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REPORT ON THE
THIRD INTERNATIONAL CONFERENCE ON THE MYCOSES

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CONFERENCE ON THE MYCOSES

by

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The Third International Conference on the mycoses was held in São Paulo, Brazil during August 27-29, 1974. Approximately 70 persons attended the sessions, which took place in the Conference Room of the Atomic Energy Institute, University of São Paulo.

The participants and invited guests came from nine countries: Argentina, Brazil, Colombia, Guatemala, Mexico, Sudan, Thailand, United States and Venezuela.

A total of seven sessions were held during which 30 different papers were presented and discussed in various languages with simultaneous translation. The material presented made significant contributions to our knowledge of the host factors in defense and susceptibility to the mycoses, of the increasing number of fungal species capable of infecting man and animals, of improved methods of serologic and cultural diagnosis, as well as the socio-economic, nutritional, and epidemiologic aspects that contribute to the incidence of the mycoses. Finally, clinical experiments with some of the newer antifungal agents were presented and discussed.

The highlights of the newer knowledge disclosed at the Conference are summarized below.

Session I - Host Defense Mechanisms in Relation to Mycotic Infections.

A basic paper was presented by Dr. Paul E. Hermans in which he discussed the function and malfunction of the immune

system in relation to infection. After reviewing the various cellular and humoral components of the immune system (T-cells, B-cells, macrophages, polymorphonuclear leukocytes and complement system), the author mentioned specific defects known to be associated with susceptibility to bacterial and fungal infections. Among these are several studied by him and his associates. (These observations were described in greater detail). A qualitative B-cell defect known as "idopathic late-onset immunoglobulin deficiency" was found in 50 patients who were victims of recurrent pyogenic respiratory infections by bacteria, but in whom fungal infections were not observed. By contrast, the author mentioned distinct correlation between T-cell dysfunction and fungal infection. This included 35 patients from whom Cryptococcus neoformans was isolated, three patients with relapsing histoplasmosis, and patients reported elsewhere with disseminated coccidioidomycosis and chronic mucocutaneous candidiasis. Various patterns of T-cell dysfunction were found in the patients unable to cope with fungal infections, and in the patients harboring Cryptococcus neoformans, a "combined late onset idopathic deficiency" was observed involving B-cells as well as T-cells. Predictably, these patients were also susceptible to pyogenic bacterial infections.

Dr. Ernesto Mendes described his earlier observations of the depression of the delayed hypersensitivity response in patients with paracoccidioidomycosis, and presented the results of newer studies with 16 other patients with this disease,

skin-tested with a variety of antigenic fractions (fungal and non-fungal). The depressed, or suppressed, delayed reaction was confirmed in these patients, and responsiveness was restored by transfer of either lymphocytes or dialyzable transfer factor from responsive patients. Therapy with amphotericin-B and sulfonamides also improved the cell-mediated response in some patients.

In-vitro methods of evaluating cellular immune response were employed by Dr. Chloe-Musatti in her studies of patients with paracoccidioidomycosis, by culturing their peripheral blood lymphocytes in the presence of phytohemagglutinin (PHA), of a sonicated soluble antigen from yeast cells of Paracoccidioides brasiliensis, and of an antigen from Candida albicans: lymphocytes from normal donors (all reacting to candidin) were used as controls.

Impaired response to PHA was found in seven of the 16 patients studied, and an inhibitory humoral factor was detected in seven of 14 plasma samples. One-third of the patients responded with blastic transformation in vitro to the Paracoccidioides antigen. This was a highly specific reaction since the lymphocytes from the normal controls responded only to Candida antigen. The leukocyte migration inhibition test showed cross reaction in the controls, but correlated well with the skin energy of the patients. The in vitro cellular immune response was found to fluctuate between normal and abnormal according to the course of the disease.

Dr. Nelson T. Mendes' study determined the relative and absolute numbers of T and B cells in peripheral blood and lymph nodes of patients with paracoccidioidomycosis. When compared with 30 normal controls, 16 patients with the disease showed a significant lowering in the percent of peripheral T-cells, and a significant increase in the total leucocyte count. There were no statistically significant differences, however, between the two groups as regards percent lymphocytes, total lymphocytes, T-cell count, percent B-cells, nor total B-cell count. Some controls, however, showed both a B-cell and T-cell reduction resulting in a lower total leukocyte count; others had elevated B-cell counts. In the lymph nodes, a depletion of T-cells was observed in the paracortical area; the follicles were reduced in size and numbers, but contained B-cells.

Dr. Librado Ortiz Ortiz studied the cell mediated immune responses in mice infected with Nocardia brasiliensis. Because human and animal infections by this actinomycete had primarily suggested that delayed hypersensitivity was not responsible for the defense mechanism, a different approach was used. This consisted in measuring cross protection against Listeria monocytogenes induced in mice infected with N. brasiliensis. This occurred at different time intervals, but an important collateral finding was that Nocardia infected mice exhibited delayed hypersensitivity as measured by foot-pad swelling when tested with Nocardia antigens. Further proof of the induction of cell-mediated protection was offered by inhibition of migration of peritoneal exudate cells and activation of blast activity in infected animals exposed to cytoplasmic Nocardia antigen.

Dr. Dorothy Windhorst discussed the initiation of candidiasis in adults as contrasted with chronic mucocutaneous candidiasis beginning in childhood. A number of underlying diseases or conditions long known to predispose to adult candidiasis, namely diabetes, steroid therapy, malignancy and immunosuppressive therapy are all known to interfere with either the quantity or the quality of the cellular immune response. A group of 10 documented thymoma patients with candidiasis were analyzed in detail (from the literature and the authors' personal experience). Two important conclusions were drawn from the analysis. First, that the initiation of candidiasis in an adult may be indicative of an underlying thymus tumor (or other serious disease) which should signal a search for the evolving problem. The second conclusion was an analogy drawn between current explanations of the immunology of cancer and of chronic mucocutaneous candidiasis. Thus, basic explanations are emerging for old practical medical observations such as pointed out long ago by J. Walter Wilson that Candida infections were like a distress flag waved by an otherwise mute (compromised) host.

The role of cellular immunity in histoplasmosis was discussed by Dr. Dexter H. Howard with emphasis on phagocytic mechanisms. Both mononuclear phagocytes (MN) and polymorphonuclear leukocytes (PML) were studied for ability to kill Histoplasma capsulatum. While the fate of microconidia (the infective particle of H. capsulatum in nature) has not been studied, blastospores were killed by at least 3 cationic proteins

besides myeloperoxidase in PMN. Of the 3 types of MN phagocytes studied, normal human circulatory monocytes, but not peritoneal monocytes from unstimulated normal mice, killed blastospores of H. capsulatum. Macrophages from immunized animals restrict intracellular growth and depress protein synthesis in this fungus. While glycogen, endotoxin, and polyanions (the usual substances that stimulate mouse peritoneal macrophages) induced no activity against H. capsulatum, this was achieved by intraperitoneal injection of 15% sodium caseinate.

Session II - Mechanisms of Fungal Pathogenicity.

Dr. Howard W. Larsh described the development of experimental cryptococcosis and histoplasmosis induced by the airborne route.

The Henderson apparatus was found effective for producing aerosols with the two mycotic agents that were employed respectively against BALB/c inbred mice, (Cryptococcus neoformans) and Hartley strain guinea pigs (Histoplasma capsulatum). Alveolar macrophages were not activated in the mice until one week after exposure to cryptococcus, but showed in vivo and in vitro phagocytosis during the second week, the activity dwindling to disappear by the end of the fourth week. Cultures were most often positive from the lungs, next in order, from the liver, spleen, brain and heart.

In animals infected by H. capsulatum, the lungs, then spleen, yielded most frequent positive retro-cultures; alveolar

macrophages in these animals were still under investigation at the time of the oral report.

The treatment of one patient with recurrent pulmonary coccidioidomycosis with transfer factor (TF) was described by Dr. David A. Stevens. Reference was made to descriptions of 5 other patients in 3 previous reports of the use of TF in this disease, and preliminary results were given of ongoing TF therapy embracing more than 50 other patients. In general, a favorable clinical response was obtained, accompanied by the development of delayed cutaneous hypersensitivity, blastogenesis in vitro, and lymphokine production in 25-60 percent of the patients.

Studies of experimental coccidioidomycosis were presented by Dr. Sotiros D. Chaparas. These included a comparison of strain virulence, mostly the Silveira, 46, and Woodville isolates of Coccidioides immitis. It is Dr. Chaparas' belief that the growth rate and other characteristics of the fungus contribute to its virulence since a rapidly proliferating strain may overwhelm the hosts' defenses. Using arthrospore preparations to induce infections, the Silveira strain was shown 25 times as virulent for mice as the 46 strain and about 14 times as virulent as the Woodville strain. The Silveira strain also gave rise to the most effective protective immunogens in mice injected with formalin-killed spherules. All strains induced the same level of sensitivity in infected or sensitized mice, but the No. 46 strain produced more skin-reactive substances than the Silveira, the Woodville strain the least. Coccidioidin derived from spherule preparations was more active in inducing

skin reactivity and lymphocyte transformation than mycelial coccidioidin.

Dr. John Willard Rippon discussed progress in correlating enzymatic activity patterns of dermatophytes with their virulence, mating type, and colonial morphology. Describing his own work and that of others, he listed the extracellular enzymes identified thus far, and added that they have been found to vary qualitatively and quantitatively depending on the isolate. A high correlation (genetic as well as phenotypic) has been shown between high elastase production and the granular colony type in Trichophyton mentagrophytes (Arthroderma benhamii). Rippon's studies of middle-western isolates confirmed previous observations (Silva-Hutner, 1954, Georg, 1954, Allen and Taplin, 1971) that granular isolates produce more inflammatory lesions than downy ones. Rippon found further that a high elastase production correlates with the granular phenotype, and that these strains were usually, though not invariably, of mating type "a".

Session III --- Recent Developments in the Laboratory Diagnosis of the Mycoses.

Dr. Hilliard B. Levine described the preparation of spherulin, and compared its behavior to that of coccidioidin, two antigenic preparations derived from cultures of Coccidioides immitis. Spherulin, derived by autolysis from the sperule rather than mycelial form of the fungus, was more than 50 percent sensitive than coccidioidin as a skin test or complement-fixing antigen in both epidemiologic surveys and clinical cases

of the disease. Spherulin also showed greater reproducibility and duration of potency from lot to lot and upon storage than coccidioidin lots have shown in the past. Freeze dried preparations of spherulin lasted up to 2 years, while reconstituted preparations stored at 22° C, 4° C and -30° C lasted a maximum of 10 months.

The advantages and pitfalls in the use of mating reactions for identification of dermatophytes and other pathogenic fungi were discussed by Dr. Arvyn A. Padhye. Attention was called to the importance of culture media, maintenance and age of the tester strains, and method of crossing and determining incompatibility when searching for fertile ascocarp production. Standardized procedures were recommended to take into account these variables.

Dr. Margarita Silva-Hutner read a paper written in collaboration with Dr. Arturo L. Carrión, reviewing the nomenclature and taxonomic characteristics of the fungi of chromoblastomycosis. After describing and illustrating the colony characteristics of five species (with emphasis on growth rate and surface topography), their microscopic morphology was discussed. This was followed by the recommendation that the generic names Phialophora and Cladosporium be reserved for the monomorphic species in this group of pathogens, namely P. verrucosa and C. carrionii. At the same time a plea was made that, in accordance with the rules of botanical nomenclature the name Fonsecaea be retained for the polymorphic species F. pedrosoi, F. compacta and F. dermatitidis.

Dr. Libero Ajello spoke on phaeohyphomycosis a name applied to a group of mycoses whose etiologic agents are dark-colored fungi (phaeohyphomycetes), which produce discrete hyphae, not organized in granules, in infected tissues. The fungi implicated thus far as agents of such lesions are: Cercospora apii, Phialophora dermatitidis, P. gougerotii, P. parasitica, P. richardisiae, P. spinifera and Phoma sp., all from subcutaneous infections, and Cladosporium bantianum, Dactylaria gallopava, and D. hawaiiensis causing systemic infections.

Session IV - New Developments in the Serology of Mycotic Infections

Some of the most significant advances in medical mycology have taken place in the development, evaluation and application of serologic procedures for the diagnosis of mycotic diseases. Thanks to the efforts of many individuals effective tests that are sensitive and specific are now available to aid in the diagnosis of the systemic mycoses, the most serious of the diseases caused by fungi. These diseases are aspergillosis, blastomycosis, candidiasis, coccidioidomycosis, cryptococcosis, histoplasmosis, paracoccidioidomycosis and sporothrichosis.

Dr. Leo Kaufman of the Center for Disease Control's Mycology Division, Atlanta, Georgia reviewed and evaluated the type of tests currently available for blastomycosis, coccidioidomycosis and paracoccidioidomycosis. He went on to point out that despite their usefulness, the tests had certain inherent limitations that were centered on the use of crude antigens. There is,

therefore, an urgent need to develop and produce purified and highly specific antigens. Other needs were the standardization of test procedures and reagents and the development of sources for the reagents required by diagnostic laboratories. The Pan American Health Organization through its Coordinating Committee for the Mycoses has begun a multinational cooperative study to standardize procedures. Toward that end it has prepared and published a:

Manual of Standardized Serodiagnostic Procedures for
Systemic Mycoses

Part I. Agar Immunodiffusion tests

Part II. Complement Fixation Tests

(English and Spanish Versions)

Dr. Morris Gordon of the New York State Department of Health took up the problem of serologic tests for opportunistic fungus infections caused by Candida albicans, various Aspergillus species, Torulopsis glabrata and Cryptococcus neoformans. The relative value of a variety of procedures as aids in the diagnosis and prognosis of these diseases was discussed. The tests found useful for candidiasis were: the immunodiffusion and the rapid immunoelectrosomphoresis tests. Other procedures considered to be of value were the slide latex agglutination test, indirect hamagglutination and immunofluorescence.

The most practical test for aspergillosis was considered to be the immunodiffusion procedure on the basis of its specificity. The immunoelectro-osmophoresis test promises to be not only specific but more sensitive.

Torulopsosis has only recently come to be recognized as a fairly common opportunistic disease. As a result serological tests for its diagnosis are not as yet fully developed and evaluated. However, precipitin tests show promise.

Serological tests for cryptococcosis, in contrast with those for the other mycoses, rely in part on the detection of capsular antigens rather than on antibody. The slide latex agglutination test for antigen is simple, highly specific and sensitive. Antigen tests should be run in parallel with antibody tests such as the tube agglutination procedure or the charcoal-particle card agglutination procedure.

Dr. El Sheikh Mahgoub of the University of Khartoum, Sudan described 10 years of experience with Ouchterlony plate immunodiffusion tests for mycetomas caused by the aerobic actinomycetes. This procedure has been found to be highly useful and reliable. The intensity and number of precipitin lines are directly related to the size of the mycetoma. The effectiveness of therapy can be objectively determined by noting whether the lines disappear.

The development and application of serological procedures for the diagnosis of bovine mastitis caused by Nocardia asteroides was described by Dr. Allan Pier of the National Animal Disease Center in Ames, Iowa. Gel diffusion precipitin tests are the most useful for this disease. Positive precipitin reactions are detectable two to five weeks after infection. To differentiate between nocardiosis and tuberculosis nocardial antigens free of cell wall antigens must be used. The use of serologic tests for

nocardiosis is limited by the lack of sources of antigens.

An insight into the newer techniques available for the serodiagnosis of mycotic infections was provided by Dr. Dan Palmer of the Center for Disease Control, Atlanta, Georgia. Among the newer techniques that aid the clinician and diagnostician are counterimmunoelectrophoresis, and the attachment of radionuclides to one of the members of the immune reactant pair. Two-dimensional electrophoretic separation of proteins is of great value in the analysis of complex antigens. This procedure lends itself to the quantitation of antigens and antibodies and the detection of cross-reacting substances. Radial immunodiffusion is a simple and promising tool for monitoring small changes in antibody and antigen levels in sera and spinal fluids.

Dr. William Kaplan of the Center for Disease Control closed this session with a review of the fluorescent antibody procedures that have been developed for the rapid and specific detection and identification of fungi in tissues. The use of fluorescent antibody reagents for the identification of cultures of pathogenic fungi was also discussed.

Session V - Opportunistic Mycoses in the Americas

In recent years there has been a significant growth of mycotic infections in individuals with compromised defense mechanisms. Dr. Amado Gonzalez-Mendoza of the Instituto Mexicano del Seguro Social reviewed what is known about the interrelationship between the aggression mechanisms of fungi and the host's defense mechanisms. The degree of pathogenicity and virulence

of opportunistic fungi has been studied but little. The ability of the fungus to survive and grow at a temperature of 37° C is one basic necessity but the mechanisms of adaptation to human tissues remain unknown. Multiple factors are obviously involved that render the host susceptible to mycotic infections. The factors involved relate to inflammatory response, phagocytosis, and immediate and delayed immunity.

The complexity of the host factors was illustrated by a case of chronic mucocutaneous candidiasis in a patient who was discovered to have a partial cellular immunity defect and an intrinsic phagocytosis defect that appeared to be an enzymatic deficiency that prevented selective intracellular destruction of Candida albicans.

Drs. Javier Pizzuto and Ruben Lopez of the Instituto Mexicano del Seguro Social Reported on their study of 270 patients with a variety of blood diseases and the development of opportunistic mycosis, chiefly candidiasis. Among a group of 170 patients 56 or 33% developed candidiasis. The opportunistic infection was unrelated to sex or age but was observed to occur in those with the most severe underlying diseases: leukemia, lymphoma and aplastic anemia. Twenty-one of these patients received specific antimycotic treatment. But treatment did not lower mortality in part due to the severity of their basic illness or the lateness in diagnosing their superimposed fungal diseases.

In 100 patients prophylactic measures were taken to prevent mycotic infections. They received 3.4 million units of nystatin orally each day from the start of their illness. Generalized

candidiasis developed in only three patients. Six others developed Candida sp. infections of no clinical significance in two sites. All the others remained free of candidiasis. The value of prophylactic treatment was stressed in the handling of immunologically compromised hosts.

Dr. Karlhanns Salfelder of the University of the Andes, in Merida, Venezuela described a variety of new and uncommon opportunistic fungal infections. The diseases presented were adiaspiromycosis, cephalosporiosis, penicilliosis, scopulariopsis and torulopsiosis. Experimental infections in mice and hamsters with Emmonsia crescens and E. parva and 22 species of basidiomycetes were also described.

Drs. Alfred M. Allen and David Taplin of the Letterman Army Institute of Research, San Francisco, California and the University of Miami, in Florida respectively presented a paper on the "Epidemiology of Cutaneous Mycoses in the Tropics and Subtropics, Newer Concepts." Their observations were based primarily on the study of tinea corporis and tinea pedis among soldiers in Vietnam. The prime disease agent proved to be a granular, pigmented form of Trichophyton mentagrophytes contracted indirectly from wild rats. A marked difference in the etiology, prevalence and severity of infection was noted between native and foreign military personnel. The difference seemed to be related to specific immunity acquired following childhood infection. In the foreign troops climate, clothing, sweat and the amount of exposure to water exerted deleterious effects through the common mechanism of occlusion of the skin.

Session VI - Social and Economic Aspects of the Mycoses

Three speakers covered these topics. Dr. Antonio Gonzalez-Ochoa of Mexico's Institute of Health and Tropical Diseases spoke on the "Prevalence, Severity, and Types of Mycotic Diseases as reflected in the Socioeconomic Status of the Patients." In this paper it was emphasized that non-opportunistic or primary fungus infections occur in the low socioeconomic groups in whom malnutrition and its resulting hypoproteinemia leads to a depression of their defense mechanisms. Opportunistic or secondary mycoses are most frequently encountered in the people of high socioeconomic development. There the people develop chronic and debilitating diseases that render them susceptible to mycotic infection.

Drs. Ruben Mayorga and Leonardo Mata presented a paper on "Nutrition and Mycotic Infection Interactions." These investigators from the University of San Carlos of Guatemala and the Instituto de Nutrición de Centro América y Panamá of Guatemala emphasized that basic studies have yet to be carried out to determine the interrelation between nutrition and mycotic infections. Experimental studies in animals have shown that nutritional deficiencies impair cellular immunity and B-cell function. Such evidence in humans has been more difficult to obtain. But it is known that protein-calorie malnutrition decreases or suppresses T-cell function. In developing countries malnutrition causes severe damage in growth and development. From this it is inferred that susceptibility to mycotic infection is

increased. But further study is needed to understand the malnourished hosts' response and immunity to mycotic infection.

Session VII - New Chemotherapeutic Agents for the Treatment of Mycotic Infections

The role of clotrimazole and miconazole, new broadspectrum antifungal compounds in the treatment of cutaneous fungus, infections was discussed by Dr. Nardo Zaias. At the Mt. Sinai Medical Center in Miami Beach, Florida and with the cooperation of physicians in several Latin American countries, Dr. Zaias has evaluated these two antifungal compounds against cases of ring-worm, cutaneous candidiasis and tinea versicolor. Of the two miconazole showed the greatest promise as a potent and effective chemotherapeutic agent.

Dr. Emanuel Grunberg of Hoffman-La Roche in Nutley, New Jersey reviewed worldwide experience with 5-fluorocytosine, a synthetic anti-fungal compound. In vivo studies with orally or systemically administered drug revealed it to be effective in aspergillosis, candidiasis, cladosporiosis, and cryptococcosis. It was ineffective against experimentally induced cases of blastomycosis, coccidioidomycosis and histoplasmosis. In humans, 5-fluorocytosine when given orally has been found to be of low toxicity and effective against systemic candidiasis and cryptococcosis. There are indications that the combined administration of this drug and amphotericin-B will be of clinical value.

The session on chemotherapeutic agents was concluded by Dr. John Utz of Georgetown University. He discussed the

effectiveness of saramycetin, an antibiotic produced by Streptomyces saraceticus. The drug was found to be highly effective in experimental animal infections caused by Blastomyces dermatitidis, Coccidioides immitis, Histoplasma capsulatum and Sporothrix schenckii. The antibiotic also proved to be highly effective in the treatment of patients with aspergillosis, blastomycosis and histoplasmosis. Unfortunately for economic reasons production of this highly promising antibiotic was discontinued by the manufacturer.

Hamycin is another antibiotic produced by an actinomycete Streptomyces pimprina. It has been found effective in mice infected by Blastomyces dermatitidis, Cryptococcus neoformans and Histoplasma capsulatum. Oral therapy with hamycin has cured patients with blastomycosis. However, it has proven less effective in cases of histoplasmosis.

As can be seen from the above, the Congress attained the important goal of bringing together a group of active investigators studying the fungi, and host, social and environmental factors responsible for the mycoses. All gained much information and stimulus from their formal and informal discussions and returned to their benches and classrooms stimulated and more informed.