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REPORT ON THE IV INTERNATIONAL CONFERENCE ON THE MYCOSES

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The IVth International Conference on the Mycoses, sponsored by the Pan American Health Organization (PAHO), was held June 6-8, 1977 at the regional headquarters of PAHO in Brasilia, Brazil. The 68 participants represented 12 of the Western Hemisphere countries (Argentina, Brazil, Canada, Colombia, Costa Rica, Ecuador, El Salvador, Guatemala, Mexico, the United States of America, Uruguay and Venezuela). An additional speaker came from Japan under the sponsorship of the University of Tokyo, and the government of France sent an observer from the Pasteur Institute of Paris.

The themes of the first three International Conferences, held in 1970, 1971 and 1974, were General Medical Mycology, Paracoccidioidomycosis and the Immunology and Serology of the Mycoses, respectively. In chronological order, these important conferences were held at PAHO headquarters in Washington, D. C.; at the University of Antioquia in Colombia; and at the University of Sao Paulo, in Sao Paulo, Brazil. This year's Brasilia conference dealt with the pathogenic black and white yeasts. These are fungi that cause mild and superficial diseases such as tinea nigra or severe and systemic diseases such as cryptococcosis. Thirty-seven papers were presented during the 3 days of the meeting.

The black yeasts or "levaduras prietas" and the diseases that they cause were discussed on the first day of the meeting. These taxonomically challenging fungi were defined as dark-pigmented, filamentous fungi that, in certain stages of their in vitro development or under certain environmental conditions, have a unicellular stage during which time multiplication occurs by a budding process. At this stage the colonies are pasty and some shade of black.

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Five fungi fit into the category of pathogenic black yeasts. From the day they were first discovered, these fungi have been variously classified, mainly because of difficulties in interpreting their modes of sporulation. The procrustean taxonomic treatment they have undergone has led to a collective synonymy of more than 50 names. Until recently these five species were generally classified in the genera Cladosporium, Fonsecaea and Phialophora and Phialophora as C. werneckii, F. dermatitidis, P. gougerotii, P. jeanselmei and P. spinifera.

Three speakers dealt with the latest developments in our understanding and classification of these fungi. Studies based on the dynamics of spore formation have provided a new and definitive basis for their classification. Drs. Gary T. Cole, Michael McGinnis and Arvind Padhye, through the use of conventional light microscopy, electron and scanning electron microscopy, Nomarski differential interference contrast microscopy, and time-lapse photomicrography, found that spore formation or conidiogeny among these fungi was fundamentally different from that encountered in the genera Cladosporium, Fonsecaea and Phialophora. All concurred that C. werneckii properly belonged in the genus Exophiala, as had been proposed by Dr. Arx of the Netherlands. Similarly, P. gougerotii, P. jeanselmei and P. spinifera were also found to be best classified in Exophiala. In addition, McGinnis and Padhye, through comparative structural and biochemical studies, concluded that E. gougerotii and E. jeanselmei were one and the same species and that the name E. jeanselmei had priority.

The genus, Exophiala, described by Carmichael of Canada in 1966, is characterized by the development of spores by conidiophores known as

annellides. These produce spores from their tips. As each spore is liberated by the splitting of the septum that joins the spore to the annellide's tip, a series of superimposed rings is formed around the conidiophore; hence the origin of the name annellide.

Fonsecaea dermatitidis, also formerly classified in the genera Phialophora and Hormiscium, was found to have a previously undescribed type of conidiophore. Dr. McGinnis described the new genus, Wangiella, to accommodate this black yeast. It is now properly referred to as W. dermatitidis. The conidiophore of Wangiella, unlike that of the genus Phialophora, lacks an apical cup. This yeast had also been improperly placed in the genus Fonsecaea. The species in that genus have three different types of conidiophores (true phialides, acrotheca and Cladosporium type conidiophores), whereas Wangiella has only one predominant type.

Borelli of Venezuela reported the development of acervuli, sporodochia and pycnidia by several isolates of Aureobasidium (Exophiala) werneckii. These are previously undescribed sporulation mechanisms for this species. Their occurrence, however, does not alter the new classification of E. werneckii, since it is based in the predominant type of sporulation by annellides.

Colony variations among a group of isolates of Cladosporium castellani, a new agent of tinea nigra, were described by Dr. Carmen Marcano de Naime of Venezuela. The classification of this species was questioned during the ensuing discussion. Drs. Cole and Padhye plan to study conidiogeny in this species in an effort to confirm or correct its current classification

in the genus Cladosporium.

Dr. Ramon Lazo described black yeast infections that have been diagnosed in Ecuador since 1971. In Guayaquil two cases of tinea nigra caused by E. werneckii were confirmed by culture, as was a subcutaneous infection by E. jeanselmei. The incidence and prevalence of such infections would appear to be low, but only recently has attention been focused on the cultural diagnosis of the mycoses of Ecuador.

Studies carried out by Dr. Ismael Conti-Diaz in Uruguay cast new light on the ecology and epidemiology of the black yeasts. E. jeanselmei, E. spinifera, E. werneckii and Wangiella dermatitidis were recovered from such natural substrates as the straw in armadillo dens, fallen palm tree trunks, bird nests and forest soils. These findings imply that these fungi live and sporulate in nature as saprophytes and that infections result from some traumatic incident that introduces fungus elements into the tissues of susceptible individuals.

In his opening address, Ajello reviewed the history of the black yeasts and the diseases that they cause. A. pullulans is considered the most common and widespread of these fungi. It occurs in nature both as a saprophyte and as a plant pathogen. Human infections have not been convincingly documented. However, an infection in a porcupine was recently reported from the state of New York. Medical mycologists commonly encounter this yeast as a contaminant of clinical material.

A. pullulans was first described in 1866. In the ensuing years it acquired a synonymy of 36 specific names that were classified in 19 different genera. Difficulties in interpreting its mode of sporulation led to

different concepts of classification.

As a disease agent E. werneckii is the most prevalent of the black yeasts. It causes a very superficial skin infection, generally in the palms of the hand. *Tinea nigra palmaris* is most frequent in the tropics, but cases have developed in the temperate regions of the world also.

In contrast to the relative high frequency of E. werneckii infections, only one infection has been attributed to E. spinifera. However, doubt exists as to the status of E. spinifera as a distinct species. Studies may prove it to be a variant of E. jeanselmei.

It was proposed that E. jeanselmei be classified as a trimorphic fungus, along with several other fungi that develop in their infected hosts in two forms completely different from their in vitro mycelial forms. When introduced into subcutaneous tissues, E. jeanselmei forms black granules made up of sterile mycelial filaments. However, in the lungs and other internal organs, the tissues are invaded by nonsporulating, dematiaceous mycelium. In culture its mycelial colonies produce conidia from simple conidiophores or coremia and ascospores in ascocarps.

Failure to realize its trimorphic nature led to the belief that mycetomas with granules produced by E. jeanselmei was caused by P. jeanselmei and that mycelial systemic infections were caused by P. gougerotii. Petriellidium boydii and some dermatophyte species are also trimorphic, their tissue forms being sterile mycelium under one set of circumstances and granular in another.

Knowledge of the existence of Wangiella dermatitidis dates back to 1934 when Kano of Japan isolated this fungus from a destructive facial

lesion. Although Kano designated this infection as chromoblastomycosis, he was aware that it was atypical. On the basis of the tissue of this fungus, it is properly classified as phaeohyphomycosis.

W. dermatitidis forms conidia from phialides without collarettes. This fact separates it from all members of the genera Phialophora and Exophiala.

Three of the speakers dealt with chromoblastomycosis, a disease common throughout Latin American. It is caused by any one of four different fungi: Cladosporium carrionii, Fonsecaea compactum, F. pedrosai and P. verrucosa.

Dr. Nardo Zaias of Miami, Florida, reviewed the histopathology of this disease with especial emphasis on the diagnostic value of microabscesses. Exudate from these abscesses generally contains the numerous muriform, thick, dark-walled cells characteristic of the tissue form of the etiologic agents.

Dr. Clovis Bopp of Porto Alegre, Brazil, presented an exciting report on the successful treatment of chromoblastomycosis, which hitherto has been notoriously difficult to cure. Dr. Bopp used a combination of intravenous amphotericin B and orally administered 5-fluorocytosine. Drug tolerance was good and the relapse rate was remarkably low. Five patients have had a follow-up period of 12 months without relapse and one has had 32 months without relapse. If its efficacy is confirmed, Dr. Bopp's therapeutic combination will represent a remarkable breakthrough in the treatment of chromoblastomycosis.

Mr. Stuart Zweibel reported on studies carried out with Dr. C. J. K. Wang of Syracuse University of New York on soil and human isolates of Phialophora verrucosa. These investigators found differences in the collarettes of the phialides produced by these two groups of fungi. The soil or saprophytic isolates had long collarettes, while the human isolates had shorter ones. These investigators believe that the long collarette isolates should be identified as P. americana and the short collared ones as P. verrucosa. Both species invaded the livers and spleens of mice when injected intravenously. Electrophoretic immunological studies revealed antigenic differences that support the concept that the two forms are distinct species.

The black yeast sessions were concluded by Dr. Everett Beneke's report on infections among toads and frogs by dematiaceous fungi. In two epizootics that took place at Michigan State University, F. pedrosoi was isolated from a group of marine toads (Bufo marinus). Histologic examination showed that these amphibians had granulomatous lesions in their livers and kidneys.

A sterile dematiaceous fungus was isolated from leopard frogs (Rana pipiens) that had developed chromoblastomycosis in captivity. This fungus could not be induced to sporulate and thus remains unidentified.

The second and third days of the conference were devoted to the pathogenic white yeasts. In his opening address, Dr. Donald Ahearn of Atlanta, Georgia, defined these fungi as being hyaline or pastel-colored fungi that reproduce by unicellular budding in culture and that do not produce profuse aerial mycelium. The most prevalent and important of the pathogenic white yeasts are Candida albicans and Cryptococcus neoformans. Recent advances in surgical and chemotherapeutic procedures, however, have predisposed patients to infection by yeasts usually considered to be non-pathogenic. The emerging yeast pathogens are primarily C. parapsilosis and C. tropicalis, along with Torulopsis glabrata. Ahearn believes that other species of yeasts will most likely be found to be pathogenic in individuals with impaired immunological defenses.

Nine speakers presented papers on the public health problems posed by C. albicans and other species of that genus. Dr. Leonor Murillo de Linares of El Salvador described her epidemiological studies on the prevalence of Candida species in healthy women and contrasted her findings with those for women with clinical signs of candidiasis. C. albicans and C. parapsilosis were the most predominant species found in healthy women. C. albicans was most prevalent in the vaginal exudates of pregnant women and in those taking oral contraceptives. C. albicans was the most common etiologic agent in patients with suspected cases of candidiasis. More blastospores and mycelium were present in these patients' exudates than those of the healthy individuals.

Dr. Antar Padilha-Goncalves of Brazil illustrated the gamut of the mucocutaneous manifestations of candidiasis with a magnificent series of color transparencies. He commented that the prevalence of candidiasis has increased because of the impairment of immunological defence mechanisms by modern therapeutic procedures. The need for training in the mycoses by both physicians and diagnostic laboratory workers was emphasized.

The high prevalence of yeast infections in temperate Canada was brought to light by Dr. Alex Bakerspigel of Canada. Among the clinical conditions caused by the pathogenic yeasts, he included cutaneous, respiratory, urinary, genital, meningeal and ocular infections. Yeasts have also caused secondary infections in burn victims, diabetics, cancer patients, premature infants and others with impaired defence mechanisms. The most frequently isolated pathogen has been C. albicans, with other species encountered being C. neoformans, Torulopsis glabrata and C. tropicalis. Frequently yeasts were the sole microorganisms involved in the infections, but in some patients they were secondary invaders. Burn victims were especially prone to infection. In Ontario, 25% of a group of 122 burn patients were infected by C. albicans within 3 to 33 days after hospitalization. The common source of infection was the patient's own mouth or bowel, or both. Histories of yeast infections in patients with such diseases as Hodgkin's disease and diabetes were used to illustrate this presentation.

Brain infections by C. albicans have been an unexpected and frequent finding among patients treated on a long-term basis with broad spectrum antibiotics. Dr. Norman Goodman of Lexington, Kentucky, found in

a retrospective survey that among 48 patients with candidiasis, 25 had deep parenchymal lesions. Thirteen or 52% of these had brain involvement. All of these patients had been treated with antibacterial antibiotics for seven or more days. Experimental studies with rats showed that brain lesions occurred in animals treated with Kanamycin, tetracycline and cortisone. The incidence of brain infection was highest among rats treated with tetracycline. These findings suggest that in the treatment and management of systemic candidiasis, the possible occurrence of central nervous system involvement must be taken into consideration.

Dr. Amado Gonzalez-Mendoza of Guadalajara, Mexico, described his studies on the fungicidal and chemotactic response of polymorphonuclear leukocytes to the cells of C. albicans in patients with leukemia and lymphoma. He found that candidacidal activity was impaired in patients with active diseases. No differences were found in chemotaxis in patients with active and inactive disease.

Continued progress in the study of glycoprotein toxins produced by C. albicans was reported by Dr. Kazuo Iwata of Tokyo. He has now succeeded in purifying the lethal toxin known as candidotoxin and has isolated other toxic fractions from C. albicans and other unrelated fungi such as species of Aspergillus. All of the toxins were similar in lethality; the tissue lesions that they caused as well as their infection enhancing and immunological activities were also similar. Current studies are designed to determine which moiety of the glycoprotein toxins (mannan or protein, or both) is concerned with toxic activity.

A direct and rapid method for the detection and identification of

C. albicans was described by Dr. Mildred Corao de Feo of Caracas, Venezuela. With the use of either a milk or an oxgall medium, both re-enforced with chloramphenicol to inhibit bacteria, C. albicans was rapidly detected and identified in a wide variety of clinical specimens. If present, C. albicans will produce its diagnostic chlamydospores within 24-48 hrs. These simple, rapid and specific media will be especially useful in areas with limited laboratory facilities and without trained medical mycologists.

Dr. John Willard Rippon of Chicago, Illinois, described five inter-related experimental approaches to the detection of antigen or antibody in patients with occult candidiasis. Immunodiffusion and hemagglutination procedures were used in parallel to evaluate their relative efficacy in the detection of antibodies. The hemagglutination test proved to be the most sensitive of the two procedures. The immunodiffusion test does not detect antibodies in patients with leukemia or lymphoma owing to the nature of those diseases and the effects of cytotoxic therapy.

In early studies carried out by Dr. Henry E. Jones of Atlanta, Georgia, it was shown that an automated microassay method was highly effective for the measurement of fungus growth. The assay is based on the incorporation of ^{14}C glucose into the cell wall of the test fungus' mycelium. The amount of labeled glucose taken up correlated well with turbidity estimates and with the dry weight of the fungal mat. Although the technique worked well with filamentous fungi, it proved to be less effective with unicellular fungi such as C. albicans. Tests with Cryptococcus neoformans are planned for the future.

A highly novel and exciting approach to the development of anti-candidal drugs was described by Dr. Jeffrey M. Becker of Knoxville, Tennessee. He and his colleagues have been synthesizing drugs that are selectively toxic for C. albicans. A peptide transport system is then used to carry the toxic agents into this fungus. Structural restrictions are incorporated into the peptide drug conjugate to prevent permeation into host tissue. Knowledge of the structural specifications of the peptide transport systems in host and pathogen is being used in synthesizing peptide drug conjugates which will be evaluated in the near future. Dr. Becker's studies point to the future directions in the chemotherapy of human diseases.

The paper presented by Dr. Awatar Sekhon of Alberta, Canada, dealt with infections by Torulopsis glabrata. He described a devastating secondary infection by this white yeast in a patient suffering from intestinal bleeding and renal failure. T. glabrata probably entered the patient's blood system through contamination of an arteriovenous cannula. Despite the administration of amphotericin B and 5-fluorocytosine, the patient died.

The importance of cryptococcosis as a public health problem was brought out in the series of ten papers that were devoted to that disease. Using diagnostic data accumulated since 1965 at the Center for Disease Control in Atlanta, Georgia, Dr. Leo Kaufman showed a significant increase in the number of cryptococcosis cases that have occurred in the United States over a 12-year period. In 1965 only 24 confirmed cases of cryptococcosis were reported. Twelve years later in 1977, 338 cases were

confirmed. The monograph on cryptococcosis published by Littman and Zimmerman in 1956 showed that only 300 cases of the disease had been recorded in the world's literature for the 55-year period of 1900-1955. In his 12-year survey, Kaufman documented 1,264 cases of cryptococcosis for the United States alone. These represent only a fraction of the number of cases of cryptococcosis that must occur in the United States since the data came from only one laboratory, that of the Serology Branch of the CDC's Medical Mycology Division. The increase in confirmed cases is not due to a higher incidence of cryptococcosis but to the development and use of specific serological procedures for the diagnosis of cryptococcosis. The maximal diagnosis of cryptococcosis is achieved through the use of a battery of serological tests made up of the latex agglutination, indirect fluorescent antibody and tube agglutination procedures. These serological tests can be used not only for diagnostic purposes but also for monitoring the effectiveness of therapy.

The high prevalence and incidence of cryptococcosis in Latin America was made evident by the reports of Drs. Donald L. Greer of Cali, Colombia, and Ferando Montero-Gei of San Jose, Costa Rica. Greer was able to document 56 cases of cryptococcosis. He pointed out that most of these cases had been diagnosed at autopsy and that most Colombian physicians are unaware of the disease's existence. Studies carried out in Colombia have shown that the pigeon droppings are infested with Cryptococcus neoformans and that the fungus is present in the environment. Greer bemoaned the lack of published information on cryptococcosis in Colombia and the rarity

of diagnostic facilities for mycotic diseases. These facts, coupled with the frequent confusion of cryptococcosis with the more common chronic tubercular meningitis, may explain the low number of recognized cases in that country.

Dr. Montero-Gei described and beautifully illustrated 11 cases of fatal cryptococcosis that had occurred in Costa Rica. He reported that 17 of 100 samples of pigeon droppings collected in 23 localities in four provinces were found to contain C. neoformans. A correlation was established between two human cases of cryptococcosis and the isolation of C. neoformans in pigeon droppings collected in the patient's neighborhood.

In the United States, Patricia Bowman found records of 87 cases of cryptococcosis that occurred in the state of Georgia. Six of 12 pigeon dropping samples collected in Atlanta were positive for C. neoformans. Some of these specimens had as many as 40,000 viable cells per gram of droppings.

Experimental studies on the dynamics of airborne C. neoformans infections were reported upon by Dr. Howard Larsh of Norman, Oklahoma. Infections were induced in mice by exposing them to aerosols of C. neoformans in a Henderson apparatus. The resultant pulmonary infection served as the primary source of subsequent dissemination of the yeast to the liver, spleen, kidney and, finally, to the brain. Dissemination occurred late in the disease course and in less than 50% of the test animals. The murine model of cryptococcal infections simulates human cryptococcosis and will lend itself to therapeutic investigations and other types of study.

Dr. Errol Reiss of Atlanta, Georgia, described an ingenious method for determining antigen-elicited delay hypersensitivity in mice infected with Candida albicans or Cryptococcus neoformans. Mice infected subcutaneously in the footpads with C. neoformans were tested with several subcellular antigenic fractions of this yeast. The most active antigen was the postmitochondrial supernate. The immune response was measured with the footpad swelling test based on volume-displacement. The procedure will permit the selection of antigenic fractions that would be most useful in developing a skin test for cryptococcosis.

A skin test survey of 198 soldiers in San Paulo, Brazil, with a crude C. neoformans antigen was described by Dr. Carlos da Silva Lacaz. Twelve or 6% of the subjects gave positive reactions with induration areas more than 5 mm wide. The positive reactors all had normal chest X-rays and negative serologic reactions. An earlier survey of 190 patients in a Sao Paulo hospital with the same antigens revealed 5 reactors. Thus out of 388 individuals tested, 43% were positive. These findings lend support to the concept that subclinical or asymptomatic infections by C. neoformans occur in the general population.

Dr. Arthur DiSalvo of Columbia, South Carolina, evaluated an automated procedure for performing the indirect fluorescent antibody test for cryptococcosis. An apparatus manufactured by the Aerojet-General Corporation was used to test split samples of sera from patients with suspected cases of cryptococcosis, and results with this apparatus were compared with results obtained at the Center for Disease Control with its automated

test. Results obtained with the two tests were comparable, and it was concluded that the apparatus would be useful in laboratories having large numbers of sera to test. The apparatus gives less subjective results and relieves the laboratory worker of much tedium.

Since carbon source assimilation is used to identify members of the genus Cryptococcus, Dr. Adhemar Purchio of Sao Paulo, Brazil, screened 90 carbon compounds. Although correlations between serotypes and carbon metabolism were not found, the patterns of utilization could aid in the identification of species.

Dr. K. J. Kwon-Chung of the National Institutes of Health, Bethesda, Maryland, spoke on the sexuality of Cryptococcus neoformans. Her genetic studies revealed that most isolates of C. neoformans are heterothallic and self-sterile. They reproduce sexually by cross-mating. A few isolates are encountered that are self-fertile and they can complete sexual reproduction without mating. These self-fertile isolates soon lose fertility and revert to one mating type. Genetic analysis showed that self-fertility was synthesized during heterothallic sexual reproduction and that self-fertility is lost during the asexual reproduction cycle.

The pathology of three yeast infections, candidiasis, cryptococcosis and torulopsosis, was surveyed by Dr. Karlhanns Salfelder of Merida, Venezuela, in a comprehensive, lavishly illustrated review. His extensive experience provided a deep insight into each disease's characteristics which enable the pathologist to arrive at a specific diagnosis.

A most informative paper was presented by Dr. William Kaplan of Atlanta,

Georgia. He thoroughly reviewed all known cases of human and animal infections caused by the pathogenic species of Prototheca. These microorganisms develop yeast-like colonies, but resemble chlorophyllless algae morphologically. In addition, Dr. Kaplan described several cases of animal infections in which the etiologic agent proved to be a green alga classified in the genus Chlorella. Electromicroscopic studies and DNA homologies need to be carried out to determine whether or not the Prototheca species are algae that have lost their chlorophyll or are a new type of fungus.

The conference came to a close after the summation was delivered by Dr. Hillel Levine of Oakland, California.