare and fatal disease. Its known incidence was limited to four well-documented cases diagnosed at necropsy between 1906 and 1926 (*29*). All these cases occurred in adults. In 1945, Parson and Zarafonetis (*27*) reviewed 64 and reported on seven cases, all of which were fatal. This is the last time that histoplasmosis has been seriously referred to in the literature as a rare and uniformly fatal disease. In 1934, Dodd and Tompkins (*7*), working at Vanderbilt University, reported the first case of histoplasmosis in an infant. This was also the first case diagnosed ante mortem, as stated earlier. It was at this same institution that Christie and Peterson (*3*) began their comprehensive investigations of histoplasmosis in children.

Clinical and pathological studies have now clearly established that this fungus disease occurs in all age groups. To be sure, certain forms may be found more frequently in particular age groups. The recorded incidence of disseminated, fatal histoplasmosis is highest in infants, with almost equal frequency among the aged. In most studies of the disease in children under ten years of age there is no definite predilection in favor of either sex. Among adult patients, however, the male predominates, and this is usually believed to be associated with exposure risk. Many reports state that there are no significant differences in racial susceptibility. These same conclusions were reached in our own recent studies on disseminated histoplasmosis (*28*). This type of disease was seen in patients ranging in age from 16 to 75, with an average age of 53. Racial distribution followed the well-recognized pattern of pulmonary histoplasmosis in that it predominantly affected the white male. The ratio of white patients over black was 24:1. Occupations of the individuals varied, but approximately 60 per cent were farmers. Frequently histoplasmosis has been regarded as a disease only in individuals who have some impairment of their immune mechanism or who suffer from debilitation. The syndrome has been found associated with such maladies as sarcoidosis, leukemia, diabetes, tuberculosis, Hodgkin's disease, and other lymphomas. In our investigations we have observed Histoplasma both as the primary organism and a concomitant organism.

Histoplasmosis has long been considered a disease of primarily rural distribution, since many of the cases have been found among farmers. This concept has changed, however, in light of the numerous epidemics observed in urban areas. Still, in many of these small epidemics the urban residents could have been exposed to the fungus during a visit to the countryside, and in some cases a fortuitous association with soil or other contaminated material from rural areas has been proved. In two or three instances, urban gardeners had used chicken manure and bat guano as fertilizer. Nevertheless, bona fide urban sources of exposure, in which the fungus has

been isolated from areas within the cities, have been documented. Mason City, Iowa (*33*); Mexico, Missouri (*14*); and Washington, D.C. (*11*) are classic examples.

Skin-test surveys have contributed significantly to a clearer understanding of the epidemiology of histoplasmosis. Prior to the availability of histoplasmin as a skin-testing antigen, the incidence of histoplasmosis was determined on the basis of cases diagnosed at autopsy. The classical investigations by Christie and Peterson (*3*) and by Palmer (*26*) established that pulmonary calcifications could result from *Histoplasma* infections. Later, skin-test surveys of human and animal population groups showed that domestic animals also react to histoplasmin, thus indicating that the fungus may be present in the environment and that infections result from local exposure (*23*).

In 1955, Loosli (*22*) estimated that as many as 30 million people in the United States have experienced some form of histoplasmosis infection. Although the largest groups are found in the north central and south central areas of the United States, a comprehensive review of the various surveys shows that the fungus is not limited in its distribution. One of the most informative papers on this subject was written by Dr. Phyllis Edwards (*8*) and was presented at the Eighth International Congresses on Tropical Medicine and Malaria in Teheran, Iran. In it, she discusses the worldwide pattern of skin sensitivity to histoplasmin and to coccidioidin.

It has long been recognized that histoplasmin sensitivity is not limited to the groups represented by isobars on the maps. Also, within a given area there may be tremendous fluctuations in the percentage of reactors to the antigen. Nevertheless, skin-test surveys serve a useful purpose, and perhaps they will lead to a more significant understanding of the epidemiology of histoplasmosis infections in the future. This will be particularly true if modern research yields a more specific and sensitive fraction that will eliminate cross-reactions with other fungal antigens. The present crude histoplasmin has permitted Furcolow (*13*) to extrapolate that "approximately 500,000 new infections occur each year within the United States." It has been estimated also that at least 800 deaths due to Histoplasma infections occur each year.

The emerging pattern of urban histoplasmosis is of considerable interest, since the disease has heretofore been thought to have a rural distribution (*14*). In the latter concept, the origin of the infecting inoculum has been most frequently designated from a "point source." Subsequent isolation of the fungus from samples collected from the "point sources" has confirmed the presence of inoculum reservoirs. The prominent feature of these epidemic studies is that soil is the natural habitat of *H. capsulatum*. Growth and multiplication of Histoplasma is enhanced in soils enriched with droppings from chickens, starlings, and bats. Individuals become infected during exposure after some unusual activity such as cleaning an old barn or chicken house or entering a cave.

In the experiments, materials from these environments usually yielded the fungus, and it was postulated that susceptibile individuals became infected following inhalation of spores or mycelial fragments from these sources. Epidemics within a family apparently result from exposure to the infecting inoculum, since no evidence exists that the organism is transmitted from man to man or from lower animals to man. Experimental epidemiological studies substantiate these conclusions, and laboratory animals can be infected with living spores and mycelial fragments. Since small numbers of infective units of the fungus cause disseminated disease, and since there has been no evidence that transmission occurs between infected and normal control animals, inhalation has been agreed to be the route of inoculation (*20*).

In urban histoplasmosis, exposures have usually been associated with the tearing down of old buildings contaminated with bird or bat droppings. A great many small epidemics have been related to such activities since as far back as 1938 (*17*). In addition, exposure to *H. capsulatum* in

open urban areas has come into prominence. A review of the patterns of urban histoplasmosis, especially in areas in which skin-test sensitivity is low, shows exposure to sources contaminated mostly with bird droppings. However, it is not uncommon to find a high prevalence of histoplasmin sensitivity near an area where the over-all sensitivity is quite low. Frequent isolation of fungus in these specific areas indicates a high degree of contamination of the soil.

Sources of infecting inoculum other than bird droppings in soil and similar natural materials are of consequence to the epidemiology of histoplasmosis. In 1958, Emmons (*10*) reported the possible relationship of house-dwelling bats to histoplasmosis. He was successful in recovering the fungus from the soil adjacent to a bat-infested house in Maryland. Later reports confirmed his findings, and bat guano was found harboring the fungus in Trinidad and Panama (*12, 18*). There have also been several authenticated cases of histoplasmosis in individuals visiting or exploring caves, as well as among professional spelunkers (*2, 15, 25*). More recently, Shacklette, Diercks, and Gale (*30*) reported the recovery of *H. capsulatum* from bat tissue. The significance of the fungus in caves was elucidated by the report of Klite and Diercks (*19*). These authors clearly showed that *H. capsulatum* was present in fecal contents and organs of bats in the Canal Zone and thus opened another area of exploration in the epidemiology and ecology of Histoplasma.

In summary, the ecology and epidemiology of *H. capsulatum* is still not fully understood. The factors that influence growth, development, and colonization of the fungus in nature have not been documented entirely to satisfaction. Because of the high rates of skin-test sensitivity in some areas of the world, it is sometimes thought that *H. capsulatum* is a common soil organism which fortuitously is pathogenic to man and other animals. Epidemics of histoplasmosis suggest that the route of inoculation is the respiratory tract and that transmission is by inhalation of infectious particles. This has not been proved unequivocally in man, but in experimental laboratory animals infection can result from exposure to the mycelial phase of the fungus. The procedures used in the laboratory simulate conditions that occur in natural infections. Evidence from experimental animals indicates that living mycelial particles and microspores are most likely the infectious inoculum, whereas the large tuberculated macroconidia fail to penetrate to the bronchioles (*20*). Transmission from man to man or from animals to man has not yet been observed under natural conditions.

The geographical pattern of skin-test sensitivity to histoplasmin raises important questions about the epidemiology of histoplasmosis and the significance of overt infections in human populations. The predilection of this fungus for "point sources," or microenvironments, has been recognized for many years. Studies of epidemics since 1938, many in retrospect, are classic examples of this specific type of association of the fungus in nature. It is difficult to accept that the millions of humans and lower animals reacting to histoplasmin obtained their delayed sensitivity merely from exposure to the fungus in microenvironments. Highly localized microenvironments associated particularly with specific epidemics would seem to account for relatively few of the vast number of human infections.

The significance of the emerging pattern of urban as compared to rural histoplasmosis has not been satisfactorily determined. Reports of *H. capsulatum* in urban areas have increased during the past fifteen years. Indeed, some of the most serious epidemics have occurred in urban areas—for example, Mexico, Missouri, and Mason City, Iowa. Rapid urban expansion has resulted in the disruption of virgin areas and the removal of trees and other structures that were previously bird habitations. Such activities have increased the number of disturbed foci in nature, which in turn means a greater number of active cases of histoplasmosis. Urbanization can also result in the addition of a geographic area in which the disease had not been reported previously.

The most recent and fascinating association of Histoplasma with its environment has to do with bats. The significance of these mammals in the over-all ecological and epidemiological picture of histoplasmosis is difficult to assess. It is common knowledge that Histoplasma has been isolated from a large number of guano samples, usually with difficulty, but not from guano in every cave. Human infections have been reported in persons entering caves in Mexico, Central and South America, and South Africa. In a few instances the number of affected individuals was large. An occasional human case of histoplasmosis has been reported from caves in the United States.

In 1958, Emmons isolated Histoplasma from soil near the foundation wall of a Maryland residence that had been infested with the brown bat, thus opening a new era of Histoplasma-bat association. Since then, there have been similar reports of Histoplasma being isolated in soil near houses infested by other species of bats. These mammals are known to harbor *H. capsulatum*, but to date it has not been demonstrated that they are important hosts of the fungus. Although there is now abundant evidence that certain bats are infected and that Histoplasma is shed in the feces of these mammals, it would seem that the recent transformation of *H. capsulatum* to a pathogen of bats is not warranted.

Finally, the evidence that an individual cannot become sensitive to dead inoculum is not yet entirely convincing. Skin-test sensitivity has been produced with killed particles of *H. capsulatum* in experimental animals exposed to large dosages of the inoculum; however, they did not become hypersensitive as rapidly as when living inoculum was used. The skin-test sensitivity waned in a few animals that were sensitized with killed mycelial particles, but the reaction persisted in most of them. Perhaps this could happen in man.

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DISCUSSION

Chairman Pappagianis: The discussion is now open. We will entertain questions concerning sporotrichosis and its epidemiology and ecology first.

Dr. Mariat: I should like to again emphasize the importance that the general condition of the host seems to have in cases of sporotrichosis, and also in chromomycosis and mycetomas. We are formulating a general hypothesis in this regard, but we do not have sufficient results yet to speak in statistical terms. We hope in the future, in cooperation with our colleagues from Mexico and Central America, to compile a large body of data and use computers to assist us in our evaluation.

The epidemiology of sporotrichosis and the ecology of *Sporothrix schenckii* are very interesting questions, and it would appear that considerable advances have been made in these fields.

We have isolated various strains of *Ceratocystis sp.*, a phytopathogenic fungus having the conidial form of *S. schenckii* and also the physiological characteristics of the pathogenic fungus —namely, ability to grow at 37°, to give a yeast form, and to require pyrimidine as a growth factor. We tried to determine experimentally the pathogenicity of one of the strains of *Ceratocystis*. This strain proved to be pathogenic for hamsters and mice, particularly on intraperitoneal inoculation of the yeast form grown at 37°. Only some of the inoculated animals showed a progressive disease, in which it was possible to note an orchitis and ganglionic involvement. The tissue forms of the fungus were the same as those in the cases of experimental sporotrichosis—namely, asteroid bodies. In all the cases of progressive disease the reisolated fungus could be considered a pathogenic, perithecial-less mutant.

Chairman Pappagianis: If there are no further questions on sporotrichosis, we can move on to chromoblastomycosis.

Dr. Mayorga: *Fonsecaea pedrosoi* is the most frequently isolated agent in chromoblastomycosis, and it seems to be present in warm, humid environments. In Venezuela, however, it has been demonstrated by Campins, Hómez, Convit, and others that *Cladosporium carrionii* has been isolated predominantly from patients living in dry, semiarid regions such as those in the states of Lara and Falcón.

I would like to ask Dr. Montero-Gei about the striking pictures he showed of double infection in a single patient, who had both South American and keloidal blastomycosis. Would this support the theory that the two fungi are closely related?

Dr. Montero-Gei: First I should like to say that so far all strains isolated in Costa Rica have been classified as *Fonsecaea pedrosoi*. Of course, there is an academic discussion about the position of the etiologic agents of chromoblastomycosis, and Emmons, for example, says they should be included in the genus Phialophora. Personally, however, I believe that the genus Fonsecaea should be maintained.

Dr. Negroni: I proposed Fonsecaea as the name to distinguish this fungus or group of fungi with multiple types of sporulation from the group Phialophora, which has only one type of sporulation. Furthermore, the type of sporulation in Fonsecaea is a little different microscopically from that in the genus Phialophora. Since I am the father of this genus, I am defending it. Please forgive me for my audacity.

As to the geographic incidence of chromoblastomycosis, I would say that most cases in Argentina apparently originate in arid or relatively dry areas. I wonder if it would not be worth our while to do here what has been done for other deep mycoses; namely, try to isolate the fungus from nature in the places of its ecology and not merely to record clinical cases in specific areas.

Dr. Montero-Gei: Returning to Dr. Mayorga's question about the cases of double infection, our purpose was to emphasize the suscep-

tibility of the host to mycotic infections, as Professor Mariat has already reported in Africa. The fact that we have had double infections of paracoccidioidal granuloma and keloidal blastomycosis or chromoblastomycosis leads me to believe that we should study the characteristics of the host very carefully, especially metabolic disturbances that might bear on the synthesis of proteins or carbohydrates, or vitamin, hormone, or enzyme problems. Keloidal blastomycosis has been found four times in my country, and paracoccidioidal granuloma approximately 12 times. Hence, the incidence of these two mycotic infections is quite low in our environment. We also have had a double infection with sporotrichosis, but I did not mention it because it is probably one of the most common mycoses in my country.

Dr. González Ochoa: We should not assume that *Cladosporium carrionii* is found only in arid areas. In Mexico it has been isolated from quite tropical regions. In other words, it is not necessarily the dry climate that conditions the ecologic factors for the existence of *C. carrionii*.

Dr. Montero-Gei: I agree fully with Dr. González Ochoa. We know that the fungi which cause chromomycosis occur everywhere, especially on plants and in soil. We have the problem of differentiating between those fungi that are not pathogenic and those that are capable of producing chromomycosis. The action of these strains can probably be studied on the proteins. A special characteristic of *C. carrionii* is that it does not liquefy the Loeffler medium, whereas the saprophytes do.

The black fungi in the group that are the ecologic agents of chromomycosis tend to grow in warm, humid environments, and this fact has been experimentally verified in tropical and subtropical areas.

Dr. Borelli: I share the position of Dr. Mayorga in regard to the *reservárea* of *C. carrionii*. As early as 1955 I wrote to Dr. Brygoo to say that he should seek the origin of his only strain of *Cladosporium sp.* in the southern part of the island of Malagasy, which is the only point on the island that is dry or arid. And, indeed, the following year Dr. Brygoo published a long list of cases and stated that all the strains of that species—which in the long run turned out to be *C. carrionii*—came from the southern part of the island, where the annual rainfall ranges from zero to 800 mm. A similar climate is prevalent in an area in Queensland, Australia, in which strains of *C. carrionii* have been isolated. Again, the same situation is found around the Kalahari Desert in South Africa and in an arid area of Venezuela that is considered part of the *reservárea* of *C. carrionii*. I cite this background so that any new facts will be considered in the light of what we have already observed. We cannot forget that most of our experience has shown *C. carrionii* to originate exclusively in arid or dry areas within the Tropics. Anything that deviates from this is a relevant fact.

With regard to the pathogenicity of the Cladosporia, two species have been incriminated: *C. bantianum* and *C. carrionii*. The only decisive test I have for identifying *C. carrionii* is injection into a cold part of the human skin. If the agent is inoculated into the skin of the trunk, for example, it may not produce disease, since many strains of *C. carrionii* do not grow above 35° to 36°. However, if it is inoculated into the skin of the knee, it always produces chromomycosis.

The idea of a host predisposition in patients with chromomycosis or sporotrichosis must be checked against our accumulated experience.

Dr. Mayorga: I would like to ask Dr. González Ochoa about the four Mexican cases of chromoblastomycosis produced by *C. carrionii*. According to the literature I have seen, they all came from the state of Oaxaca. It is my understanding that the state of Oaxaca is dry or semiarid.

Dr. González Ochoa: Oaxaca is definitely an arid region, but I do not know of any isolations made there. We Mexicans who know the situation are in agreement that all these strains have been isolated from the mountains in the state of Puebla, which is a humid and hot region.

Dr. Mariat: The ecology of the fungi and actinomycetic agents of mycetomas is very complex. Dr. Segrétain and I studied samples of soil, water, plants, and the like in three separate surveys conducted in West Africa in 1966, 1968, and 1970, and we found several agents of mycetomas in the soil.

With regard to the paper by Dr. Macotela-Ruíz, I would like to ask what epidemiological value can be assigned to a survey of 300 persons shown to be nocardin negative in the endemic area described.

Dr. Macotela-Ruíz: As regards the negativity of the nocardin cutaneous tests in the endemic area described by Atala, it is interesting to point out that these healthy subjects came from areas in which we had detected cases of mycetoma. This result is interesting, since the persons who did not show a lesion gave a negative reaction, whereas those persons having a mycetoma gave a positive reaction to the polysaccharides of *Nocardia brasiliensis*. This bears out the diagnostic value of the polysaccharide described and isolated by González Ochoa and Baranda. Our findings differ somewhat from those of Rodríguez, who found an average reactivity of 47 per cent to a protein antigen. However, this antigen, which comes from *N. brasiliensis,* is similar to that obtained from *M. tuberculosis* (PPD). It would be interesting to make a survey from this viewpoint of skin reactions to both antigens. I was referring only to the polysaccharide antigen.

Chairman Pappagianis: We can move on to coccidioidomycosis now.

Dr. Levine: I would like to say that recovering *Coccidioides immitis* arthrospores from the air by artificial means, such as by a high electrical charge or by sampling, is difficult. Actually, we have the best samplers of all: man and animals. Thus, when Dr. Pappagianis refers to one spore in that volume of air which a human being would breathe over a two-week period being a low figure, this is probably because of the inadequacy of the sampling methods.

We know that airborne arthrospores acquire electrical charges. If one shakes them in a flask one sees this phenomenon illustrated by the way the spores are repelled or attracted, in different circumstances, to the glass. They are difficult to trap, however. John Converse's study using monkeys held in cages well above the ground, showing that they contracted coccidioidomycosis, points to the likelihood that the arthrospores are in the air in large numbers. Perhaps there is too much concern over the fact that we have not been able to trap them mechanically.

I think the thesis that Dr. Pappagianis presented relating to the airborne route is well proved epidemiologically.

Dr. Mayorga: The 0.2 per cent prevalence of coccidioidin reactors in Honduras could well give an erroneous picture of the situation. One might think the disease does not exist in that country. However, the results of studies conducted in the valley of Comayagua show that 10 to 50 per cent of the residents there have been infected with *C. immitis*. The thousands of reactions recorded by Guzmán are for the whole republic of Honduras, whereas the tests I am referring to were carried out only in the valley of Comayagua.

Chairman Pappagianis: This suggests that we should look for more restricted locations of *C. immitis*. I am curious about the existence of disease in the Central American countries, and I wonder if those of you who have worked in that area feel that much clinical disease is going unrecognized, or whether there is no particular interest in looking for coccidioidal infections.

Dr. Mayorga: I think the reason that clinical cases are very seldom reported is that the hospitals which are located in the endemic areas are small and do not have specialized personnel. These infections are therefore frequently overlooked.

Chairman Pappagianis: If there is no more discussion on this, we can pass on to cryptococcosis.

Dr. Shadomy: I would like for Dr. Muchmore to comment on the possible incidence of the be-

nign primary form of cryptococcosis, the potential threat this disease may constitute for individuals who are troubled either hormonally or immunologically later in life, and possibly the need for treatment of the disease in its subclinical form.

Dr. Muchmore: I don't think we know anything about benign primary infections in cryptococcosis. We assume, by extrapolation from other diseases, notably coccidioidomycosis and histoplasmosis, that these forms probably occur. The iceberg I drew on the board suggests, if we accept that cryptococcin is even remotely specific, that benign primaries, or inapparent infections, occur in a reasonably large number of people—at least in Oklahoma, and probably throughout the areas where cryptococcosis is found.

Dr. Seabury: There have been, of course, a number of observed cases of pulmonary cryptococcosis in recent years that have not been treated, and the patients have not exhibited any increase or exacerbation in their disease. How long these patients need to be observed is quite another matter. We have also seen patients with apparently the same form of limited lobular pulmonary cryptococcosis whose disease reactivated several years after surgical biopsy—that is, pulmonary biopsy—to prove existence of the infection. I do not think there is any doubt that primary pulmonary infections of cryptococcosis do occur, and they probably occur in considerable numbers.

I am not sure we should call them benign. I do not think we know enough to attach a label. I do know that most of the cases recognized clinically are meningitic, and these are very serious forms of the disease. We assume that they arise after previous infection, and in some instances we have long years of documentation of proven pulmonary lesions prior to the development of meningitic infection.

I think we should ask ourselves what is the risk of serious disease following primary infection. If we pose the same question in regard to tuberculosis, most of us will agree that the majority of individuals who contract a primary infection with tuberculosis recover spontaneously without treatment, and in many cases, if not most, without developing subsequent active clinical disease. Still, I would not hesitate to give INH to a patient with a primary pulmonary tuberculosis lesion. By the same token, a short course of amphotericin B in low dosage for a patient with a documented primary pulmonary cryptococcal lesion is probably quite sufficient and certainly as justifiable as a year of treatment with isoniazid.

Dr. Macotela-Ruíz: According to Dr. Muchmore, the data to be found in the literature do not give anything like a true picture of the morbidity of cryptococcosis in Latin America. Dr. González Ochoa has studied several cases among persons, most of them males, who went to him for study and who had a previous history of good health. Dr. González-Mendoza has also reported cases of cutaneous cryptococcosis in Mexico. In addition, I might add that my own studies include five such cases. Two of these five were diabetic patients who were admitted to the hospital in a diabetic coma, the third patient was a woman who had a glioblastoma, the fourth was a patient with meningeal-associated tuberculosis, and only one case was a pulmonary cryptococcosis as we observed it.

Dr. Muchmore: I have emphasized that the published reports undoubtedly fail to reflect a large proportion of the actual number of cases. Of the 45 that I know have occurred in Oklahoma, only four of them have been published. I agree that *C. neoformans* makes itself known in debilitating conditions. I might point out that cryptococcosis at present occupies the third position in frequency among the iatromycoses occurring in patients who receive immunosuppressants, organ transplants, steroids, etc. The first in frequency is candidiasis, and the second is aspergillosis.

Dr. Huppert: Within the past six months we have had experience with three kidney transplant patients who came down with dissemi-

nated coccidioidomycosis while on immunosuppressive agents. As recently as Monday of this week we discussed at the University of Pittsburgh the case of a nine-year-old boy in whom disseminated coccidioidomycosis was diagnosed on postmortem examination. In retrospect, it was realized that this child had been in Arizona. When he returned to Pittsburgh it was necessary for him to have a kidney transplant, and during the period that he was on the immunosuppressive agent he had the mycosis from a previous asymptomatic infection incurred in Arizona.

I predict we are going to see a lot more of these situations with coccidioidomycosis in the near future.

Dr. Furcolow: I have a general comment. The iceberg Dr. Ajello was talking about in the first place did not refer to normal people; it referred to people who were infected. We did three studies on blastomycosis over a period of five years in three different states. From the results, it looks as if we have about five new cases of the disease per 100,000 population each year in the states of Kentucky, Arkansas, and Mississippi. These are highly populated states, and the figures and reporting run more or less continuous from the Great Lakes to the Gulf of Mexico and from the central United States eastward to the Atlantic Ocean. We have not made an estimate of the population in this endemic area, but we intend to do so. The general ecologic conditions appear to be similar. In the three states in which careful figures were developed, the incidence of disease in dogs is about 10 times as great as it is among humans.

Dr. Mackinnon: We are currently engaged in looking for the agents of chromoblastomycosis in nature. Our attention has been drawn to the frequency with which we isolate *Phialophora verrucosa*. Some time ago we obtained 20 strains of *P. verrucosa* which for the most part had been isolated from the wood of tree trunks, from the soil, from plant debris, and from pieces of wood. We also isolated the species from wasp nests. Rarely, however, did we find *F. pedrosoi*.

Chromoblastomycosis is a very rare disease in Uruguay. We only know of two cases contracted in Uruguay, and *P. verrucosa* was found in both. Some people attribute this low incidence to the use of footwear, but I am not satisfied with this explanation. I believe there are other factors.

Dr. Montero-Gei: I would say we have only isolated *Fonsecaea pedrosoi* from soil. We have not found any *Philalophora verrucosa* as yet. Dr. Trejos inoculated himself with a strain of *F. pedrosoi* isolated from nature, and he showed that it was capable of producing chromomycosis.

Dr. Conti-Díaz: In Lexington, Kentucky, I recently isolated four strains of *P. verrucosa* from the soil.

I would also like to say that I was impressed by the case presented by Dr. Montero-Gei with symmetrical gluteal lesions. I wonder if he considered the possibility of another portal of entry in this instance.

Dr. Montero-Gei: We know that in chromomycosis the lesions are unilateral and there are very few cases described in the literature in which you find bilateral lesions. This is an exceptional case of a 16-year-old female who was infected in both buttocks. The aspect of the lesions would be of interest to Dr. Conti-Díaz; they appear to have cauliflowerlike characteristics. Here is something different from leishmaniasis or sporotrichosis. It is a proven case of chromomycosis with positive culture, and exceptionally a case of bilateral lesions.

Session V

Thursday, 26 February 1970, 9:00 a.m.

MEDICAL MYCOLOGICAL TRAINING

Chairman

Pablo Negroni

Rapporteur

Leanor D. Haley

THE TRAINING OF PHYSICIANS IN MEDICAL MYCOLOGY

Pablo Negroni

This paper is addressed not to mycologists but rather to faculty members and officials of medical centers interested in mycological training programs.

The Latin word "fungus" means sponge, and indeed the larger fungi have this appearance. The Greeks and Romans recognized and distinguished various edible and poisonous mushrooms. Pier Antonio Micheli was the first to study these organisms under the microscope, and his great work *Nova Plantarum Genera,* published in 1729, contains a description of one of the commonest microscopic fungi, the Aspergillus.

The discovery of the filamentous microscopic fungi as agents of tinea took place during the period 1836 to 1841. In 1857 the fungous nature of Madura foot was established, and between 1877 and 1908 the deep mycoses were discovered and their agents isolated in artificial culture media. The development of improved antigens for skin tests led to the knowledge that there may be mycotic infection without disease and that about 90 per cent of the healthy adult population in some areas of America react positively to coccidioidin or histoplasmin. Further, the introduction of serological techniques contributed greatly to the diagnosis, prognosis, and follow-up of patients under treatment.

Some of the superficial mycoses are found predominantly in children, but the deep mycoses, although they can be seen in children, occur most frequently in adults between 30 and 50 years of age. Fungus diseases of medical interest are common to man and animals, domestic or wild, and their reservoir is often the soil. Their geographical distribution depends on the nature of the soil, the climate, the "habitat" and ecology of the respective parasites, and the nutritional and socioeconomic standards of the population. Some mycoses may be acquired as occupational diseases and give rise to legal claims. We also know that fungi are responsible for many allergic conditions and that a number of fungi considered common contaminants can produce opportunistic mycoses when predisposing factors such as blood dyscrasias, metabolic diseases, anatomic disorders, or new methods of therapy are involved.

Toxic disorders of man and animals due to the ingestion of mouldy foods have been widely studied in recent years. Ergot poisoning was probably the first to be known. The Russians observed and studied toxic aleukia, and the Japanese worked on the "yellow rice disease." The aflatoxins, first extracted from cultures of Aspergillus by Allcroft *et al.* and by Sergeant *et al.* in 1963, are considered to be among the most potent carcinogens for animals.

The clinical diagnosis of the superficial mycoses presents no difficulty in most cases, but mycological confirmation is often still required. The deep mycoses, on the other hand, can mimic tuberculosis, silicosis, sarcoidosis, and even hydatidosis. Microscopic diagnosis is frequently made by pathologists during the examination of formaldehyde-fixed specimens. In such cases, the

opportunity of obtaining cultures and animal inoculations is lost. X-ray examination and serologic tests should be used more often in the diagnosis and follow-up of patients.

Medical treatment is usually sufficient to cure the mycoses. Nevertheless, surgery may be formally indicated in some cases—for example, mycetomas, pulmonary mycotic cavities, abscesses, and granulomatous lesions.

Prophylaxis is one of the most important public health goals of medical mycology. Although for the superficial mycoses no special preventive measures are required, for the deep mycoses they are badly needed. These may consist of such steps as employing adequate vaccines or changing the ecology of the parasite.

The importance of training different groups of physicians in medical mycology is emphasized if we group the mycoses according to their localizations, as follows: (1) dermatophytoses; (2) mycoses of the central nervous system; (3) ocular mycoses; (4) mycoses of the ear, nose, pharynx, and larynx; (5) pulmonary mycoses; (6) abdominal mycoses; and (7) mycoses of the extremities, joints, and bones.

The panorama of medical mycology is broad indeed, and it is important to accept the following premises: (1) the training of physicians in this field cannot be postponed; (2) teaching programs should vary in scope and duration depending on the medical groups and countries involved; (3) in most cases, short-term programs will meet the prime needs of the group in question; (4) a long-term program is essential for individuals who intend to practice mycology as a profession; (5) suitable short-term intensive programs can be carried out on a basis of four to six hours a day for five days a week; (6) for teaching purposes, the medical specialists should be separated into the following groups: dermatologists; pneumonologists; epidemiologists and physicians engaged in public health activities; internists and pediatricians; specialists in otolaryngology, ophthalmology, and stomatology; pathologists; and surgeons and radiologists.

Mycological training of dermatologists

The dermatophytoses account for 20 to 50 per cent of all consultations in dermatology, depending on different climates and social groups. Moreover, mucocutaneous manifestations are often seen in the course of deep mycoses. A short intensive course of about one month would take care of the needs of this group. Based on a total of 110 hours, the work would be distributed as follows: 20 lectures, 30 hours devoted to the examination of patients and the collection of clinical material, and 60 hours in the laboratory. The following subjects should be developed: morphology, biology, and classification of fungi, three lectures; superficial mycoses, four lectures; treatment of the superficial mycoses, one lecture; deep mycoses, eight lectures; opportunistic mycoses, one lecture; immunity and serology, one lecture; pathology, one lecture; and treatment of the deep mycoses, one lecture.

Mycological training of pneumonologists

This group should have a course of about 18 days' duration, with 60 hours of work distributed as follows: 12 lectures, 24 hours for the examination of patients and the collection of clinical material, and 24 hours in the laboratory. The following subjects should be covered: basic mycology, three lectures; superficial mycoses, one lecture; deep mycoses, five lectures; opportunistic mycoses, one lecture; immunity (serology), one lecture; and treatment, one lecture. Special emphasis should be placed on the source of infection, portal of entry of the parasite, epidemiology, symptomatology, pathology, diagnosis, and treatment of the lung mycoses.

Mycological training of epidemiologists

The needs of this group could also be met with a short course of about 18 days' duration with 60 hours of work distributed in the same way as for the pneumonologists. The following subjects should be developed: basic mycology, two lectures; superficial mycoses, two lectures; deep and opportunistic mycoses, five lectures; zoonoses and occupational mycoses, one lecture;

poisoning by fungi and mycology of foods, one lecture; immunology and treatment, one lecture.

Mycological training of internists and pediatricians

For these specialists, it would be appropriate to offer a short course of about 10 days' duration, with 50 hours of work distributed in the following way: 10 lectures, 20 hours dedicated to the examination of patients and the collection of clinical material, and 20 hours in the laboratory. The following subjects should be covered: basic mycology, two lectures; superficial mycoses, three lectures; deep mycoses, three lectures; opportunistic mycoses, one lecture; and immunology and treatment, one lecture. Special emphasis on the opportunistic fungal infections, particularly with Candida, should be given to the pediatric group.

Mycological training in otolaryngology, ophthalmology, and stomatology

A short course of about 10 days' and 40 hours' work would meet the needs of specialists in these fields. The work should be distributed as follows: eight lectures of 45 minutes each, 16 hours devoted to the examination of patients and the collection of clinical material, and 16 hours in the laboratory. The following subjects should be dealt with: basic mycology, two lectures; superficial mycoses, one lecture; deep mycoses, three lectures; opportunistic mycoses, one lecture; and treatment, one lecture. Special emphasis on the mucocutaneous manifestations of deep mycoses would be given to the otolaryngologists and stomatologists, and on opportunistic mycoses to the ophthalmologists—for example, the fungal endophthalmitis that can be seen after cataract surgery.

Mycological training for pathologists

A short course of about 10 days' duration, with 40 hours' work, would be sufficient for the mycological training of pathologists. Special emphasis on the microscopic characteristics of the parasites in tissue and on experimental pathology should be given.

Mycological training for surgeons and radiologists

For this group it is recommended to offer a short course of about one week's duration and 25 hours of work, distributed in the following manner: 5 lectures, 10 hours devoted to the examination of patients and the collection of clinical material, and 10 hours in the laboratory. The following subjects should be covered: basic mycology, one lecture; deep mycoses and opportunistic mycoses, four lectures. Special emphasis on the clinical and radiological characteristics of the deep mycoses would be given to this medical group. In reference to the opportunistic mycoses, it should be remembered that endocarditis is often observed as a complication of open heart surgery, and that with the increasing use of transplantation techniques and concomitant immunosuppressants such opportunistic mycoses will become more frequent.

Until 35 years ago, medical interest in human diseases due to fungi was restricted mainly to the dermatophytoses. Sabouraud became famous all over the world as the father of medical mycology. It is really only in the last three decades or so that physicians devoted to this specialty have had to have a solid knowledge of botanical and applied mycology and of soil and water fungi.

The present author used to teach basic and applied mycology at the National University of La Plata, Argentina, as a long-term postgraduate course. In 1948 the Mycology Center was created at the Faculty of Medicine, and a B.A. degree was offered in three areas of activity: research, service, and teaching. Its staff consists of a director, three mycologists (one of these a physician, one a dentist, and one a biochemist), two assistants (both of them physicians), one secretary, a laboratory technologist, and a laboratory assistant. The physical plant is distributed as follows: three areas for research; one office for the examination of patients and the collection of clinical material; one laboratory large enough to accommodate 12 to 13 persons; an office for the secretary; an office for washing, sterilization, and

culture media preparation; and an area for keeping experimental animals. Lectures are held in the library of the Faculty of Medicine.

Pursuant to the advice of Dr. Baldó, a Commission for the Study of the Mycoses was established in 1962 with technical support from the staffs of the Mycology Center and of the Muñiz Hospital for Communicable Diseases of Buenos Aires, where the patients with deep mycoses are hospitalized. The Mycology Center and the Muñiz Hospital have access to an abundance of human fungal infections that provide a unique opportunity for research and teaching.

Since 1966 a Symposium on Mycology has been held once a year in different cities of Argentina. The teaching of medical mycology and the creation of a Mycology Center have been the subject of discussion at some of these meetings, and the creation of chairs of medical mycology has been proposed to the authorities of various faculties of medicine.

Since 1965, short mycological training courses have been offered at the Mycology Center for dermatologists, pneumonologists, and physicians working in otolaryngology, ophthalmology, and stomatology. They have been attended by graduates from various parts of Latin America and from Spain.

AN AUDIO-TUTORIAL KIT FOR TRAINING IN BASIC MEDICAL MYCOLOGY

John H. Krickel and Leanor D. Haley

The need for better laboratory training of medical technologists in the area of diagnostic medical mycology is a critical problem. Proficiency tests in mycology sent out by the U.S. National Communicable Disease Center, Atlanta, have shown that a number of laboratories actively engaged in diagnostic medical mycology are unable to correctly identify some of the most common pathogenic fungi seen in the United States. And, indeed, many students taking advanced courses in medical mycology at NCDC are unfamiliar with some of the basic procedures employed in the identification of common fungi despite the fact that they have all been doing mycology in their respective laboratories. An examination of the curricula in a number of schools of medical technology has revealed significant deficiencies in the amount of time students actually spend in the mycology laboratory.

Why is education and training in medical mycology so inadequate? Several explanations can be offered, but perhaps the most important is that the schools of medical technology and microbiology frequently lack faculty and technologists with adequate training themselves in this specialty. Consequently, medical mycology may be omitted entirely, presented very briefly, or taught by someone who does not have the proper preparation or experience.

In order to assist schools and laboratories in the teaching of this subject, the Mycology Training Unit and the Education Specialist of the Laboratory Training Section at NCDC have developed an audio-tutorial kit consisting of laboratory exercises in basic medical mycology. It is designed so that the schools can essentially maintain their autonomy and flexibility in instruction while at the same time they are relieved of the tedious detail of the laboratory periods.

This kit is intended to be incorporated into a curriculum for medical technology students who have completed their studies in basic microbiology. It may also be used by laboratory technicians who wish to strengthen their background in medical mycology or simply refresh their earlier training.

The term *audio-tutorial* reflects the use of audio tapes that capture the style, the frequent questions, and the personal warmth of a one-to-one teacher-student relationship. The tapes discuss methods for identification of commonly encountered dermatophytes and subcutaneous and systemic mycotic agents and guide the students through their laboratory exercises. The kit is designed to systematically organize these laboratory experiences through a carefully chosen sequence of clinically significant fungi. It is not meant to be completely self-instructional, however. Students are expected to attend lectures by a competent instructor and to complete the specified reading assignments. In the laboratory, their work should be supervised by a technologist. There, individual students or very small groups listen to the information and directions on the

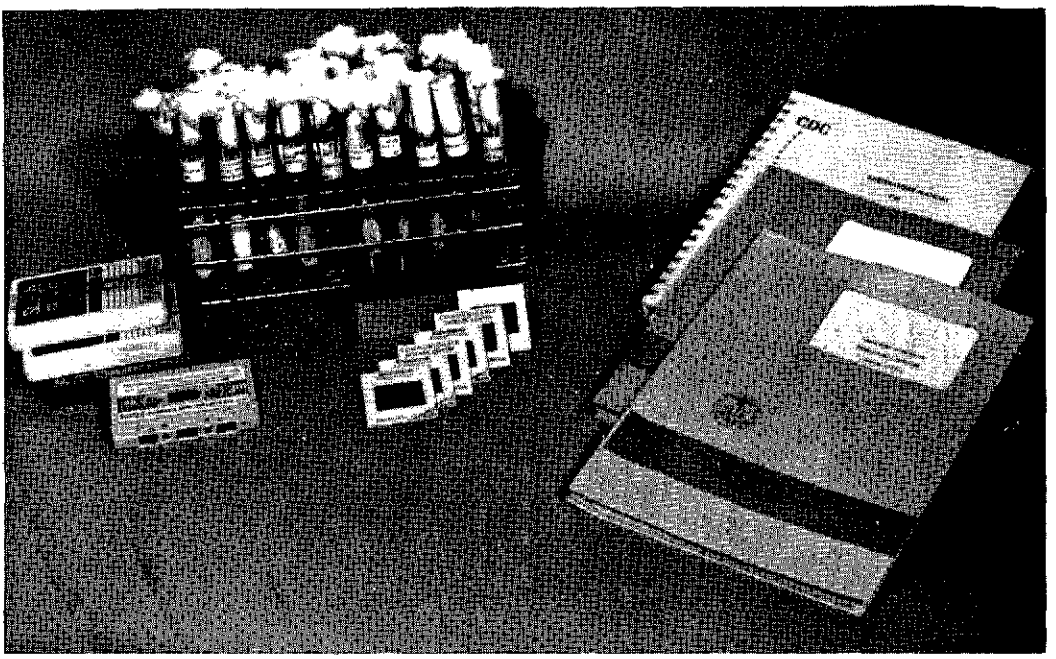

Figure 1. NCDC audio-tutorial kit in basic medical mycology, consisting of stock cultures, audio tapes, 2 x 2 slides, and manuals. All other equipment and supplies are furnished by the using institution.

tapes, stopping the recorder frequently, as directed, to answer questions, record gross observations of cultures, prepare for microscopic studies, take notes, and make drawings.

Two manuals have been developed for use with the kit. One is an instructor's guide with sample outlines of eleven lectures. Each outline is accompanied by a list of references of the most

Figure 2. Student desk set for a typical laboratory exercise with the audio-tutorial kit, showing many of the required supplies furnished locally by the using institution.

recent and basic material available on the subject covered. Also in this manual are lists of media, reagents, and equipment that will be needed for each laboratory session, as well as instructions for the laboratory supervisor. The second manual is for the student. It contains detailed instructions for performing each laboratory experiment, reading assignments, written quizzes, practical examinations, and a glossary of many mycological terms.

Kodachrome slides showing the identifying features of each of the fungi in question are included in the kit. These slides are discussed in detail in the tapes.

Finally, if the class is small, cultures are furnished for each student. For larger groups, two sets of stock cultures are provided with instructions on how to prepare individual sets. These cultures are discussed in the tapes and they are also used in the student exercises.

Since the NCDC *Manual for Medical Mycology* is no longer available from the U.S. Government Printing Office, copies of this publication are made specially available and shipped as part of the package.

The institutions using the kit must furnish their own tape recorders (tapes are on 3M cassettes), as well as media, reagents, laboratory bench equipment, and texts. When the course is completed, they are asked to return the tapes, the Kodachrome slides, and three copies of the *Manual for Medical Mycology* to NCDC in Atlanta.

NEED FOR BASIC RESEARCH IN THE TRAINING OF GRADUATE STUDENTS IN MEDICAL MYCOLOGY

Luis M. Carbonell

The volume of basic research on the pathogenic fungi is small compared to what has been done on nonpathogens. Most of our knowledge on the molecular biology, genetics, and ultrastructure of fungi comes from the study of nonpathogens. This is probably because the latter are easier to handle—in the first place they do not harm the researcher, and in the second place they can readily be used as experimental models to explain a basic or specific function.

Owing to the special characteristics of the pathogenic fungi, the studies we are doing on them are related mainly to their identification in the patient or in the environment. However, it is in the study of the biology of these organisms that we will be able to make progress that is applicable to epidemiology, serology, and therapy. For example, although sexual characteristics have been extensively studied in the nonpathogenic fungi, the research in this area recently started with the pathogens is already beginning to yield important new taxonomic information. Fungi once believed to be separate species have now been found to be variants of the same species, as in the case of *Tricophyton quinckeanum,* where the mating reaction has shown that it must be equated with *Tricophyton mentagrophytes* (*2*).

We are all aware of the tremendous new horizons opened up by the discovery of the helical structure of nucleic acid and of the great contribution this finding has made to studies on viruses, bacteria, and fungi. Nevertheless, in regard to the pathogenic fungi, a look at the literature over the period 1964 through 1969 reveals only one very preliminary report on the nucleic acid of *Blastomyces dermatitidis* (*9*) and another small study on the genus Candida (*8*).

Medical mycology is also slow in putting the latest technology to work on its own problems. Ultrastructure, which is used extensively in other biological sciences, has entered our field only recently. The last review on the ultrastructure of fungi by Bracker (*5*) and on cell wall chemistry by Bartnicky-García (*3*) show the enormous amount of work that has been done on nonpathogens as compared to pathogens. Edwards and co-workers (*6*) wrote about the ultrastructure of *Histoplasma capsulatum* in 1959, and the first report on the ultrastructure of a nonpathogenic fungus (*Sacharomyces cerevisae*) was published by Agar and Douglas (*1*) in 1955. At first glance it would appear that only a few years elapsed between these two works, but a search of the literature thereafter shows that the ultrastructure of the pathogenic fungi is only now beginning to be worked out.

Before proceeding further, it is well to distinguish clearly between basic and applied research. Basic research consists of the studies done to gain new knowledge, regardless of its application; applied research, on the other hand, is the work that is pursued with the clear understanding that the acquired knowledge will be used for a specific purpose. Thus, basic research is centered on the researcher, who must *look* for the problem, while applied research concentrates on a particular area. Applied research will give us a technology which, if the proper environment is provided, will stimulate basic research

by means of a feedback mechanism. These are the two extremes, however. There is a kind of research in between that we might call "oriented basic research." It is a kind of research that can help toward the solution of applied problems. For instance, the work done on protoplasts of pathogenic fungi (*4*) does not have an immediate application, but in the long run, with more applied research, it could be helpful in the development of a substance with increased antigenic properties (*7*). Most of the "basic" research done in medical mycology is of the oriented type; practically none of it is pure basic research.

The medical mycologist is not to be entirely blamed for the lack of emphasis on basic research. Since medical mycology has a very definite purpose—namely, to cure and prevent mycotic diseases—it has concentrated on this objective. Existing funds and personnel, which are already meager, are channeled mainly into this effort.

Basic research suffers also because of the way in which training is given to would-be medical mycologists and because of the little attraction that this field holds for nonmedical specialists such as biochemists, molecular biologists, and the like. Postgraduate training in medical mycology is given either as an intensive course lasting several weeks or else as part of the teaching program for other related specialties—for instance, microbiology. When included with other specialties, the training is not as thorough as the intensive course devoted to medical mycology alone. Usually the student who takes the latter has a real interest in the subject and is likely to continue in this kind of work.

The content of the courses in medical mycology is generally standard. It includes the laboratory diagnosis of pathogenic fungi and the study of their pathology, immunology, epidemiology, clinical aspects, and treatment. Laboratory work concentrates mainly on diagnostic mycology. The general purpose of the intensive course is to provide the necessary expertise so that the student in the laboratory can identify fungi recovered from patients or from the environment. The graduate students who take these courses are usually medical doctors; only seldom are they from other fields such as biology, plant physiology, or genetics. Thus, even though the purpose of the course is to provide technology, in the long run we are preparing people who will not have the necessary tools to improve on this technology.

The pathogenic fungi hold little attraction for the biologist: in the first place he stands the chance of becoming infected, and in the second place he believes that other fungi are better models to work with experimentally in order to explain basic metabolic or genetic functions. The first reservation could be dispelled by showing the low infectivity of these fungi in the laboratory when they are properly handled. In regard to the second point, it is difficult to know how well the pathogenic fungi serve as models since they have seldom been tried.

In addition to improving and expanding the courses in medical mycology, we need to foster the establishment of laboratories devoted to training for basic research on the pathogenic fungi and to try to interest molecular biologists, plant physiologists, geneticists, electron microscopists, and other specialists in working on these fungi. We must also provide equal opportunities to the nonphysician researcher in medical institutions, which are the only places where there is sufficient interest in medical mycology. Finally, and most important, we need to encourage a positive attitude toward research in medical mycology—a *sine qua non* for progress in this field.

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PROFICIENCY TESTING IN MYCOLOGY

David Kirsh

As an extension of various training and refresher courses, the U.S. National Communicable Disease Center (NCDC) carried on a program over the period 1963 through 1968 involving the submission of unknown samples to state laboratories for testing. The purpose of this activity, called the Evaluation Service, was to assess the effectiveness of NCDC training courses and at the same time to reveal any deficiencies or problem areas that should be corrected.

During the six years that the Evaluation Service was in effect, an average of 42 state and local public health laboratories participated. Shipments consisting of five cultures were submitted by NCDC twice a year. The laboratories' average responses to each shipment ranged from 65 to 90 per cent correct. The majority of the problems involved the identification of yeasts and dermatophytes—the groups of organisms most frequently encountered in clinical mycological material submitted to public health laboratories. Other problems were with the saprophytes, or laboratory contaminants, which are commonly isolated and are a source of confusion to many laboratory workers.

A possible explanation for the difficulty in identification of dermatophytes may be that most of those fungi are usually classified on the basis of subjective or microscopic morphology rather than by objective methods—as, for example, biochemical reactions in the case in bacteriology.

In the U.S. Public Health system, the State Health Laboratories serve as a reference resource for laboratories in a particular geographic or governmental region, and the cultures or specimens that are difficult to identify or are beyond the capabilities of smaller laboratories are sent to them. They, in turn, frequently refer cultures that are unusual or difficult to identify to the NCDC for confirmation or identification. NCDC functioned mainly in a secondary reference resource capacity until three years ago, and it therefore had little definite information available on the competency required or the type of work performed in clinical diagnostic laboratories.

This situation was changed by the Clinical Laboratories Improvement Act of 1967 (CLIA), which provided a means of assessing the over-all performance of clinical diagnostic laboratories. The CLIA specifies that all licensed laboratories must participate in a proficiency testing program. The Act applies to any laboratory that solicits or accepts specimens in interstate commerce for the purpose of providing information for the diagnosis or treatment of any disease or the assessment of the health of man. A laboratory may be licensed in any or all areas of microbiology as well as in serology, chemistry, hematology, immunohematology, cytology, pathology, and radiobioassay. It should be stressed that it is not the purpose of the CLIA to "police" laboratories. Rather, the objective is to improve their performance so that ultimately the usefulness of patient data may be increased. At present, 146 laboratories, including licensed laboratories and voluntary agencies such as state and territorial public health laboratories and those of other federal agencies, participate in this program.

Each quarter, NCDC sends five pure cultures

to the laboratories in question. The cultures sent during the past year were considered to be representative of the pathogenic and saprophytic fungi frequently encountered in clinical material. Before the samples are distributed, they are sent to three independent laboratories, which characterize and identify them, or, in the terms of the clinical chemist, "determine the target values." If the findings of the three reference laboratories are in agreement, the samples are then submitted to the participants and to ten referee laboratories. The referee laboratories represent a peer group. All responses, whether from a licensed or a voluntary participant, are graded against the reports of the reference laboratories. A grade of satisfactory performance for a participating laboratory is defined at present as no lower than 10 points below the lowest score of a referee laboratory. The grades of 10 per cent of the referee laboratories may be excluded, however, if the results are obviously deviant.

The results from all the reference and referee laboratories are compiled and then analyzed. In this way the most frequently occurring errors are determined and attempts can be made to define the source of the problem. An individual summary analysis prepared by NCDC for each participating laboratory reviews the specific errors, emphasizes procedures and methods that could have yielded better or more meaningful results, and discusses quality control. This last element is an important part of laboratory improvement, and it is given even more stress than the findings themselves. Good quality control means taking into account all those procedures or techniques that are necessary for accuracy. It means carefully controlling incubator or water bath temperatures; properly preparing reagents, media, and other materials; and thoroughly checking the reagents or media with known cultures so as to make sure they will yield the target values. These points may seem elementary, but the results of NCDC's proficiency testing series indicate that they are often overlooked and that lack of attention to one or more of these factors is usually the cause for failure to correctly identify the organisms submitted.

For purposes of handling, the agents are divided into three classes. Those in Class I are of minimal hazard at most under ordinary conditions and do not require any particular competence in handling or special containment facilities. Those in Class II are of ordinary potential hazard; they include agents that may produce disease of varying degrees of severity through accidental inoculation or injection or other means of cutaneous penetration but can be controlled by ordinary techniques. Classes I and II include all fungal agents except *Coccidioides immitis* and *Histoplasma capsulatum*. These last two organisms involve significant hazards and require special conditions for containment. They are regarded as Class III agents. During the past year, agents in Classes I and II were sent out.

One deficiency in the program is that pure cultures rather than clinical or "simulated clinical" material are used. Pure cultures test only the ability to identify an organism and do not test the methodology for isolation of a particular agent from clinical sources. Some laboratories often identify a sample incorrectly because they have taken short cuts which, however useful for pure cultures, are inappropriate for routine diagnostic material. For example, *Sporotrichum schenckii* was once submitted in the yeast-like form. Because of the temperature variation during shipping, the organism reverted to the mycelial form. Many laboratories incorrectly identified the specimen as "yeast cells contaminated with a mold." Obviously, even with the clinical history that was provided indicating a systemic fungus infection, these laboratories relied only on a direct microscopic examination and did not subculture the fungus. NCDC is now trying to prepare simulated specimens and hopes to have them ready in a few months.

Many laboratories find it difficult to assemble a representative stock culture collection unless they purchase it or obtain it as a donation. The cost of purchasing a representative stock culture

collection can be prohibitive, and donations frequently create problems because the cultures may not be truly representative. The participants are encouraged, therefore, to retain a subculture of the sample or isolate until a copy of the summary analysis, showing the correct identification of the isolate, is received. In this way, they can accumulate a stock culture of fungi with known characteristics.

In summary, the objective of proficiency testing is laboratory improvement. Hopefully, viewed in this perspective, the NCDC effort will materially contribute to better patient care in the total health program.

DISCUSSION

Chairman Negroni: We can start the discussion with questions on the paper presented by Dr. Kirsh.

Dr. Borelli: I should like to ask Dr. Kirsh which species, in order of frequency, have been sent out to laboratories.

Dr. Kirsh: Dr. Haley, who is the resource person on what organisms are submitted can probably answer that question better than I can.

Dr. Haley: Right now, in the Candida group, Dr. Kirsh's team is sending out *C. albicans, C. tropicalis,* and *C. parapsilosis.* In the Cryptococcus group he sends out *C. neoformans* and *C. albidus.* In the dermatophytes, any and all. In the contaminants, many representing those most frequently isolated from clinical materials, particularly from the upper respiratory tract and skin lesions.

Dr. Restrepo: I would like to ask Dr. Kirsh whether this type of service is available to foreign laboratories, or whether it operates only within the United States.

Dr. Kirsh: It is only operating in the United States at the present time. We have had some requests from foreign laboratories to participate in our program. Chemical samples can be received from abroad without difficulty, but there are customs problems when it comes to transporting infectious agents across international boundaries.

Dr. Ajello: I would like to ask Dr. Martins da Silva whether PAHO has a mechanism through which potentially infectious agents can be sent across international boundaries without running into customs problems.

Dr. Martins da Silva: PAHO has agreements with all its member countries which in practice provide free customs clearance for equipment and supplies used in connection with official programs or projects. In cases involving the shipment of infectious materials, some countries require special import permits, which are normally issued by their quarantine departments.

Under these conditions, the answer is yes.

Dr. Kirsh: We would be happy to cooperate in this project if at all possible. One of the difficulties is the delay in the mails—I am not specifying whether this is the United States mail or the mail of other countries. The cultures may be late being submitted, and there may be a delay in receipt of the reports. As a general rule, we allow the participants five weeks' time from receipt of the cultures to submission of their reports. After that time they are classified as "no participation." Although people from abroad would be voluntary participants and would not be graded, they could still get copies of the summary analysis for the final answer and any pertinent tricks of the trade that might be applicable.

Dr. Mayorga: To my knowledge, in the Americas—and please correct me if this is not true—only the Mexican, Costa Rican, and Guatemalan universities teach complete one-semester courses in medical mycology at the undergraduate level. Our students are microbiologists, rather than medical students, and they naturally have a good background in general microbiology, biochemistry, histopathology, and immunology. I wonder if there are any other American countries that have had a similar experience in medical mycology.

Dr. Montero-Gei: I would like to make it clear that in Costa Rica the training is given at the rate of nine hours a week for one semester.

Dr. Borelli: We must emphasize the basic need for A-1 mycologists—physician mycologists, mycoimmunologists, and medical mycologists—with thorough preparation. The continued orientation of physicians and mycologists will depend on the availability of guidance from these experienced persons. The main usefulness of short courses will be to attract and single out candidates for more thorough training. The formation of a first-rate mycologist calls for a minimum of three years devoted exclusively to study in this field in close cooperation with a well-trained teacher.

Dr. MacKenzie: I would like to ask Professor Negroni for his views on the teaching of medical mycology to groups other than those he already specified. What about the recent medical graduate? What about the medical student? What about undergraduates?

Chairman Negroni: In my country, the third-year medical students have 8 lectures and 15 hours of laboratory work devoted to mycology as part of their work in microbiology.

Dr. Mackinnon: In Uruguay there is not enough demand to justify offering a special course in mycology, so we leave the laboratory open for consultations. Many people come to us, most of them microbiologists working in other areas. We train them in various practical situations that may arise, and we give them a few brief lectures on basic mycology. The guidance we offer varies depending on the circumstances. Uruguay is a small country with only one large city, and we have to adjust to the situation.

Dr. Pollak: In Venezuela we have had experience with two kinds of physicians: chest physicians and pathologists. We do not have special courses for either of these groups, but training is given within the over-all curriculum of each of the two specializations. Lectures are given on the theory of such subjects as the deep mycoses, and we have a chest clinic for non-tubercular pulmonary diseases. Every physician pursuing the postgraduate course on chest diseases receives practical training at our clinic. Each trainee or candidate also spends a month in our mycology laboratory, where he practices mycological diagnosis. The training is similar for pathology students. During the graduate course in morbid anatomy, they are given theoretical lectures, after which they are provided practical training with autopsies and histopathology. Finally, they spend some time in the microbiology laboratory, where they practice the identification of fungi, particularly those of the deep mycoses.

What we do not have, and urgently need, is a regular training course for mycologists.

Dr. Huppert: I have a question for Dr. Haley. How do you train the technicians to handle cultures of *Coccidioides immitis*? Are they included in your kit?

Dr. Haley: We do include *C. immitis* in the kit, but we formalinize it before it goes out. However, the students at NCDC work with it in Petri dishes, because we give very careful training in plate reading and the handling of primary isolates.

Dr. Huppert: Are the students skin tested with coccidioidin before and after the course?

Dr. Haley: They are not. Personally, I have been doing this for many years, and I am happy to say I still have a negative skin test.

Dr. Mayorga: A few years ago I was invited to go to Paris to give a lecture on coccidioidomycosis. I took two cultures with me, one of them a primary culture from a dog that had died of coccidioidomycosis, which still had a cotton swab on it.

In order to be safe, I put the tube in one of those flasks I use for CO_2 cultures, and I added some formalin in the bottom. I then put it in the incubator at 37° for approximately ten days. After ten days I looked at it and decided to leave it there for five or six days more. After that, I figured it had to be dead.

I put the two tubes in my pocket and went to Paris. In Paris, Dr. Mariat said, "This is a very dangerous fungus, so we had better be sure." He himself then took a small amount of the colony and put it on Sabouraud's medium. It grew very nicely.

Dr. Haley: We have several reasons for teaching the Petri dish technique. One is that in our part of the country we see a good deal of coccidioidomycosis in the chronic cavitary form, and in many instances these cultures of *Coccidioides immitis* are picked up in the bacteriology lab. This organism usually grows very nicely at 37°, and I therefore feel it is essential to teach bacteriologists as well as mycologists how to handle such cultures when they come up unexpectedly. We do kill ours. We use a cotton plug, and we put 0.1 cc of concentrated for-

malin into the tube, down the side. Then we immediately put in a rubber stopper, and in two weeks we study the culture.

Dr. MacKenzie: I would like to say to Dr. Haley that I think this is a most imaginative teaching device. How many of these kits are in existence now, and how many are planned?

Dr. Haley: Right now they are still on field trial, and we have three kits out. The field trials will be over by the middle of June, and the response we have had will determine how many kits are prepared.

Mr. Krickel, the coauthor of the kit, tells me that if there is really a demand for it there will probably not be too much difficulty in setting up an adequate number. Obviously, if a school wanted 65, this would be impractical for us to handle. We would send several kits, and the school would be free to copy the tapes for wider use.

Dr. Huppert: I do not want to belabor the point, but I am still concerned about the *Coccidioides immitis.* Some time ago we were involved in a case of disseminated coccidioidomycosis at the Riverside County Hospital. A young girl, who had draining sinuses in the leg, had been diagnosed as having osteomyelitis, and the leg had been put in traction with a window cut in the cast. As it turned out, the draining material contained viable *Coccidioides immitis.* It grew on her stockings and on the inside of the cast just as though it were in an open culture. Every time they opened the window to change the dressing, they were spreading *C. immitis* spores into the environment. There were six diagnosed cases of coccidioidomycosis deriving from this particular case. In addition, we skin-tested the entire hospital staff and found among presumably nonexposed personnel an incidence of about 5 per cent positive reactors to coccidioidin. Among individuals who worked on the pediatric ward but who had no direct contact with the patient the incidence was about 20 per cent.

The other case I have in mind is the one I mentioned earlier from the University of Pittsburgh, who was not diagnosed until autopsy. The laboratory used Petri dish cultures. One technician who did the culture work now has a severe, primary case of coccidioidomycosis.

In short, you can get away with culturing *C. immitis* in Petri dishes for a while, but if you run into one case that could have been avoided, then there can be no justification for using them. I think the point can be made just as well by warning students that they may encounter these things on Petri dishes and teaching them what precautions they should take. As far as the audio-tutorial kits are concerned, I would seriously object to putting the material out in Petri dishes.

Dr. Haley: Let me make it clear that under no conditions are we sending out viable *C. immitis* in the kits. I was referring only to the NCDC courses.

Dr. Huppert: Are these cultures tested to see if they are dead before they go out?

Dr. Haley: They are.

Dr. Furcolow: I think we are being unduly cautious. The only sensible way to do cultures is in the Petri dish. The problem is grossly exaggerated. The history of disseminated disease with coccidioidomycosis is no worse than with histoplasmosis.

Dr. Shadomy: I would like to add my comments to those of Dr. Huppert. We have a situation that is not unique in the southeastern part of this country, in that we have have several Negroes among our technicians and housekeeping staff. Thus we have a potential problem in our laboratory with organisms such as *C. immitis,* which have been shown to have a definite racial predilection in terms of virulence. And we do not want to take the chance of being responsible for accidental infections in these lay or nonprofessional people.

All our cultures that are possibly pathogenic, and these include *H. capsulatum* and *C. immitis,* are kept in screw-cap bottles. I believe in this firmly, and I agree with Dr. Huppert. There is no justification for taking risks in the laboratory, particularly with nontrained personnel.

Accidents can and do happen, and I do not want to be responsible for a person's death.

Dr. Levine: There are at least two people sitting around this table who have gotten laboratory infections and will attest that coccidioidomycosis is not a pleasant disease.

Moreover, I have done the following experiment in a completely sealed hood. I had a plate with *C. immitis* that had sporulated, and around this plate I placed four Petri dishes without lids. Then, very carefully, I removed the lid of the plate containing the *C. immitis* culture, as slowly as I could—a millimeter per second, as it were—and then replaced it. *C. immitis* grew on two of the other dishes.

I believe that if a culture of *C. immitis* has sporulated on a plate, it is quite impossible to uncover that plate without generating an aerosol. At the very least, one would be exposed to a potentially dangerous aerosol.

Dr. Huppert: There is one more point, Dr. Furcolow. The student or technician working with the Petri dish is in danger of inhaling massive doses of spores, and this can precipitate a very serious infection. It is not as though the person were being infected from a natural exposure in the environment.

Dr. Haley: Although I do not wish to prolong this debate, to defend NCDC I want to say that obviously we do not let the cultures in the Petri dishes go to sporulation. We are teaching the students what *C. immitis* looks like when it begins to come up. We plate-read every morning before the students come in, and on weekends as well. As soon as the culture begins to come up, the plates are removed, and the students are then given a formalinized test tube culture.

I might say to Dr. Levine that if you do the same thing with *Cryptococcus neoformans* you will be startled to see how fast this organism disseminates from Petri dishes, too.

Dr. Negroni: In my course I do not use this dangerous culture for teaching purposes. I only use a *Myxotrichum sp.* culture, which in its mycelial form provides spores that look like those of *C. immitis*.

Mr. Taplin: Many of you know that Gerbert Rebell in Miami wrote a manual on the identification of dermatophytes. The first edition is now completely out of print, and we have a backlog of something like 800 requests, many of them from Latin America. It appears to have found a place in the teaching of mycology as far as the dermatophytes are concerned. He is now rewriting this manual, and it should be out within two months. It will be larger, with more pictures, and we think it will be a workable key for the identification of dermatophytes.

Dr. Pappagianis: The question of the instruction of medical students was raised a little earlier, and I really think this matter deserves a great deal of attention.

The persons who will be in contact with mycotic infections at first hand are going to be the new physicians produced by the medical schools. While *we* have been infatuated with the fungi, I think it is very difficult to convey this sense of enthusiasm to medical students, especially if one attempts to teach medical mycology as a solid block of what to them seems like a very dry subject.

We have attempted to avoid some of this in our new medical curriculum by teaching medical mycology, as well as other medical microbiologic topics, in the context of other subjects. During the first and second years, we devote attention principally to the various organ systems, with the emphasis during the first year on the normal and during the second year on the abnormal. When we reach the integumentary system, for example, we have an opportunity to teach about candidiasis and the superficial fungus infections as problems related to the skin, rather than as problems related to fungi. Study of the central nervous system permits us to deal with cryptococcosis and several other mycotic infections involving the CNS. Introduction of the hematopoietic-reticuloendothelial system allows us to discuss intracellular parasites and histoplasma. Study of the respiratory system

gives us an opportunity to repeat some of the instruction on histoplasmosis and to devote primary attention to coccidioidomycosis and to the opportunistic fungi. And so on. I think this approach generates a great deal more interest and makes the students more receptive to problems concerned with medical mycology.

Dr. Schmitt: There are several points from Dr. Carbonell's paper to which I would like to speak.

First, about ten years ago I attempted to identify institutions in which a student could earn a Master's or Ph.D. degree, or both, with research in medical mycology. Such programs were reported in about 30 states. The majority were encompassed in microbiology or bacteriology departments: only three were in botany departments. It is perhaps time to conduct another survey and ascertain what the present-day opportunities are to specialize in medical mycology.

Second, I must take exception to the statement on the paucity of biologists taking medical mycology courses. Over the past 15 years, enrollment in my course at the Ohio State University has gone from 5 microbiology graduate students in the first class to 69 students in the present quarter, including 52 microbiology seniors or graduate students, 4 premedical technology girls, 4 non-M.D. clinical pathology graduate students, 4 mycology graduate students, 2 zoological parasitology majors, 1 resident dermatologist, and 2 poultry science graduate students interested in mycotoxins. Among the present class members, 5 have already been accepted as 1970–71 freshman medical students, and about 20 more have applications still pending. Nevertheless, medical doctors do not generally constitute too large a proportion of the enrollment. I believe these statistics substantiate the fact that, at least at Ohio State, the interest in medical mycology is stronger outside than inside the medical school. I might add that Dr. Everett Beneke at Michigan State quite consistently has enrollments in excess of 100 in his medical mycology course, and Michigan State is just starting its medical school.

Third, among my 12 medical mycology graduate students, 3 were undergraduate microbiology majors, 7 were botany majors, and 1 each were majors in biology and zoology, respectively. Among the 5 doctoral students, 2 received the M.Sc. in microbiology, 2 in clinical pathology, and 1 in medical mycology. Five are doing ecological projects in an effort to fill in the gap in field data on human-pathogenic fungi in Ohio environments. Quite incidentally, a doctoral student has recovered several such organisms from central Ohio streams and finds that the chlorination treatments used in water purification and in the handling of sewerage are inadequate *in vitro* to inactivate these pathogens.

For years I have held the position that what a person calls himself may not really be a reflection of his training. I think this would be abundantly evident if we were to make a survey in this room today, where we all consider ourselves medical mycologists.

Dr. Cozad: I quite agree with most of the comments of Dr. Carbonell. We have a rather unique situation in medical mycology, in that almost every finding from basic research can be applied immediately. We constantly see this in the work we do on the pathogenesis of certain systemic mycotic infections. Our findings on the basic immune mechanisms, or actions of these diseases on the basic immune mechanisms, helps us to better understand the progression of the disease, how it might be diagnosed, and how it might be controlled.

I think we have very much to offer the graduate student in terms of research pertaining to the mechanisms of mycotic infection. This is true for immunology and biochemistry and for certain other fundamental areas as well.

I also feel that more emphasis should be directed toward study of basic mechanisms of pathogenicity and other work at this level. It is really only through a better knowledge of these areas that we can put our best foot forward in terms of epidemiology, understanding

characteristics of the organisms, diagnostic procedures, and control of the diseases.

Dr. Furcolow: I think we are getting away from the main issue here. The problem is not that we do not have good places that teach mycology. The main question is how to get people to recognize that we have big health problems. Histoplasmosis, South American blastomycosis, and, to a much lesser extent, coccidioidomycosis are serious threats to public health, and people do not realize this. Money is not going to be given for research on specific projects when the grantor is not convinced that these are important problems. This may be one of the reasons why PAHO has been relatively inactive in the mycoses over the last eleven years.

Dr. Seabury: In connection with what Dr. Furcolow has just said, I approached PAHO some ten years ago about doing studies on histoplasmosis in Central and South America and was told, quite legitimately I think, that they had to deal with problems in the order of their priority. So long as nutrition and tuberculosis were tremendous problems in Latin America, the funds available would have to be assigned to these and to other areas of major public health significance. After these problems were brought under control, they would be able to look further afield. This may not be what you want, or what I want, but economically, and I think humanely, it does make good sense.

Dr. Carbonell: I am from a country in which we need to do much more about public health problems. You say that ten years ago the main problems were A, B, and C. After ten years, we are still pointing to the same kinds of problems in my country.

The main thing is to have the right mental outlook. I do not think we should ask for large amounts of money to do research in pathogenic fungi while critical public health conditions go unattended. However, if we can have this money and the other health problems could be solved at the same time, then that would be so much the better.

Dr. Pollak: As to the statement by Dr. Seabury, it is true that tuberculosis has been a severe problem in the Latin American countries, and in many it still constitutes a serious threat. I wish to recall, however, that at a public health conference in Caracas in 1956 Dr. Baldó presented a paper on proposed new areas of public health activity. In other words, already in 1956 the need for making advances in other areas was pointed out, and among the fields mentioned was that of mycoses. Since 1956 we have progressed quite a bit in tuberculosis. Currently almost all the countries provide ambulatory treatment facilities, so that this problem has been greatly reduced, and we are now at a stage where we have to think of expanding into other areas of activity, such as the mycoses.

Dr. Furcolow: The fact that PAHO is having a conference right now shows that progress has been made.

We have to get back to the main point, however, and think about ways to impress on people that this is a real problem.

Dr. Schmitt: Perhaps one thing we could do—particularly those of us who are in a position to reach second-year medical students and other embryonic physicians—is to create a much higher index of suspicion with regard to the fungi.

Dr. Carbonell: I think it is interesting, and important, to know all the places where facilities exist for the training of medical mycologists. For instance, I did not know about the program at Ohio State University. It seems to be good. This information should be available to us so that, when the opportunity comes, we know where to send people for training.

Dr. Larsh: The University of Oklahoma's program in medical mycology has been in existence since 1946. It was instituted after I spent the summer with Professor Norman Conant at Duke University retooling myself in medical mycology. We have an enrollment of 15 to 23 graduate students each year, over half of whom are candidates for the Ph.D. degree. Primarily, the students are trained in medical microbiology,

with a year's course in medical mycology and a minimum of 30 hours of research in this specialization. We would be happy to assist the programs in Latin America in any way.

Dr. Furcolow: I think what the South American countries need—and PAHO has the means to do it—is to concentrate on the training of ten people in their own institutions for every one they would send to the United States at a large cost. Once that was proved to be productive, the problems of money would be less serious and we could then talk about where the students should go for training.

Dr. Pollak: The teaching of mycology in the medical schools is very imporatnt, and much greater stress needs to be placed on this aspect. Students at that level should have an understanding of the importance of the mycotic diseases.

With regard to Dr. Furcolow's last point, I believe that in Latin America we already have several centers which are in a position to provide mycology courses, and I believe that it is preferable for Latin American students and graduates to receive their training in these places, because quite often the language problem is a very serious obstacle indeed. These centers could be selected by PAHO.

Session VI

Thursday, 26 February 1970, 1:30 p.m.

FUTURE DIRECTIONS

Chairman

Fred E. Tosh

Rapporteur

Milton Huppert

STANDARDIZATION OF IMMUNOLOGICAL REAGENTS

Milton Huppert

One of the major problems plaguing investigators concerned with immunological reactions in the mycoses has been, "How does one relate results reported from one laboratory to those of another?" This is particularly difficult, if not impossible, when methods and reagents differ and when there is no common basis to serve as a reference. One obvious solution would be to insist that all investigators use the same methods and reagents. This, however, would be stultifying and unacceptable, for it would restrict the evolution of new ideas, prevent the testing of hypotheses, and abort the progressive accumulation of information and knowledge. The problem to be solved, therefore, requires that freedom to conduct independent investigation be maintained while still providing a mechanism by which results from different laboratories can be interpreted and related. Logic would dictate that a reasonable resolution of this problem can be achieved by providing a standardized reference system as a common entity linking the work of independent investigators.

When considering immunological reactions, there are basically three factors involved: the host response to be measured, the reagent to be used for eliciting or measuring the response, and the procedure to be employed. The responses to be considered here are delayed type hypersensitivity and humoral antibody. The characteristics of these two responses have been defined by many investigators, and the procedures used in routine practice for demonstrating them in mycotic infections will be discussed by other participants in this Symposium.

The present paper will focus on a discussion of the reagents used for eliciting or measuring these responses and offer recommendations for an approach to standardizing reference systems within the general framework of immunological reactions in the mycoses. Attention will be concentrated primarily on problems arising from the complex composition of antigen preparations currently available, citing examples from coccidioidomycosis and histoplasmosis rather than attempting to cover the entire field of mycotic infections.

Dictionary definitions of the act of standardizing refer to ". . . conforming to, or comparing with, a standard . . . ," and a standard is defined as ". . . anything recognized as correct by common consent, by approved custom, or by those most competent to decide." There are two elements involved: the standard substances, and the method by which comparisons can be made. The ideal standard antigen would be a single, homogeneous, molecular species with full potential for producing the specific activity to be studied. A pure reagent of this type could be characterized by chemical, physical, and immunological properties, and its biological activity could be assayed reproducibly by a dose-response curve based on activity per unit of weight.

A separated fungal antigen fulfilling these criteria has not yet been achieved, but recent reports indicate that the goal is near. For example, Sprouse (*28*) has separated two electrophoretically pure protein fractions from histoplasmins. One of these elicited delayed type sensitivity reactions in guinea pigs infected

either with *Histoplasma capsulatum* or with *Blastomyces dermatitidis*. The second fraction was positive only in animals with histoplasmosis, and assay of the dermal induration produced by increasing amounts of the protein yielded a reasonably smooth dose-response curve. Similarly, in earlier work with serologically active fractions from *H. capsulatum*, Salvin and Smith (*21*) isolated a glycopeptide substance that was homogeneous by ultracentrifugation and by immunochemical analysis, but not by moving boundary electrophoresis.

Since antigens fulfilling the criteria for a pure substance are not yet available, any currently proposed standardization system would have to use a mixture of molecular entities with a potential for producing multiple antigen-antibody reactions. Such mixtures may contain only soluble substances or both soluble and particulate materials.

While physicochemical analyses are useful and necessary to determine whether or not an isolated substance is homogeneous, these procedures are of relatively little value for standardizing a new preparation that is known to contain more than one potentially reactive component. In fact, the incautious use of these analyses has led to imprudent conclusions in some cases. It is not uncommon to find statements in the literature that polysaccharide fractions from fungi elicit delayed type sensitivity reactions (*3, 6, 8, 14, 15, 17*). In each instance, however, the possible presence of small amounts of protein or polypeptides was not excluded. This is important, because more careful studies have shown that delayed sensitivity reactions can be evoked in humans with as little as 0.06 μg of a protein-arabinose fraction from histoplasmin (*29*), and that delayed hypersensitivity in guinea pigs can be induced and elicited with synthetic hapten-oligopeptides containing as few as seven amino acid residues (*22, 23*). Furthermore, the dissociation of reactive glycoprotein fractions derived from several fungi has resulted either in nonreactive material or in only the protein moiety retaining the capacity for evoking delayed sensitivity (*1, 5, 20, 21, 27, 29*). Several reviews have emphasized the consistent reports by many investigators that a protein or polypeptide moiety is required to induce or to elicit delayed sensitivity (*4, 7, 10*). Physicochemical values for a preparation containing multiple antigens can be very misleading, since the immunological system is exquisitely sensitive to even small determinant groups of single molecular species.

Similar arguments can be presented to document the limited value of physicochemical criteria for characterizing preparations for serologic tests if multiple antigens are present. The critical points here are that the size of an antigenic determinant can be as small as a four amino acid component, that the specificity of the antigen-antibody reaction can be altered by a change in only one of these amino acids, and that inhibition of the reaction can be achieved with a peptide dimer (reviewed in *24*). Interpreting a relationship between physicochemical criteria and a specific immunologic response is even more complicated for serologic reactions because circulating antibody is inducible by pure polysaccharides as well as by proteins.

Since the fungal preparations generally available at present are not pure single antigen solutions, and since physicochemical determinations have only limited value for standardizing these preparations, the only remaining reliable characteristic that can be employed is immunological activity. Standardization can be, and has been, accomplished by comparing the immunological activity of a new reagent with that of a reference preparation for which the specific reactivity has been established. The value and reliability of these reference systems depends in large part on their availability and on meticulous attention to the detailed procedures for performing the required tests. Some practical considerations will be discussed in the following examples illustrating standardization of reagents for skin testing and for serologic tests.

Skin test reagents

A good approach to the standardization of

a new lot of antigen for skin testing can be illustrated with data reported by Smith and his colleagues (*27*) for coccidioidin skin tests done on U.S. Army recruits newly arrived in the San Joaquin Valley of California. These investigators had found occasional positive, and many equivocal, reactions among personnel who never had been in a known endemic area, and, in addition, they had noted great variability with different lots of coccidioidin in the frequency of these presumably nonspecific reactions. Therefore, they deemed it necessary to standardize each new lot of antigen for potency, specificity, and sensitivity in comparison with their best previous lot of coccidioidin.

Potency determinations were done first on three or four human subjects with known sensitivity. After appropriate adjustment of the new lot to a potency level matching that of the reference coccidioidin, simultaneous intradermal tests were done on large numbers of recruits, and residence histories were obtained for the positive reactors. As shown in Table 1, the sensitivity of the new lot (i.e. its ability to detect minimal reactors) was equivalent to that of the reference coccidioidin, and the results in residents from the midwestern and southern regions of the country indicated that the specificity of the new coccidioidin was as good as, if not better than, the reference material.

Specificity determinations are important with both coccidioidin and histoplasmin because of the cross-reactions occurring in these two diseases. Evidence will be presented indicating that these cross-reactions may result either from infections by both fungi or from the sharing of common antigens by these fungi. The reports by Smith and his associates (*25*) are particularly useful for illustrating these points, since their results are based on the unique opportunity (1) to study individuals newly arrived in a coccidioidomycosis endemic area, and (2) to follow the changes occurring in personnel who became infected.

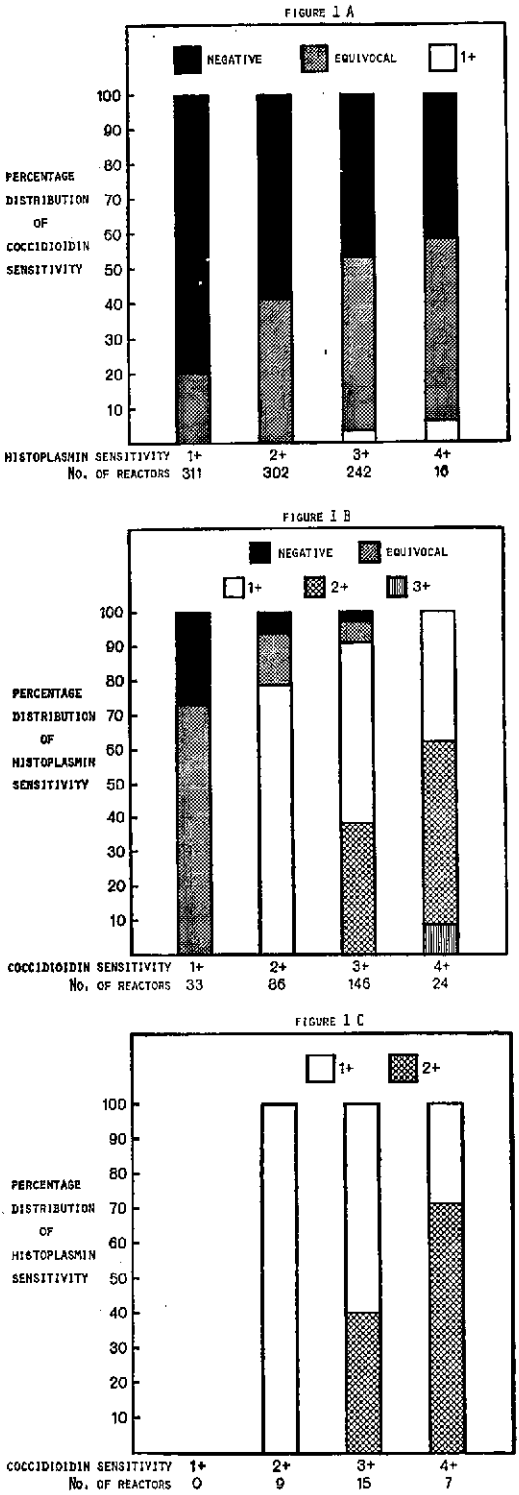
Figure 1 A shows that, among the recruits who were predominantly sensitive to histoplasmin at the time they arrived in the coccidioidomycosis endemic area, a large number exhibited negative or equivocal reactions (<5mm induration) to coccidioidin, but only a few (1 per cent) had definite positive reactions. None of the latter exceeded a 1+ degree of sensitivity, and all occurred in persons with the highest levels of sensitivity to histoplasmin. In contrast, as shown in Figure 1 B, a large number (78 per cent) of the individuals who were residents exclusively in the coccidioidomycosis endemic areas, or who were undergoing coccidioidal infections, exhibited definite positive reactions to histoplasmin of a degree only slightly less than those to coccidioidin. An indication that at least some of these cross-reactions were caused by antigens

Table 1

Comparison of a new lot (1:100) with a reference lot (1:100) of coccidioidin for skin test reactivity in Armed Forces recruits newly arrived in endemic area

Reaction	No. reacting to:		Total tested	Residence history
	Reference	New lot		
Negative	182	182	182	
Equivocal	17	13	18	Midwest and south
1+	7	7		
2+	8	9		Coccidioidomycosis
3+	8	7	24	Endemic area
4+	1	1		

Modified from SMITH, C. E., *et al., Amer Rev Tuberc,* 1948.



common to both fungi can be inferred from the results illustrated in Figure 1c. Among 31 individuals who were originally negative to both antigens but who subsequently were infected by *C. immitis,* all developed some degree of cross-sensitivity to histoplasmin along with significant sensitivity to coccidioidin. Therefore, one must have an evaluation of the specificity of the antigen solution to derive valid clinical interpretations.

Now one can consider the critical question, "How can one accomplish standardization of antigens for skin testing with acceptably accurate assays for potency, specificity, and sensitivity?" The first requirement is a reference preparation that has been characterized for the specific immunological activity to be studied. Apparently, a reasonably accurate assay for potency can be obtained by titrating the new preparation in comparison with the reference reagent on at least four human subjects with proven sensitivity, provided the range of dilutions yields a spectrum of dermal reactions from negative to those exceeding in degree that which is obtained with the reference reagent. Specificity can be determined by simultaneous tests on humans, but this would require larger groups with determinable and restrictive residence histories. Even under the best conditions, such as those available to Smith's group, the results would be subject to the reservation that cross-sensitization to as yet unknown microorganisms might exist, as indicated in the excellent analysis by Palmer, Edwards, and Allfather (*19*).

A valid experimental animal model would be most desirable, since it would provide controlled groups with known specific sensitization and would offer the flexibility of adding new groups as our knowledge of the spectrum of cross-sensitizations expands. At the present time, any experimental animal studies should precede, and

Figure 1. Dermal hypersensitivity cross-reactions with coccidioidin and histoplasmin. (A) Coccidioidin reactions among dominant reactors to histoplasmin. (B) Histoplasmin reactions among dominant reactors to coccidioidin. (C) Coccidioidin and histoplasmin reactions among patients with coccidioidomycosis who were initially negative to both antigens. Modified from Smith *et al.* (25).

must be confirmed by, tests in humans until it is established that a valid interpolation can be made from results in animals. The difficulty with experimental animal studies has been the variability existing even within inbred strains. Recent work with the Hartley strain of guinea pigs has shown that "responsiveness" to synthetic hapten-polypeptide antigens is controlled by a single dominant gene and that inheritance of this "responsiveness" follows Mendelian patterns (*2*). It should be possible, therefore, to breed these animals selectively to obtain a strain with minimal variability. Such a uniformly responsive strain of animals might serve as a valid experimental model for assaying the potency and specificity of skin-testing reagents, but sensitivity can be evaluated only by simultaneous tests with both a new preparation and the reference lot in humans with known degrees of sensitivity. In the future, one might hope that results with the *in vitro* correlates of delayed hypersensitivity (i.e. macrophage migration inhibition and lymphoblast formation) might parallel skin testing in humans and, if so, these *in vitro* procedures would provide simpler and more practical methods for standardizing such reagents.

Serological reagents

The requirements for standardization of serological reagents are, in general, similar to those for skin-testing materials, including a reference system and determinations of potency, specificity, and sensitivity. In contrast to testing for hypersensitivity, the methods available for serologic tests are more numerous, the procedures for performing the tests are more varied, and the reading of test results is more objective. The entire scope cannot be covered here, but two examples, complement fixation (CF) and Ouchterlony-type immunodiffusion (ID), can serve to illustrate some of the principles involved (*18*).

Practical serological testing for mycotic infections has made extensive use of the CF test. Unfortunately, the results obtained with this highly sensitive method are affected considerably by even slight changes in the procedures used (*26*). A Laboratory Branch Task Force of the National Communicable Disease Center has published the details and the laboratory evaluation of their proposed uniform procedure for CF, referred to as LBCF (*16*). Many laboratories participated in this program, in which results by the LBCF technique compared favorably with those obtained by several routine CF procedures for a variety of infectious diseases, including histoplasmosis. Furthermore, good agreement among results obtained by different laboratories with the LBCF procedure indicated that the technique was very reproducible. We have obtained equivalent results with the LBCF and older Smith CF procedures using sera from patients with coccidioidomycosis, and we endorse the recommendation that the LBCF be adopted as the standardized procedure for all CF testing.

Satisfactory results with the CF procedure require careful titration and standardization of all reagents employed. Most of the steps involved are common to CF tests for antibody to any infectious agent, but the elements related to a specified disease are the antigen and a known positive control antiserum for that disease. Since the fungal preparations used currently for serologic studies contain multiple antigens, standardization must be done by comparison with a reference system characterized for the specific immunological reaction to be used. It has been our practice to accomplish standardization of the antigen in three stages (*13*), as illustrated in Tables 2, 3, and 4.

The first stage involves preliminary potency determination for a new lot of antigen for CF testing and is made by titrating it with a specific antiserum of known titer (Table 2). This assay must be accompanied by a simultaneous test with a previously standardized reference antigen to verify that the titer of the specific antiserum has remained constant and that the same antiserum titer is obtained with the new lot of antigen. The results for the example in

Table 2

Preliminary titration of new lot of coccidioidin by LBCF Procedure [a]

Coccidioidin dilution	Serum dilutions [b]					
	1:2	1:4	1:8	1:16	1:32	1:64
1:20	0	5	30	100	100	100
1:40	0	0	20	100	100	100
1:80	0	0	25	100	100	100
1:160	0	0	0	10	100	100
1:320	0	0	0	10	70	100
1:640	0	10	20	40	50	100
1:1280	100	100	100	95	95	100
Reference coccidioidin	0	0	0	10	100	100

[a] Controls were satisfactory for the hemolytic system and for serum and antigen anticomplementary activity.

[b] Previous titer = 1:16. Results in % hemolysis with endpoint at 30% or less.

Table 2 indicate that the optimum titer for the new lot of antigen lies between 1:160 and 1:320.

In the second stage, the final determination of antigen titer is made (Table 3). The results for this example show that the optimum titer of the new coccidioidin is at a dilution of 1:300, according to the criteria for the LBCF protocol, even though endpoints can be read through the 1:500 dilution of antigen.

Table 3

Final titration of new lot of coccidioidin by LBCF Procedure [a]

Coccidioidin dilution	Serum dilutions [b]					
	1:2	1:4	1:8	1:16	1:32	1:64
1:100	0	0	25	100	100	100
1:200	0	0	0	10	100	100
1:300	0	0	0	5	50	100
1:400	0	0	0	30	60	100
1:500	0	0	10	30	60	100
1:600	0	0	20	40	90	100
Reference coccidioidin	0	0	0	10	100	100

[a] Controls were satisfactory for the hemolytic system and for serum and antigen anticomplementary activity.

[b] Previous titer = 1:16. Results in % hemolysis with endpoint at 30% or less.

The third stage is the determination of the sensitivity and reproducibility of the new preparation compared to the reference antigen (Table 4). In our experience, a minimum of five of the ten specimens employed must have low CF titers in order to ascertain that the antigen has adequate sensitivity. For reproducibility, a twofold dilution difference is considered within the limits of the experimental error inherent in the technique, and at least nine of the ten results must be within these limits. If they are not, the test is repeated with a second set of ten sera, with the requirement that results for at least 18 of the total of 20 specimens must be within the limits of a twofold dilution difference. If these conditions are not met, the new antigen preparation should be either assayed again for optimal titer or discarded. Determinations of specificity can be performed by a protocol similar to that for sensitivity and reproducibility, but using sera from individuals with known heterologous reactions.

The ID method has been chosen as the second example for standardization of serological reagents. This technique is particularly useful because it has the potential for separating and distinguishing particular antigen-antibody reactions from among a mixture of such systems. If any of these single antigen-antibody reactions can be correlated with specific infections, one has an easily performed and highly reproducible laboratory aid for diagnosing the disease. This

Table 4

Determination of sensitivity and reproducibility of new lot of coccidioidin by LBCF procedure

Low titer sera		High titer sera	
Reference antigen	New antigen	Reference antigen	New antigen
1:4	1:2	1:128	1:128
1:4	1:4	1:64	1:32
1:2	1:2	1:64	1:128
1:4	1:2	1:64	1:64
1:4	1:4	1:256	1:128

has been accomplished for coccidioidomycosis (*11*) and also, to a more limited degree, for histoplasmosis (*9*). In previous reports (*11, 12*) it has been demonstrated that one of the several lines of precipitate formed by coccidioidin with serum from a patient with coccidioidomycosis is correlated with a positive CF test for this disease. This particular antigen-antibody reaction in the ID test has been called the F line, just as two similar precipitation lines in histoplasmosis have been referred to as the M and H lines.

While the Ouchterlony type of ID test provides conditions for detecting an antigen-antibody precipitation reaction in which either one of the reagents may be in considerable excess, maximum effective use of this method is achieved only when the procedure is standardized carefully and appropriate reference systems are included.

The problems necessitating careful standardization and use of an appropriate ID reference system for coccidioidomycosis are presented in Figures 2 and 3. If the concentration of coccidioidin is too low, then a serum with a high level of antibody might be missed (Figure 2). Conversely, if the coccidioidin concentration is too high, it may be impossible to detect a specimen with a low antibody content (Figure 3).

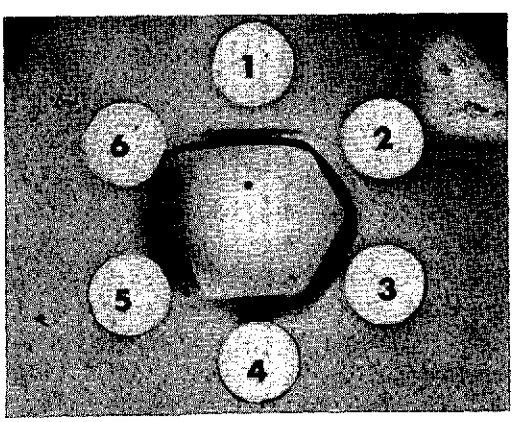


Figure 2. Effect of high concentration of antibody on titration of coccidioidin for use in ID test. Center well contains an antiserum with CF titer = 1:512. Peripheral wells contain coccidioidin dilutions as follows: well 1 = undiluted; well 2 = 1:4; well 3 = 1:8; well 4 = 1:12; well 5 = 1:16. Well 6 contains saline.

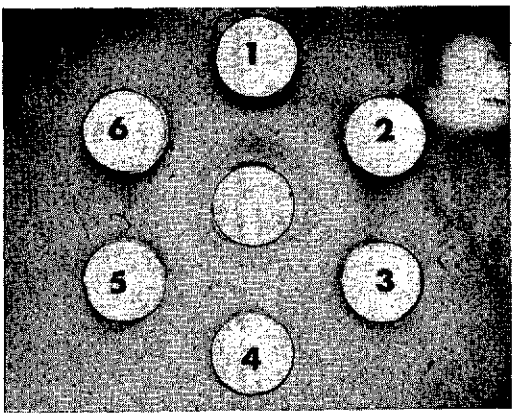


Figure 3. Effect of low concentration of antibody on titration of coccidioidin for use in ID test. Center well contains antiserum with CF titer 1:2. Contents of peripheral wells are the same as in Figure 2.

When the coccidioidin is titrated against a serum with a median level of antibody content, then an antigen dilution can be selected that will produce a reference system capable of detecting virtually all concentrations of antibody that might be encountered.

The results shown in Figure 4 indicate that a serum with a CF titer equal to 1:64 combined with this particular lot of coccidioidin at a dilution of 1:8 should produce a reference system with this capacity. The F line is about midway between the antigen and antiserum wells, leav-

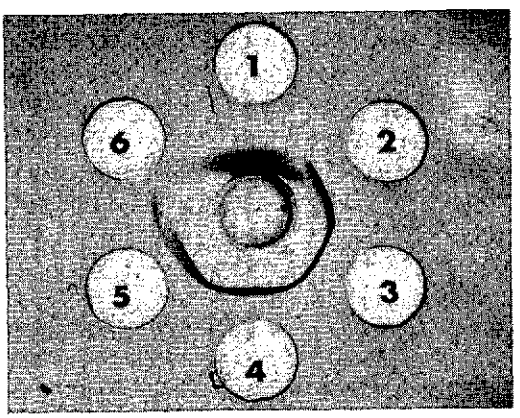


Figure 4. Effect of appropriate concentration of antibody on titration of coccidioidin for use in ID test. Center well contains antiserum with CF titer = 1:64. Contents of peripheral wells are the same as in Figure 2.

ing a relatively large area closer to the serum well for reacting with lower concentrations of antibody and a similar area closer to the antigen well for detecting reactions with higher concentrations of antibody.

The effectiveness of this approach is demonstrated in Figure 5. The seven-well ID pattern in routine use contains antigen in the center well and the reference antiserum in wells 1 and 4, with the remaining wells for the sera to be tested. In this pattern, each of the test sera can be compared with a known positive reference system for a reaction of identity, and it is obvious that the arrangement readily detects specimens with CF antibody titers ranging from 1:2 to 1:512 and higher.

The standardization procedures described above will characterize the potency and sensitivity of the reagents used in the ID method. The versatility and practical value of this test, as well as the specificity of the reagents employed, are demonstrated in Figures 6 and 7. In a square pattern with wells at the corners and with both the coccidioidomycosis and histoplasmosis antigen-antibody systems, respectively, in opposite wells (Figure 6), one finds that the

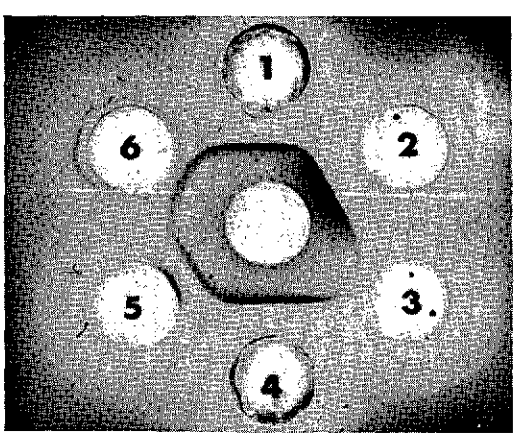


Figure 5. Standardized pattern for ID tests for coccidioidomycosis. Center well contains coccidioidin at 1:8 dilution. Wells 1 and 4 contain reference control positive antiserum (for F line) with CF titer = 1:64. Remaining peripheral wells contain sera to be tested for F line. The final quantitative CM titers for these four sera were as follows: well 2 = 1:512; well 3 = 1:2; well 5 = 1:32; well 6 = 1:4.

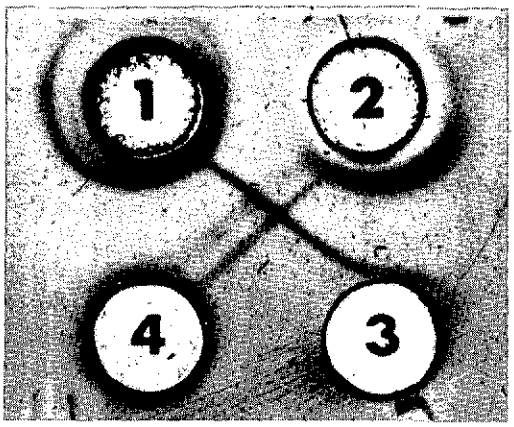


Figure 6. ID test demonstrating that coccidioidomycosis F line is nonidentical with histoplasmosis M and H lines. Contents of wells are as follows: well 1 = histoplasmosis antiserum; well 2 = coccidioidomycosis antiserum; well 3 = histoplasmin; well 4 = coccidioidin.

three principle reactions, F for coccidioidomycosis and M and H for histoplasmosis, are lines of nonidentity. Additional precipitation reactions can occur with each system, but these three principal lines can be identified readily when appropriately standardized reference reagents are included, as shown in Figure 7. The serum tested against the coccidioidomycosis reference system (wells 1 and 3) and the histoplasmosis reference system (wells 2 and 5) is from a patient with

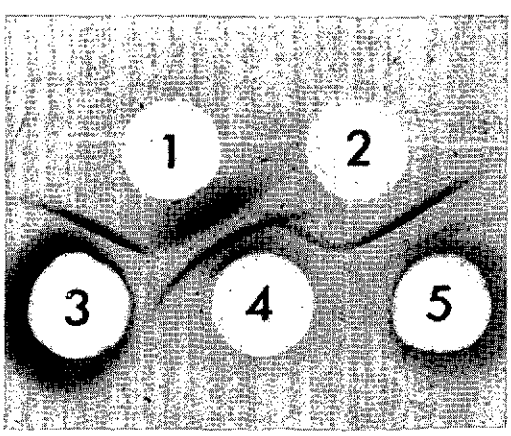


Figure 7. ID test comparing a cross-reacting serum (in CF tests) with standardized reference patterns for coccidioidomycosis and histoplasmosis. Contents of wells are as follows: well 1 = coccidioidin; well 2 = histoplasmin; well 3 = coccidioidomycosis antiserum; well 4 = tested serum; well 5 = histoplasmosis antiserum.

acute primary coccidioidomycosis. This serum had been positive in CF tests for both infections. Both the coccidioidomycosis F line and the histoplasmosis M line are present. The second precipitation line formed against the coccidioidomycosis reference system was identified as the antigen-antibody reaction which corresponds to the older tube precipitin test for coccidioidomycosis. It was apparent, therefore, that the patient's coccidioidal infection was superimposed upon earlier histoplasmosis. The diagnostic problem created by the cross-reactions with the CF tests was resolved by use of the unique characteristics of the ID method with the appropriate standardized reference systems.

Conclusion

In summary, we have presented the opinions of others as well as ourselves concerning the need for, and the satisfactory approach to, standardization of reagents for immunological tests in certain mycoses. Pure homogeneous antigens would be the ideal reagents, and current work indicates that these will be available soon. Meanwhile, valid interpretations and comparisons of results from different laboratories can be realized only by common use of appropriate reference reagents that have been standardized for potency, sensitivity, and specificity.

This requirement for available, immunologically characterized, reference reagents is being fulfilled. Histoplasmin and coccidioidin skin-testing antigens are available from the Division of Biologics Standards of the U.S. National Institutes of Health (Bethesda, Maryland 20014), along with instructions listing the minimum requirements for potency determination of new preparations in comparison with the reference antigens. In addition, a number of investigators are depositing characterized antigen preparations and antisera for serologic testing with the Scientific Resources Branch, Laboratory Branch, U.S. National Communicable Disease Center (Atlanta, Georgia 30333), with the Fungus Antigen Study Group of the American Thoracic Society designated as the advisory committee. These reagents are available to qualified investigators as well as to commercial manufacturers.

This presentation was opened with the definition of a standard as ". . . anything recognized as correct by common consent, by approved custom, or by those most competent to decide." It is submitted, in conclusion, that the individuals gathered at this Symposium are among the most competent to decide what should be the approved custom for establishing reference standards by common consent.

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SURVEILLANCE PROGRAMS FOR THE MYCOSES

Fred E. Tosh

The purpose of a surveillance program is to systematically collect information that can be used to assess a problem, help determine whether preventive and control measures are needed, or evaluate the effectiveness of existing control programs. At the present time in the United States, and probably in most other countries, effective surveillance systems for fungal diseases do not exist, and consequently there are no accurate statistics on their annual incidence.

This author believes there are several reasons why the mycoses have not received as much attention as some other diseases by official health agencies. In the first place, they are not considered a significant health problem because of the small number of cases that are recognized and brought to attention. Knowledge and awareness of fungal infections, as compared to diseases such as tuberculosis and syphilis, are relatively recent developments.

At present, requirements for the notification of mycotic infections in the United States are only spotty. Reporting of ringworm infections is required in 18 states, histoplasmosis in 15, actinomycosis in 14, coccidioidomycosis in 11, blastomycosis in 11, and cryptococcosis in four. However, if indeed the estimates are correct, and 200,000 cases of histoplasmosis and 33,000 cases of coccidioidomycosis do occur annually (*5*), then these illnesses certainly constitute a significant health problem in the United States. In addition, diseases caused by opportunistic fungi are becoming more prevalent as the population increases in age and individuals with chronic conditions survive longer because of improved therapeutic procedures. The 1968 *Cumulative Index Medicus* lists more than 20 articles describing *Aspergillus fumigatus* infections complicating conditions such as heart surgery, burns, neoplasms, lung cavities due to tuberculosis, and peritoneal dialysis in eclampsia. And the cases reported in medical journals probably represent only a small proportion of the total number that occur.

A second reason why fungal diseases may not have received too much attention as a public health problem is that they are commonly believed to be self-limiting, only rarely producing serious illness. While it is true that the illness associated with most primary infections lasts only a few days, the time lost from work and school is significant. Moreover, the disseminated, chronic pulmonary, and central nervous system forms result in considerable morbidity and mortality. The therapy available for these serious forms is far from ideal, often involving extended hospitalization and other expensive measures.

The present attitude toward fungal infections might be compared to the earlier approach to measles in this country. Until recently, measles was considered a childhood disease that was inevitable and not too serious. The development and use of a vaccine for measles stimulated workers in this field to calculate the savings resulting from immunization. They estimate that by averting 9.7 million cases of measles over the period 1963–1968 it has been possible to avoid 3,244 cases of mental retardation and to save 973 lives, 555,000 hospital days, 291,000 years of normal life, more than 1.6 million workdays,

32 million school days, and 423 million dollars (*1*). It is conceivable that mycotic diseases could be shown to be equally costly if accurate information on their annual incidence were available.

Also contributing to the lack of interest in fungal diseases is the common belief that the fungi are so widespread in nature that there is little hope of developing effective control methods. While this may be true for some of them, evidence is accumulating that preventive measures can be applied in histoplasmosis. Sites responsible for outbreaks of histoplasmosis in two cities have been successfully decontaminated and should pose no further threat to the communities in question (*6*). Data have been collected suggesting that starling-blackbird roosts harboring *Histoplasma capsulatum* are responsible for infections with this fungus even though the sites are not disturbed (*4*). This author believes decontamination of the point sources of infection could help considerably in reducing the incidence of endemic infections.

Still another reason for the lack of surveillance of fungal diseases is that official health agencies are obliged to concentrate their resources on diseases and public health problems that have greater implications for more people than the mycoses do.

Methods of surveillance

Case reporting

We already have the knowledge needed to develop effective surveillance for fungal diseases, since surveillance schemes for certain other diseases are well established. In such programs, physicians are required to periodically report the cases they see to their local health agency, which transmits data on the cases within its jurisdiction to the State Health Department. The latter, in turn, compiles the information received and sends it on to the U.S. Public Health Service.

Although reporting is incomplete for most notifiable diseases, baselines have been established that are useful in detecting unusual increases in prevalence and also in evaluating the extent of the problem. Physicians would probably be more conscientious about reporting if they felt that their efforts would stimulate action toward prevention and control of the disease.

Since only the more severe fungal infections are likely to be recognized and reported, these cases should be investigated. When four cases of histoplasmosis in a city came to the attention of the health officer, an inquiry was begun. The results revealed a total of 28 cases, two deaths, and thousands of infections (*2*). The investigation of one severe case often leads to the diagnosis of mild or subclinical forms of the disease in other members of the family and may pinpoint the source of infection.

Review of laboratory tests

The systematic review of laboratory diagnostic tests may provide data useful in surveillance programs. The number of tests performed for a particular disease may reflect awareness on the part of physicians, and the ratio of positive tests may be an indication of the prevalence of the disease. A sudden increase in the ratio of positives to the total number of tests performed may be the sign of an outbreak or an increase in prevalence.

Follow-up of patients having positive diagnostic tests may lead to increased reporting of cases. In the investigation of an outbreak of histoplasmosis, a letter was sent to physicians asking if a diagnosis of histoplasmosis had been made on their patients having a positive complement-fixation test on serum submitted to the State Hygienic Laboratory. This inquiry led to the reporting of 183 additonal cases of histoplasmosis, about two thirds of the total number recorded during the outbreak (*3*).

Figures on fungal serology testing by State and Territorial Public Health Laboratories in the United States during the fiscal year 1967 revealed that more than 33,000 specimens were tested. Although there were 1,262 specimens positive for coccidioidomycosis, only 859 cases of this disease were officially reported during the calendar year 1967. Likewise, there were 5,456 specimens positive to the yeast antigen of *Histoplasma capsulatum* during fiscal year 1967, but only 206 cases of histoplasmosis reported in cal-

endar year 1967 (5). Thus, for the latter disease there were 25 times as many positive serologic tests as reported cases. This figure, of course, does not allow for the possibility that more than one specimen may have been from the same individual. An additional factor contributing perhaps to the wide discrepancy is that histoplasmosis is not a reportable disease in some of the states where the serologic tests were performed.

Surveys

Under special circumstances, periodic surveys may be used to provide surveillance data. For example, since the decontamination of a site responsible for two outbreaks of histoplasmosis, we have been performing histoplasmin skin tests on the first-grade schoolchildren in the community in question. The prevalence of infection has declined from 33 per cent in 1965 to 12 per cent in 1969, and it is expected to decrease to less than 3 per cent this year. Had the gradual decline not occurred, we would have been concerned about the effectiveness of the control procedure, or we would have considered the possibility of another source of infection in the community.

Establishing surveillance programs

The reports presented in earlier sessions of this Symposium have stressed the magnitude of the problem of fungal diseases and leave little doubt that surveillance programs are needed. The first step in establishing such programs is to get the cases reported to official health agencies. How this can be accomplished may vary from one country to another. In the United States, each individual state must take the step to include mycoses in the list of reportable diseases.

This may meet with opposition, since an attempt is made to keep the list to a minimum. It is doubtful that any state would be willing to list each of the fungal diseases that those of us in this specialized field think should be included. As an alternative, the states might be encouraged to incorporate a general heading such as "Fungal diseases—specify." This would not limit the diseases that the physician should report, and it might reveal that certain fungal infections are more common than is now realized.

Until official surveillance programs are established, we should make sure that the cases of fungal diseases we see are reported, and we should encourage other physicians to report the cases that they see. If those of us present at this Symposium did nothing more than that, the number of cases reported to health agencies would probably more than double. In April 1969 the U.S. National Communicable Disease Center, Atlanta, published its first *Mycoses Surveillance Report.* The primary purpose of this publication is to encourage the development of a more adequate system of national surveillance of fungal diseases. Another aim is to stimulate more active reporting of cases and outbreaks.

We must continue to collect data on the public health significance of fungal diseases. Until better reporting is accomplished, information on morbidity and mortality must be collected by whatever means possible. Such data are necessary to focus attention on the fungal diseases.

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SURVEY PROGRAMS IN THE MEDICAL MYCOSES: FUTURE DIRECTIONS

Phyllis Q. Edwards

During the past two decades dramatic progress has been made in the control of bacterial, viral, and parasitic diseases, including many of the great killer diseases of the past. With this decline has come an awareness that the mycotic infections, long considered inconsequential, are of increasing public health significance. This has been clearly brought out by the papers presented during the course of the present Symposium.

Considerable information on the mycoses has been accumulated, but much still remains to be acquired, particularly in regard to the systemic mycoses. What we have yet to learn may far outweigh what we already know. To tolerate such gaps in the existing body of knowledge on any disease can be hazardous, regardless of how mild or innocuous its course in the human host appears to be.

Survey programs can be an effective and economical means of gathering certain kinds of information about mycotic infection and disease in human populations. But surveys should be undertaken only when there is a definite well-defined reason for them. In this respect they may be likened to the physician's examination of a patient, which is undertaken in response to a specific need. For example, the person may consult the physician because he is ill, or he may come seeking assurance that no insidious or occult physical condition threatens his well-being. By analogy, the survey may be regarded as a "physical examination" of the community, a means of getting the information needed to diagnose and treat a public health problem.

In the case of the systemic mycoses, the stimulus for a survey may be the recognition of a case or cases of a disease seldom or never before seen in the area. It may be serologic or other laboratory or clinical evidence of the disease, as when tests are made on a group of individuals from a particular area; or it may be the result of an epidemiologic inquiry or some other condition or event that directs attention to a need for additional information.

Assuming that such a stimulus has motivated the investigator to undertake a survey, we should then ask what *specifically* is wanted to be known, and how one goes about finding it out. Essentially, there are four steps or stages in a survey. They are as follows:

Step 1: Definition of the specific purpose—that is, a decision as to what particular question is to be answered and what information will be needed in order to do so.

Step 2: Selection of the test or method that will yield this information.

Step 3: Choice of a suitable survey population and application of the chosen test or measure.

Step 4: Interpretation of the results.

Purpose

In the systemic mycoses, the purpose of a survey, first of all, may be to look for cases of a particular disease. Something—some incident or event, as mentioned earlier—may have suggested

that the disease might be present in a certain area. A survey will be appropriate for this purpose if there is a diagnostic test or a characteristic sign that can identify clinical cases or that can single out suspects who can then be included in a smaller group for further examination.

On the other hand, the purpose of a survey may be to find out whether there are inapparent or subclinical cases, i.e. individuals with low-grade or latent disease, possibly with symptoms but maybe without. In order to do this, there has to be some sign or symptom that can be detected through a test procedure, by examination of the individual, or by questioning him or his associates.

Finally, the purpose of the survey may be simply to identify infected persons—individuals who are not sick, persons who do not have symptoms, people in whom the only detectable effect of an encounter with the etiologic agent is an altered host response such as hypersensitivity or immunity. The question to be answered might be, "Do such persons in fact exist?" In other words, can infection be completely benign, or does it inevitably progress or develop into clinical disease?

Information from a survey may lay the groundwork for additional studies to answer related questions of epidemiologic and medical importance such as the following:

- Are there immunologic differences among the three groups mentioned—namely, the clinical cases, the subclinical cases, and the infected?
- What is the ratio of infection to disease? What percentage of the persons who become infected will develop active disease?
- What is the prevalence of infection in the total population? Is there an "iceberg" configuration of the disease, as was found to be true for histoplasmosis? If so, what percentage of cases comprises the visible part of the iceberg? And what proportion corresponds to the submerged base—the inapparent cases or the benign infections that give rise to the clinical cases?
- What are the characteristics of the population affected—their age, sex, race, occupation, place of residence, and other relevant factors?
- Are there any leads as to the possible source or sources of infection?

Such information as this is needed for understanding the natural course of infection or disease, for recognizing the frequency of the disease in a given area, and for determining the need for preventive measures—to prevent transmission of disease in a susceptible population, or, possibly, to prevent the development of disease among persons already infected.

Method

In conducting a survey of a human population, one generally looks for a characteristic sign of the disease or infection, asks about a characteristic symptom, or uses a test that detects a characteristic response. The decision of what procedure will be appropriate depends on which particular mycosis is being studied and on the kind of information being sought. One of the big problems in surveys may be the lack of test procedures and materials that are specific and dependable. Whatever procedure is finally chosen, it should be one that technicians can be trained to perform, it should be harmless, reasonably painless, and acceptable to the people being tested; it should be relatively specific for whatever characteristic the test is meant to detect; and it should be reliable (replications should give the same results).

In some instances when a completely satisfactory test is not available, it may be worthwhile to use a less-than-precise test to weed out those subjects who are of no interest in the study, thus leaving a much smaller group for further examination, perhaps by a more time-consuming or technically more difficult procedure. In this smaller group, then, will be the persons with the condition under study plus a variable admixture of "false positives."

Skin testing with presently available fungal antigens well illustrates the point that a test may be of value despite certain limitations. Take, for example, the results of skin testing for sensitivity to coccidioidin in young adults in the United States (Figure 1). Using crude antigenic

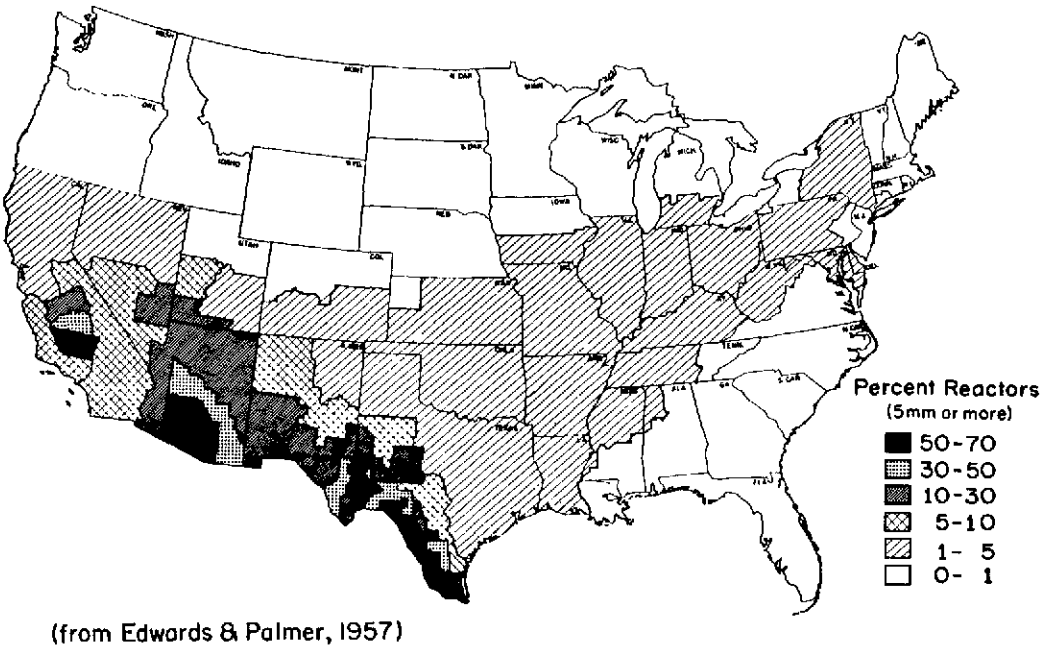

Figure 1. Pattern of coccidioidin sensitivity among young adults in the United States.

preparations, primarily C-24 at a dilution of 1:100, to test large numbers of student nurses, college students, and naval recruits, we obtained reactor rates ranging from zero for some parts of the country to as high as 70 per cent in the Southwest, where coccidioidomycosis is known to be endemic (*1*). The broad band running diagonally across the country from southwest to northeast, with reactor rates of 1 to 5 per cent, may well represent "false positives" or cross-reactions. Most of these reactions were only 5 or 6 mm in size—just over the borderline for "positive." Moreover, this excess of small reactions occurs in the geographic area where histoplasmosis is endemic.

Another illustration is the results of histoplasmin testing with H-42, a crude antigen developed more than 22 years ago and still used as the reference standard histoplasmin in this country (*4, 6, 8*). At the critical dose of 1:100 dilution it is useful for surveys, distinguishing people *with* skin sensitivity from those *without*. Since 1958 this antigen has been used in a program for the testing of young naval recruits. The results obtained during one six-year period, in which more than 250,000 of these young adults reported that they had always lived in the country of their birth, gave the picture shown in Figure 2. The geographic pattern in this map reveals a wide variation in the prevalence of reactors—from none in some areas to as high as 80 per cent in others.

The Americas, Asia, Western Europe, and parts of Africa have been surveyed fairly extensively during recent years, the results indicating the presence of endemic foci of sensitivity throughout much of the Americas and in scattered localities on other continents as well. Even so, great portions of the world still remain entirely unstudied.

From the corresponding maps it can be seen that skin tests are useful as a survey method when the purpose is to detect skin sensitivity in a sample of a total population. However, the

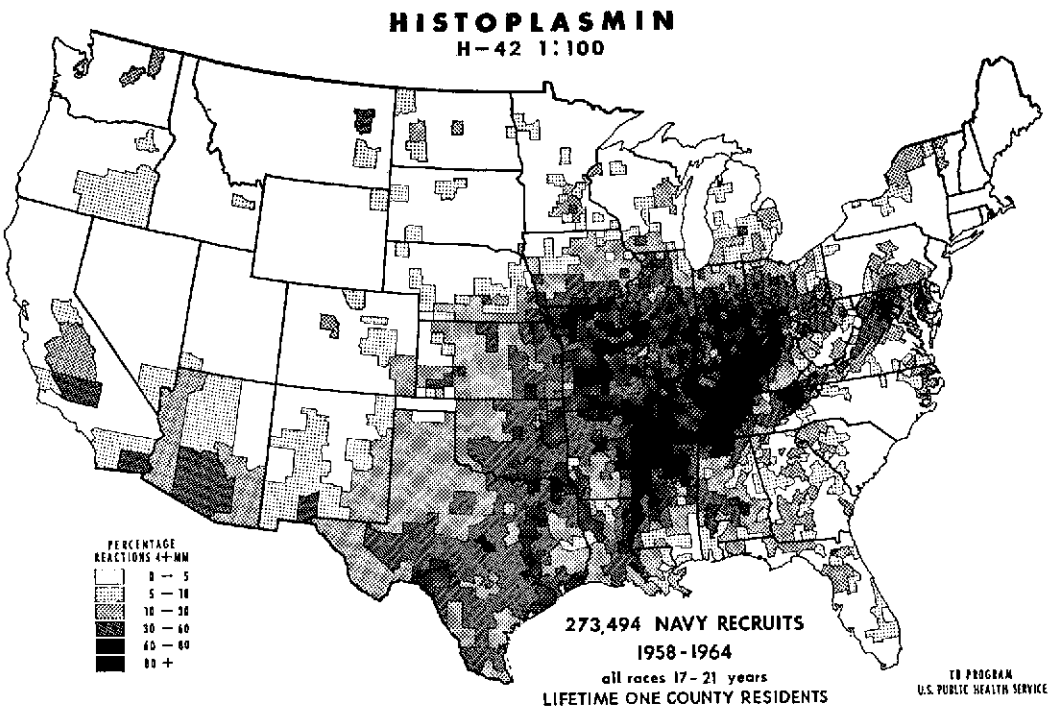

Figure 2. Histoplasmin sensitivity in the United States, as found through a wide sampling of young adults representing the entire country.

results are given only as the percentage of "positive" reactions; they tell little about the specificity of the test—that is, they give no clue as to how many reactions may, in fact, be cross-reactions. The question of specificity is a difficult and complex matter, as has been so clearly pointed out by Wijsmuller (*7*) and others (*2, 5*).

Population

Assuming that a survey procedure is available and has been deemed appropriate, the next step in planning a survey is to decide *who* is to be tested. This will depend in large part on the purpose of the survey. If it is to find people sick with the disease, the investigator will want to look among sick people—among hospital patients or individuals going to outpatient clinics or to private physicians for treatment. Needless to say, sick people who do not seek medical care will not be found in this way.

If, on the other hand, the purpose is to find inapparent cases or benign infections, ideally the entire population—*all* the people living in a village, or a county, or possibly even an entire country—should be surveyed. Such comprehensive coverage, however, is usually a practical impossibility. Thus, some segment of the population or some sample group—preferably a group that is readily available for survey purposes—is chosen to provide the data needed.

Armed Forces recruits and schoolchildren are both good examples of appropriate survey groups for certain purposes. As a rule, recruits include a high proportion of individuals who are lifelong residents of one place, which is an important factor when studying the geographic distribution of the mycoses. And although they represent only a narrow age range—generally only young adults—they can provide a good sampling of the entire country. Schoolchildren, on the other hand, usually provide a wider age range—as much as 12 years when ages 6 through

18 are included. Furthermore, when data from a survey of schoolchildren are supplemented by samples of younger and older ages, the prevalence in the total population of a particular area can be estimated with a reasonable degree of accuracy. Thus, the results from recruits would give information as to the geographic distribution of infection, whereas the findings from schoolchildren would be more useful in learning the age frequency of infected people within a given area.

Interpretation of results

In the systemic mycoses, perhaps more than in any other group of diseases, survey results must be interpreted with caution. The investigator must bear in mind the reliability and validity of the test materials and procedures, the adequacy of the survey population in terms of size and suitability, and other factors that may affect the survey results. For example, in the map showing sensitivity to histoplasmin among U.S. Naval recruits (Figure 2), it may be asked what proportion of the reactions represented here indicates sensitivity caused by infection with *Histoplasma capsulatum,* and what proportion corresponds to cross-reactions to other mycotic infections. Mention has already been made of the cross-reactions to *Coccidioides immitis* in the Southwest, where coccidioidomycosis is endemic. Are there areas where some of the sensitivity is caused by infection with *Blastomyces dermatitidis?* Or with other mycotic agents? Recently, Goodman (*3*) reported studies suggesting that a number of fungi in addition to Blastomyces and Coccidioides should be considered as possible causes of cross-reactions to histoplasmin in naturally infected human and animal populations. Several fungi, including *Aspergillus fumigatus, Aspergillus terreus,* and Penicillium, produced as many cross-reactions to histoplasmin as did Coccidioides in experimentally infected animals—namely, 60 per cent. The strains used to infect groups of guinea pigs were isolated largely from routine sputum specimens of patients admitted to two tuberculosis hospitals.

This recent experimental work, as well as earlier epidemiological studies, leaves little doubt that our concept about the sources of cross-sensitivity to histoplasmin and coccidioidin in human populations must be broadened to include a possible array of other fungi not hitherto considered important or even likely causes of cross-reactions. The clinical significance of such infections also remains to be evaluated.

Future trends

What lies ahead in the field of surveys for the medical mycoses? Dr. Huppert has pointed out the great need for skin-test and serologic antigens having a high degree of potency, specificity, and sensitivity. Dr. Tosh has reminded us that official health agencies will establish surveillance programs when there is evidence to show that the benefits will justify the resources required. Case-finding and surveillance-reporting both hinge on the development of better materials and test methods.

Our most urgent need is for skin-test antigens that will be (1) effective for mapping out the areas and regions in which the various mycoses occur, and (2) free of the complications of cross-reactions. This is a problem that has hampered the studies with our present antigens, which have now been in use for some 25 to 30 years. When new antigens are developed, it is hoped that they will be both sensitive and highly specific, and, in addition, that they will be available in critical dosages expressed in measurable amounts of the active principle, rather than as a dilution factor of a crude material, as is now the case.

We need serologic antigens and techniques that will detect the presence of a specific infection and that ideally will also indicate the stage of infection—i.e. whether it is early, latent, active, or waning.

We need more precise diagnostic procedures for clinical disease. If satisfactory antigens and test procedures could be made available to all clinical laboratories, this achievement could stimulate more complete case-reporting, which in turn could lead to the designation of at least the more common mycoses as reportable diseases. Finally, we need much better and more complete data on the prevalence and incidence of mycoses throughout the world, both in humans and in animals. Through imagination and ingenuity we must find better ways to get these data.

There are vast areas of the world that have been studied very little or not at all. Until they are surveyed, we cannot know the true extent of the health problem of the known mycoses; and until we examine the possibility that additional fungi are pathogenic, we cannot know all of the sources of mycotic infection and disease to which human populations may be exposed.

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DISCUSSION

Chairman Tosh: The papers presented in this session are now open for discussion.

Dr. Pappagianis: I would like to comment on Dr. Huppert's presentation regarding the adoption of a standard method for complement fixation. I think this is to be applauded. And his selection of the Laboratory Branch complement fixation (LBCF) test, which was developed and standardized by the National Communicable Disease Center may well be the most appropriate.

I want to point to two problems that I am sure Dr. Huppert would have dealt with if he had had more time. The first concerns the selection of antigen. I believe that in his own evaluation of the LBCF test he utilized an antigen different from the one that NCDC had used. This in itself constitutes a variation from a "standard" method. The second has to do with dilutions. You may recall that yesterday Dr. Kaufman felt that at a 1:2 and 1:4 dilution of serum the CF test for coccidioidomycosis, at least in their hands, was not useful. We do believe that CF titers of 1:2 and 1:4 are significant, and I think Dr. Huppert demonstrated with his slides that one can rely on titrations with serum diluted 1:2 and 1:4 and utilize data from that as diagnostic in coccidioidomycosis. I feel that the inclusion of these two questions—namely, the difference in antigen used and the difference in dilutions of serum—indicates again the kind of problem Dr. Huppert was trying to focus on. Thus, even though those of us involved in coccidioidal serology communicate very well, there are still methodologic differences among laboratories that might be quite significant.

Dr. Kaufman: Dr. Pappagianis misinterpreted my statement of the other day. We do believe that 1:2 and 1:4 titers with coccidioidin are useful. I consider that such low titers are possibly diagnostic of early or meningeal coccidioidomycosis. However, I also realize that, in the LBCF test, concentrated sera, i.e. 1:2 and 1:4 diluted specimens from individuals suffering from histoplasmosis and blastomycosis, may demonstrate cross-reactions with coccidioidin antigen. Such reactions may result in false positive reports.

I prefer to perform the immunodiffusion test described by Dr. Huppert on those sera that show 1:2 and 1:4 CF titers. Only sera that are positive in the CF test at 1:2 and 1:4 dilutions and also positive in the immunodiffusion test are considered diagnostic for coccidioidomycosis. If I do not perform the immunodiffusion test along with the CF test, I find it difficult to interpret these low titers accurately.

Dr. Pappagianis: I would like to ask Dr. Edwards whether the adoption of 5 mm area of induration as representing a positive reaction, as has been customary over the years in mycotic skin testing, is still acceptable as far as you are concerned, or whether the current tendency to read 5 to 9 mm as equivocal when using mycobacterial antigens should be carried over into the mycotic antigen field.

Dr. Edwards: This has been one of the most serious problems we have had in interpreting skin test results: 5 mm has been a magic number that we accepted as positive; 4 mm was negative. Frequently there was very little scientific evidence to back this up. For example, if 5 mm is considered positive to a dilution of 1:100 or 1:1,000, this does not make good sense in a lot of cases. We have to have—and this ties in with the subject of standardization of antigen—a way of titrating the potency and specificity of an antigen in homologously infected groups. Normally, this would give you an indication of the size range within which 95 per cent of the subjects will fall, and that, in turn, would help to define the lower limit of what is positive.

One can adjust the potency to give a reaction range that is within acceptable limits, so that you do not get too many strong reactions or too many weak reactions. If you can encompass around 95 per cent of homologously infected individuals with a particular dose of antigen, then this is a very useful antigen. The

standardization testing must be done not only with homologously infected animals but also with humans. What one obtains in animals may not necessarily be what one obtains in people. This is particularly true with respect to sources of cross-sensitivity in characterizing an antigen.

To answer your question, I think we have to look at the size of reactions obtained with a particular antigen in a homologously infected group of persons. This is not easy to come by in the fungus field. We cannot readily test a group of infected individuals of any reasonable size at the same time and in the same place, as is necessary to avoid variations in technique. So we have settled on 5 mm for want of something better.

Happily, the 5 mm criterion has turned out to be not too bad with the histoplasmin H–42 Public Health Service antigen. But if in a large sample the bulk of reactions are smaller than 5 mm, with only 1 or 2 per cent trailing off at 5 and 6 mm, the chances are good that these 5 and 6 mm reactors are not specific either; they are probably just the tail end of a negative distribution. I speak in terms of a frequency distribution of sizes of reactions, because that has to be the way one evaluates the usefulness of a test, and it also has to be how the criterion for a "positive" reaction is arrived at.

Dr. Furcolow: We should not forget that there is a difference between the fungal antigens and tuberculin. As Dr. Carroll Palmer showed long ago when he was doing calcification-related sensitivity to histoplasmin, the 5-to-9 mm reactors to histoplasmin showed almost as much calcification as did the 10 and over. On retesting, the small reactors, by and large, were found to be positive to histoplasmin. With tuberculin this is not so; the 5-to-9 mm reactors are rightly called questionable.

Mr. Taplin: I would like to make a few comments on the general aspects of the value of surveys.

In listening to some of these presentations, one might be forgiven for believing that by going out into the field and conducting surveys this will result in some kind of remedial action. For example, tinea capitis and pyoderma can become an enormous drain on resources, and although they do not carry the same impact as the life-threatening deep mycoses, they nevertheless can and should be considered an important public health problem. We could quote prevalence figures in some populations in the United States that would equal those given from foreign countries by Dr. Ajello at the beginning of the Symposium.

Once the extent of a problem has been established by field surveys, one is then faced with the often complex task of delineating responsibilities, generating funds, and assigning personnel to tackle the problem. I would merely suggest that it is not too early to begin planning to make the best use of the data once they are collected, and to consider the logistics and particularly the economics of prevention and treatment.

Dr. Seabury: I would like to go back to what Dr. Furcolow said about retesting subjects with small calcifications and small reactions. My question is this: Just what is the significance of a pulmonary calcification? At one time we really took it quite seriously, and, of course, originally it indicated a healed TB primary. Then we added coccidioidomycosis, and we added histoplasmosis. We see it in blastomycosis. We even see it occasionally in cryptococcosis with subpleural calcifications. It occurs in *Dirofilaria immitis* infestations of man with larvae. It is found sometimes in pulmonary rubeola infections, and certainly in pulmonary varicella infections, and there are findings now indicating that it may occur after certain other viral infections as well. Thus, I think care should be taken in attaching any significance to the presence of pulmonary calcification in interpreting the results of the skin test.

Dr. Furcolow: An advantage of being an epidemiologist is that you look at groups of people. If you take a group of people who are negative to tuberculin and positive to histoplasmin and you do chest x-rays, as Dr. Palmer did long ago, about 30 per cent show pulmonary

calcifications. If you take people with positive coccidioidin, and negative tuberculin, you get about 14 per cent calcification.

Dr. Edwards: I was involved with Dr. Palmer's work at that time, and I recall that 1 to 2 per cent of the people who had pulmonary calcifications reacted negatively to all the skin tests—tuberculin, histoplasmin, and coccidioidin. There are always some you cannot account for, but this was not exactly an insignificant percentage.

I should also like to mention the work of Dr. Robert High and others on the Aronson Indian material, which showed that the development of pulmonary calcification was related to the age at which tuberculosis infection took place. With the infection rate in this country decreasing to levels of lower than 1 per 2,000 a year, we will now be seeing fewer calcifications in the younger age groups.

Dr. Lazo: In regard to the planning of surveys, I wish to call attention to the work of the National Commission on the Study of Mycoses in Ecuador, which was established by the Department of Parasitology and Mycology in the "Leopoldo Izquieta Pérez" National Institute of Hygiene and involves a group of professionals who are actively concerned with these problems. I belong to this group, which is directed by Dr. José D. Rodríguez, and I can say that we have received gratifying support and response from the Medical Corps of Guayaquil and from other government agencies that consult us daily. We are currently engaged in a rural health planning effort, organized by the Ministry of Health, and it would be highly desirable if we could include, as part of the program, a plan for mycology coordinated with over-all efforts in this field being undertaken by the Pan American Health Organization.

FUTURE TRENDS IN THE MYCOSES IN LATIN AMERICA

Michael L. Furcolow

The present discussion will focus on future trends from the point of view of the epidemiologist, presenting a combination of facts and of inferences drawn from facts.

On the basis of the scattered reports from the literature and other sources that have been presented in the course of this Symposium, it may be assumed that the endemic areas of histoplasmosis and South American blastomycosis encompass most of the great river basins of South America (Figure 1). If one applies the recently cited case rate for North American blastomycosis —namely, 5 per million per year—to the estimated population of 40 million in these areas, the annual incidence of South American blastomycosis would be 200. Figure 2 shows the probable areas of coccidioidomycosis in South America, which are again very extensive. From these two maps it may be deduced that the fu-

Figure 1. Principal river basins of South America.

Figure 2. Probable areas of histoplasmosis, South American blastomycosis, and coccidioidomycosis in South America.

ture will reveal increasing rather than decreasing problems of prevalence of disease, of prevention, and of all the other public health aspects with which we are concerned.

If the true endemic area of histoplasmosis, South American blastomycosis, and coccidioidomycosis even approximates what has been indicated, then it will be necessary to learn a great deal more about the spread of infection in these areas over the coming years. Here the use of skin testing as an epidemiological tool takes on importance. An effort must be made to carefully define, using the best measurements possible, the endemic areas of histoplasmosis, of South American blastomycosis, and of coccidioidomycosis.

The next step is that the state of disease caused by these infections must be more clearly understood. It is quite evident from even casual observation that illness accompanying infection may be fairly prevalent. Indeed, if one takes the clinical symptoms outlined in studies that have been done on coccidioidomycosis and histoplasmosis, perhaps 20 to 30 per cent of the persons infected show some sort of clinical illness at the time of infection. Moreover, something like one in 1,000 persons who become infected develops severe disseminated disease, which is usually fatal unless it is treated. To these figures could be added an unknown number of persons, undoubtedly hundreds or thousands a year, who are breaking down with the cavitary type of disease and require diagnosis and therapy. Indeed, between 2 and 4 per cent of the admissions to tuberculosis hospitals in the endemic area are patients who have histoplasmosis with or without tuberculosis. This means 3,000 cases a year of cavitary histoplasmosis alone.

If one then looks at the tremendous land mass and numbers of persons involved in the probable endemic areas in Latin America, it is quite clear that the clinical problems of the mycoses need to be more accurately defined. For example, of 88 cases of histoplasmosis recorded in Uruguay and Venezuela, 76 to 86 per cent were of the disseminated variety and only 14 per cent were chronic cavitary, while at the same time of 309 cases in the United States only 46, or 15 per cent, were disseminated and 85 per cent chronic cavitary (*1*). Thus, it is possible that many chronic cases are being missed. Another fascinating clinical problem is the reported mortality of one fourth in epidemics of cave sickness in Mexico as compared to less than 1 per cent in the United States. It may be asked whether this is another type of disease.

In particular, the clinical spectrum of South American blastomycosis needs to be defined. The animal reservoirs of the causative fungi need to be studied in South America, since they are probably quite different than in North America. The prevalence and occurrence of the disease in nature and the existence of reservoirs in the soil and other areas need to be investigated before effective control can even be thought of. Finally, if we presume that the occurrence of severe disease will approximate and perhaps exceed the levels found in North America, extensive clinical facilities will be needed for the care and treatment of patients.

It must be remembered that patients with advanced forms of some of the systemic fungus diseases resemble in every way advanced cases of tuberculosis. With the acute need for tuberculosis hospital beds, it is a great loss to have these beds occupied by patients with mycotic disease who receive no benefit. Antituberculosis therapy is ineffective and may even be detrimental to such patients.

Perhaps one of the most important future needs is for clinical personnel with an understanding of the problems of mycotic diseases to train and develop medical students and other young scientists. The physicians and mycologists engaged in research and treatment of mycotic disease in South America are relatively few in number and are found in small, isolated groups. Moreover, many physicians involved in mycologic research are also engaged in private practice and thus have only a limited time available for studies and teaching. There are a few scientists engaged solely in the study of mycotic disease, and unfortunately a considerable proportion

of them are now close to retirement. In view of these problems, one can see the importance of developing more centers for the training of mycologists and the encouragement of mycological research. These two facets, teaching and research, must go together. There must be young physicians who are able to recognize the disease, and there must also be researchers to study the distribution of the mycoses, the relationship of one disease to another, the reservoirs of the soil, the eradication of the organism from the soil, and other urgent problems. This is a critical time for mycology, and it is of the utmost importance that organizations concerned with training and education and with future trends in health see that urgent steps are taken now to provide trained physicians and scientists in this field.

Summary

It is evident that the mycotic diseases are of tremendous importance to the health of the people in the Americas. Moreover, the number of infections and the prevalence of the diseases probably far surpasses current estimations. The following future steps are therefore considered necessary.

Definition of the extent of the problem of infection

Skin-test surveys employing standardized antigens will need to be conducted extensively in order to define the areas in which mycotic diseases are prevalent. In addition, improved antigens will have to be developed for South American blastomycosis, sporotrichosis, chromoblastomycosis, and some of the other less well-defined mycotic diseases. These infections probably affect major portions of the three great river basins in South America, as well as large areas of Central America.

Definition of the extent of the disease problem caused by the mycoses

This undertaking calls for widespread medical education and for the broad application of such tools as serologic tests and cultures. It is very important that the uses and limitations of such procedures be understood. Studies should be done of each disease itself and also of the frequency with which it occurs, the time sequence in relation to infection, and other pertinent aspects.

Treatment

Hospital planning and management must necessarily be closely tied in with any extensive use of amphotericin B, since this treatment involves lengthy confinement. The question of the efficacy of amphotericin B and the sulfas in treating the South American varieties of fungus diseases is extremely important. Priority should also be given to the exploration and development of new treatment procedures. The application of known methods of therapy to mycotic diseases other than the pulmonary forms will depend, of course, on accurate diagnosis. The clear separation of histoplasmosis, coccidioidomycosis, and South American blastomycosis from pulmonary tuberculosis, in terms both of hospital patients and treatment, is an urgent necessity.

Training

The chief problem in Latin America and in many other places is the lack of adequately trained personnel in the following categories:

- Medical school teaching staff: Professors competent in the recognition, diagnosis, and treatment of these diseases should be available in every medical school to train young physicians. Only a few of the medical schools in Central and South America currently have adequate teaching staffs in this regard.
- Medical mycologists: The South American countries have an eminent history in medical mycology, but unfortunately this reputation is owed to a few individuals, many of whom are approaching retirement age and some of whom have already retired. A serious question arises as to where the new generation of medical mycologists will come from, and, more important still, where they will be trained.
- Research workers: It is clear that the develop-

ment of many new tools, the application of epidemiologic methods, the clinical recognition of diseases, and the answers to many other problems depend largely on knowledge that is not now available. Only through research by trained mycologists can these needed solutions be found.

REFERENCE

1. Comparison of treated and untreated severe histoplasmosis: a Communicable Disease Center cooperative mycoses study. *JAMA* 183:823–829, 1963.

SUMMATION[1]

J. I. Baldó

I am honored that the Pan American Health Organization, through its Department of Research Development and Coordination, has asked me to present the final summation of this International Symposium on Mycoses.

At first I wondered whether I was in a position to accept such a difficult task, for on reading the agenda and the list of participants I saw that I am the only health expert present who is not a mycologist. Then it occurred to me that my selection, rather than having a scientific purpose, was an indication that PAHO, the highest regional organization for health matters in the Western Hemisphere, is ready to open up more direct fields of action and to help initiate a concrete health policy for the mycoses. For this reason—and for the fact that I, as a Latin American, am in a position to speak for a part of the Hemisphere in which conditions are entirely different from those in the United States—I decided to accept.

At the same time, I recalled that the first official document of the Pan American Health Organization on the subject of mycoses was Resolution XXIV of the XI Meeting of the Directing Council, approved in plenary session on 29 September 1959. Allow me to quote the text in full:

Resolution XXIV
Study of Mycoses in the Americas

THE DIRECTING COUNCIL,

Considering that the type of agency known in Venezuela as the Coordinating Committee for a Nation-wide Study of Mycoses has proved to be extremely useful in introducing the valuable collaboration of private initiative into the official institutions in charge of public health programs; and

Bearing in mind that the most recommended method for ascertaining the magnitude of the mycoses problem in the Americas is through epidemiological surveys that are carefully planned and evaluated,

RESOLVES:

1. To recommend to the Member Countries of the Pan American Health Organization that they promote the creation of coordinating committees, of nation-wide scope, for the study of mycoses.

2. To recommend to Member Countries of the Pan American Health Organization the undertaking of epidemiological surveys as a means of ascertaining the magnitude of the problem of mycoses in the Americas.

Thus, in 1959 formal international support was first given to the idea of coordination and epidemiological surveys for these diseases. Unfortunately, however, eleven years later, the situation in Latin America is unchanged.

Factors that have weighed negatively in the past

It amazes me that, despite the progress achieved to date in the understanding of some of the mycoses, especially in terms of their significance as a public health problem, these diseases have not yet been given consideration at the level of officially organized health campaigns. This phenomenon is, I believe, without precedent in any other field of medicine. Several factors may account for it. The present Symposium, thanks to a most complete and carefully

[1] Original text in Spanish.

worked out agenda covering the entire subject of medical mycology, has given us a clear panoramic picture of the magnitude of the problem.

In the first place, the most dissimilar diseases that can attack the human organs and systems are found under the heading of mycoses. Indeed, the term is really a common denominator for all the numerous and varied diseases caused by fungi, grouped together much in the way one speaks of diseases caused by bacteria, by parasites, or by viruses. The complex picture presented by the various mycoses—cutaneous, subcutaneous, and systemic—calls for a concomitantly wide range of training on the part of professional and paramedical personnel. Hence, control and treatment are administratively difficult.

Another great drawback is the lack of laboratory services for mycological and mycopathological diagnosis. In past years, the Pan American Health Organization has directed its attention toward the most urgent health problems, giving priority to the development of bacteriology and parasitology in relation to acute infectious-contagious diseases and gastroenteritis; to the control of very serious endemics such as tuberculosis, leprosy, and parasitosis; and to the eradication of diseases such as malaria. Although the situation of the general laboratory is still very deficient in Latin America, it is satisfying to note that a favorable change has taken place in many of the important specialties just mentioned. Work in mycology, however, has been restricted for the most part to isolated institutes or services. Despite high levels of excellence, these centers have never been sufficiently organized so as to be able to extend their radius of action. Meanwhile, in the regional laboratories mycological activities either play a secondary role or are not carried out at all.

The incompleteness of official records has also been an impediment. I do not wish to go into the question here of compulsory notification, for that will have to be considered by each individual country in the light of its diagnostic capabilities. However, it is a fact that mortality records, which more often than not show only the immediate cause of death, are an inadequate source of information on the mycotic diseases that can have fatal complications. For example, a case reported as "cor pulmonale" on the corresponding death certificate had started with a paracoccidioidomycosis that ultimately led to residual pulmonary fibrosis and finally over many years resulted in general cardiorespiratory insufficiency.

Perhaps the most significant negative factor of all has been the lack of coordination of existing resources. This was the main concern expressed by Venezuela when the Coordinating Committee for a Nation-wide Study of Mycoses was created in 1959. If we say that in the United States—a country with tremendous resources—there has been very little coordination in terms of public health measures at the national level, then we must admit that the results in Latin America have been extremely limited indeed. Despite the initiatives of various international congresses, factors within the individual countries have kept the coordinating committees from achieving the results that were expected. There is a strong need at this point for intervention on the part of an organization such as PAHO, with recognized authority before the governments, to recommend and direct a new area of health action.

Factors that weigh positively for the future situation

Mycology is one of the fields of medicine in which valuable and abundant information is available and in which ceaseless research is being conducted in ecology, epidemiology, immunology, and therapeutics. Efforts in this direction are currently being carried out at numerous scientific centers both in the United States and in Latin America. The present Symposium has clearly illustrated this fact. Everything is moving forward. In the United States, officially in the Mycology Section of the National Communicable Disease Center at Atlanta, Georgia, leaders such as Dr. Libero Ajello have made

every effort to see that the numerous groups dealing in one way or another with this subject project their activities throughout the Hemisphere. In Latin America, on a more reduced scale, initiatives of varied scope are being taken by institutions and public agencies. However, they will need the impetus of technical assistance and of recommendations from international bodies such as PAHO to carry them forward.

Over the past ten years the Pan American Health Organization has been providing assistance through visits of experts, as requested by the respective official health departments, in the field of serology and in other needed areas of mycology. The Director, Dr. Abraham Horwitz, has shown special concern, doubtless because of his long experience in laboratory work in previous years, over the difficult situation of general microbiology in Latin America. However, from time to time—as for example at a 1964 seminar in Venezuela which considered, among other things, the relationship of paracoccidioidomycosis to pulmonary tuberculosis—PAHO has prudently let it be known that questions such as these had to be considered in the light of given priorities and possibilities. As Dr. Horwitz pointed out in his letter of invitation to this Symposium, it is with progress in the control of diseases caused by bacteria, parasites, and viruses that the mycoses have come to public attention.

We consider that this Symposium, the first such meeting to be sponsored by the Pan American Health Organization, is indeed of great importance, not only because it is bringing together needed information on the over-all problem, but also because it is laying the groundwork for a health policy for the mycoses. The suggestions made here will have far-reaching significance for the countries of the Hemisphere.

Conclusions

Now to my conclusions—or "summation." As you can see, in spite of the difficulties and stumbling blocks that are inherent in some of the questions discussed here during the last three days, I have great faith in the future and my attitude is optimistic. However, I am somewhat less optimistic about the specific prospect of putting into public health practice a given set of duly planned measures for the control of this complex group of diseases. I have attended many scientific meetings, and it is frequently the case that administrative steps are not taken to put into action such programs as might be a logical outgrowth of the information received. My practical judgment has therefore led me to translate the "summation" entrusted to me here into a series of suggestions based on what I have heard during the deliberations of the Symposium.

We must not forget, as I said before, that we are here before the Pan American Health Organization, the regional international organization that is responsible for dealing with the Ministries of Health in the respective American governments. Its functions are stipulated in a "code" made up of all the Basic Documents, and it is to Official Document No. 88 of March 1969 that we refer ourselves now.

Let me begin by making two very broad suggestions:

- First, taking into account the desire I have felt among the participants here to assure some lasting and stable results from this meeting, I propose that the present dialogue be continued, and that studies related to the mycoses be recognized as being of interest and concern to the Pan American Health Organization, inasmuch as these diseases have been shown to constitute a public health problem.

- Further, in order to establish an effective liaison between the community of medical mycologists and the Pan American Health Organization, I should like to suggest that PAHO study the possibility of creating a Technical Commission for the Study of the Mycoses, in accordance with Article 23, Chapter V, of the Constitution of the Pan American Health Organization, which says: "The Director of the Bureau may appoint such permanent technical commissions as are authorized by the Conference or the Council, as well as such nonpermanent technical com-

missions as are authorized by the Conference, by the Council, or by the Executive Committee." With the assistance of such a commission, PAHO could consider the advisability of carrying out the following specific activities, if it so deems appropriate, in keeping with the considered judgment of its Department of Research Development and Coordination:

1. Sponsorship of studies leading to a better understanding of the mycotic diseases;

2. As a first step toward this objective, training of personnel from all related fields in the use of laboratory methods for clinical diagnosis, as well as in epidemiology and ecology, through the granting of fellowships and the provision of personnel and technical assistance to carry out courses in those Latin American countries where conditions are suitable;

3. Studies toward the standardization of antigens and techniques used in mycological diagnosis, including serologic and skin tests, so that results may be readily compared;

4. Facilitation of the acquisition of antigens, both for skin tests and for serologic tests, that meet the above-mentioned requirements;

5. Encouragement of compulsory notification of the mycotic diseases in those countries in which the development of mycological studies has reached a sufficiently advanced level to make such an effort useful and practicable;

6. Development of specialized bibliographic collections on medical mycology and of related library services;

7. Formulation of recommendations to medical schools in the sense that they integrate the teaching of medical mycology into their curricula at all levels;

8. Promotion of broad exchanges between the Latin American and North American institutions engaged in work on the mycoses;

9. Continuation of the scientific dialogue on medical mycology at intervals not in excess of three years and at a level as high as that of the present Symposium, as an effective means of furnishing health administration departments with needed information;

10. Provision of assistance to the proposed Symposium on Paracoccidioidomycosis, to be held in Medellín, Colombia;

11. Exchange of visits by experts to help improve existing mycology centers in Latin America; and

12. Promotion of mycological research in Latin America through technical and economic assistance.

The suggestions above could constitute the doctrinal basis for a program to be recommended to the respective Ministries of Health.

CLOSING STATEMENT[1]

Abraham Horwitz

I am indeed sorry I was not able to be present at the opening session of this Symposium, whose success I am pleased to note has already been reported in the *New York Times*. This "newspaper of newspapers" has not only honored us with daily articles but has also taken the trouble to mention the name of the Pan American Health Organization in its statements.

The distinguished speakers today, especially Dr. Baldó, have asked us what is going to happen now, with so much valuable opinion mobilized, so many fine papers presented, and so many transcendent proposals set forth. Dr. Baldó has warned us, in passing, that the results of international meetings of this kind may frequently be consigned to the technical literature and not reach the hands of decision-makers who are in a position to put the suggestions into practice. There was, indeed, a resolution in 1959, as Dr. Baldó has mentioned. But unfortunately the progress achieved in the intervening years is reflected only inside specialized institutes; the public as a whole has not yet been able to benefit. I want to assure you now that we intend to put into practice a good part of the program called for by Drs. Furcolow and Baldó.

The moment has come to carry out the steps that are already classic in the analysis of any situation that affects a large number of human beings. I am speaking of that part of a "health policy" that refers to the organization and administration of the resources available to a particular society at a given time in its history for solving a problem to which it has assigned priority. In the case of the mycoses, as in others, it is necessary to identify the various infections, describe each of the diseases precisely, apply the knowledge available, and continue to conduct research on the unknowns. The professionals and their properly trained technicians make up the essential infrastructure, and for each of these two components the task takes on different emphasis.

We are in a position to carry out several of the measures that have been proposed today. We have no difficulty in offering opportunities to interested colleagues to prepare themselves at the best centers in any country of the world. We have no difficulty in promoting steps to secure antigens for comparative studies. We have no difficulty in arranging for specialists in the various aspects of the mycoses to advise governments on solutions to the problems that these diseases bring about. And, although we may have financial restrictions, we certainly have no technical difficulty in developing surveys that will tell us the true magnitude—both current and potential—of these problems.

But we cannot guarantee that the knowledge that exists today or will be produced in the future is going to be applied by the governments. We cannot guarantee this because the governments are sovereign. Moreover, we cannot guarantee it because we live in an era of planning, and in planning we preach that since the needs are greater than the resources the latter must be carefully invested so as to deal effectively with the greatest number of harmful consequences. The most important of these is mor-

[1] Original extemporaneous remarks in Spanish.

tality; afterwards comes morbidity; and only in the last instance do we consider the detrimental effects on the economy and on development. Thus we have a vicious circle. In the case of the mycoses, for example, we know of their existence, but we do not know of their extent, and it will not be easy to find the resources with which to remedy the situation. For this reason, it might be preferable, before we begin to urge that governments include these problems in their lists of priorities, to emphasize right now such efforts as may help to define the problem more precisely and hence permit application of the knowledge that already exists.

To begin with, we propose to introduce the topic of mycoses at the Pan American Sanitary Conference to be held here in Washington at the end of September 1970. In addition, we plan to disseminate the report of this meeting broadly —to the health ministries, to universities and research centers, and to many other interested institutions, groups, and individuals. In the circular that accompanies it we are going to tell the governments that we are in a position to carry out the activities I have just summarized for you. It is hoped that these steps will contribute to a better definition of the quantity and quality of the problem, and that they will facilitate the application of existing knowledge, while research, in the meantime, is finding new means of prevention and new prospects for the cure of these diseases.

Sometimes the comment is made in Latin America that we have too many serious concerns in regard to population, poverty, and the alternatives of politics to be able to devote ourselves also to complex problems like the question of the mycoses. The record, however, does not bear this out. Ten years ago no one spoke about community psychiatry—to cite another scientific and practical entity that is equally complex—and yet today we have at least a hundred psychiatrists in the Hemisphere who are intensely interested in community-centered studies, and we have the pleasure of seeing that in many departments of psychiatry this approach has become the basis of the curriculum. Similarly, ten years ago no one talked about nuclear medicine, and now the Organization is convening a group similar to yours that will attempt to define the framework of this new discipline in the field of public health. A decade ago, finally, people reacted with surprise when we ventured to state that there would be a vigorous movement of capital in order to supply water to the people of the Hemisphere. But today we can say with pride that 60 million inhabitants of Latin America have, or are about to have, water at their disposal, and that national and international capital in the amount of 1,300 million dollars—in great measure from the Inter-American Development Bank, but some of it also from the Government of the United States and private capital—has been mobilized. The Pan American Health Organization has helped to advise in this task—in this undertaking which is primarily humanitarian in nature and yet at the same time carries us forward toward our economic goals.

We feel justified, then, in the belief that we should go on working in a field of growing complexity, attuned to the accelerated pace of economic and social development of the Hemisphere. We share Dr. Baldó's optimism. I believe that the time has come when governments and universities are beginning to take up a continuing concern for the mycoses—to do everything they can to apply what is known on behalf of those who are afflicted and to prevent these diseases from occurring in persons who are now well. But we are counting on you, because without the advice of the best talent existing today in the Hemisphere and in the world, the job cannot be done.

I hope that this first meeting will be followed by others. Because if there is anything dynamic, it is science today. And I hope that in future encounters we may be able to present to you signs of the progress that we had not been able to achieve up to now. We look forward always to the pleasure of having you with us and to being favored by your experience, advice, and suggestions.

I thank you very much, and I assure you that we are going to put into practice very soon the immediate steps to which I have referred