WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 7, 287-311. Research Article ISSN 2277– 7105

THE MEDICAL MYCOLOGY AND THE LEGACY OF ANTONIO GONZALEZ OCHOA

Cudberto Contreras Pérez[1] and José D. Méndez[2]*

[1]Mycology Laboratory. Instituto de Diagnóstico y Referencia Epidemiológicos. Secretaría de Salud. México City, México.

[2]Medical Research Unit in Metabolic Diseases. Cardiology Hospital. Instituto Mexicano del Seguro Social. México City. México.

Article Received on
12 April 2019,
Revised on 02 May 2019,
Accepted on 23 May 2019
DOI: 10.20959/wjpr20197-15163

***Corresponding Author**
Dr. José D. Méndez
Medical Research Unit in Metabolic Diseases. Cardiology Hospital. Instituto Mexicano del Seguro Social. México City. México.

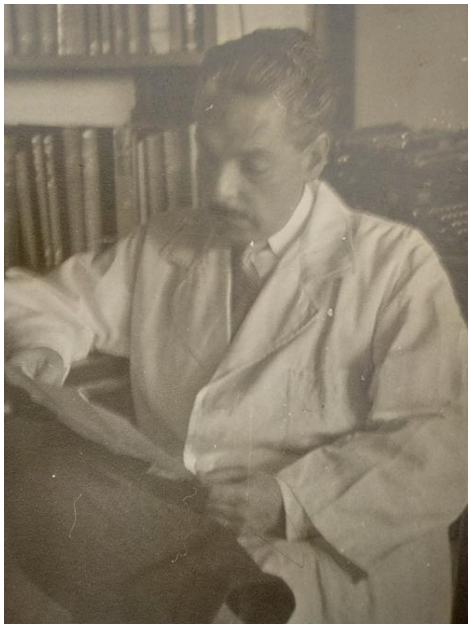
ABSTRACT

This work presents an approach of the scientific work of Dr. Antonio González-Ochoa, prominent Mexican mycologist and dermatologist. His research had a deep transcendence in the national and international medical mycology. Highlights the isolation and identification for the first time of *Histoplasma capsulatum*, *Fonsecae pedrosoi* var. *cladosporioides* and *Actinomyces mexicanus*, synonym of *Actinomyces brasiliensis*. The study of actinomycetes contributed to the reclassification of species and confirmation of the four current genera of medical importance: *Actinomyces*, *Actinomadura*, *Streptomyces* and *Nocardia*. His clinical classification of mycoses, extended to histoplasmosis, coccidioidomycosis, paracoccidioidomycosis, candidosis and sporotrichosis, for a better understanding of these mycoses. In histoplasmosis and coccidioidomycosis, his contributions on the epidemiology and geographic distribution are classic, applying about 40, 000 and one million tests with histoplasmin and coccidioidin. In the dermatophytosis showed in the histopathology, that the lesions are of the allergic eczematous dermatitis type, produced by an allergen released by fungi. He was a pioneer in obtaining polysaccharide antigens in actinomycete mycetoma and sporotrichosis, along with metabolic antigens in histoplasmosis, coccidioidomycosis, aspergillosis, candidiasis and paracoccidioidomycosis, allowed to establish immunological tests, which facilitated the diagnosis and prognosis of these mycoses. The development of the first experimental model in animals on the reproduction of actinomycetic mycetoma and chromomycosis, was determinant for the investigations on pathogenicity and virulence of the

Nocardia species, and continues to evaluate drugs in the treatment of these mycoses. In the most relevant mycoses for Mexico, histoplasmosis, coccidioidomycosis, cryptococcosis and dermatophytosis, we have updated the epidemiological panorama with the recent investigations developed in recognition of his work as a researcher and his contributions to medicine.

KEYWORDS: Coccidioidomycosis, Cryptococcosis, Dermatophytosis, Histoplasmosis.

1. INTRODUCTION



Dr. Antonio González-Ochoa (1910-1984)

Dr. Antonio González-Ochoa, graduated from the Universidad Nacional Autónoma de México prominent mycologist and dermatologist, founded the Laboratory of Mycology in 1940 and organized the Department of Mycology and Tropical Dermatology of the Institute of Health and Tropical Diseases, now the Instituto de Diagnóstico y Referencia Epidemiológicos (InDRE). He developed a brilliant work as a researcher and teacher, most of the mycologists and dermatologists were trained with him.[1] He left as a legacy important contributions to national and international medical mycology, highlighting research in actinomycetic mycetoma, sporotrichosis, cocidioidomycosis, histoplasmosis, dermatophytosis, candidosis, paracoccidioidomycosis and cryptococcosis.[2-8] In this work, we present a brief description of the main investigations by area of medical mycology and the epidemiology of the most important mycoses, as well as of the activity that is currently carried out in the InDRE since its foundation to date.

2. TAXONOMY OF PATHOGENIC FUNGI

2.1. Chromomycosis

In 1941 the first case of a mycosis confirmed by the isolation of *Fonsecaea pedrosoi* var. *cladosporioides*.[9] The patient presented a dermatosis of 30 years of evolution, it had been studied in 1940 and in the histological sections of the lesions fumagoid cells had been observed, publishing the case as a probable verrucose dermatitis.[10]

2.2. Dermatophytosis

In 1934 the dermatophytes grouped three genera: *Trichophyton*, *Microsporum* and *Epidermophyton*. The genus *Trichophyton* was divided into five groups, Gypseum, Crateriforme, Faviforme, Rubrum and Rosaceum. The Crateriforme group comprised four species: *tonsurans*, *epilans*, *sabouraudi* and *sulfureum*. The study of the trichophytic tinea reduced to a single species the crateriform group: *Trichophyton tonsurans*, contribution that was accepted internationally, based on the morphological characteristics.[11]

2.3. Actinomycetic mycetoma

In 1942 a pathogenic actinomycete was isolated and identified in a Mexican mycetoma patient living in Los Angeles, California, USA, who had some differences with the *Actinomyces mexicanus* described by Boyd and Crutchfield. The comparative study of several Mexican strains with other pathogenic actinomycetes of reference, allowed to identify that this group of species had characteristics of *Nocardia* and not of *Actinomyces* establishing that the specie *Actinomyces mexicanus* was synonymous with *Actinomyces brasiliensis* and different from *Actinomyces asteroids*,[12] later these species would be reclassified in the genus *Nocardia*.

In 1943 there was a huge confusion in the species grouped in the genus Nocardia. The serological study of 11 species of *Nocardia*, 1 of *Actinomyces* and 3 of *Streptomyces*, clearly allowed to identify 4 different groups that had international acceptance.[13] The results then contributed to the reclassification of species and together with other investigations in 1955,[14] as well as other mycologists,[15,16] allowed to define the 4 groups of medical importance of actinomycetes, which currently correspond to the genera *Actinomyces*, *Actinomadura*, *Streptomyces* and *Nocardia*, in the latter genus several species ended up being synonymous with *Nocardia brasliensis* or *Nocardia asteroids*.[17]

2.4. Candidosis

In 1943, the first study was performed, isolating Candida for the first time in patients from Mexico City. *Candida albicans*, *Candida guilliermondii*, *Candida psudotropicalis*, *Candida parakrusei* and *Candida deformans*. These yeast-like fungi showed great variations and it was necessary for their identification to combine the study of the morphological and biochemical characteristics, since some prominent mycologists of the time only considered the morphological characteristics.[18]

3. CLINIC

3.1. Clinical classification of mycoses

In 1956 he proposed a classification based on the special affinity that pathogenic fungi have for the cutaneous tegument.[19] It divides mycoses into three groups: exclusively tegumentary fungal infections (superficial mycosis), fungi that affect the skin and its annexes, include tinea or dermatophytosis, pityriasis versicolor, erythrasma, tinea nigra, otomycosis and stones. Initially tegumentary mycoses (subcutaneous mycosis) group the mycoses where fungi penetrate the skin or external mucous membranes, invading the dermis, subcutaneous tissue, muscles, aponeurosis, bones, lymphatics and even viscera. They include chromomycosis, mycetoma in its two modalities, sporotrichosis, rhinosporidiosis and tegumentary candidosis. The third group, secondarily tegumentary mycosis (systemic mycoses), includes those mycoses where fungi frequently enter the respiratory tract, grouping histoplasmosis, coccidioidomycosis, paracoccidioidomycosis, blastomycosis, cryptococcosis, nocardiosis and deep candidosis.

3.1.1. Histoplasmosis

To better understand the clinical behavior of histoplasmosis in Mexico, in 1957 he proposed some variants in the clinical classification proposed by Furcolow in 1956, grouping the disease into non-progressive primary and secondary progressive, the primary in turn was divided into asymptomatic that comprised the most of the cases, while in the symptomatic it was divided into mild, moderate and severe. The secondary histoplasmosis comprised the acute type and the chronic type. The first includes cases of endogenous reinfection and the primary cases of severe progressive symptoms. The acute type is often fatal in the short term and affects older children, while the chronic type is often confused with tuberculosis due to the pulmonary location of the lesions, its evolution is several years and is practically fatal.[20]

In 1957, the importance of primary acute pulmonary histoplasmosis in Mexico from 1948 to 1957 (8 epidemic outbreaks and 1 isolated case) was highlighted, with cases in the States of Coahuila, Durango, Tamaulipas, San Luis Potosi, Queretaro, Guerrero, Nayarit and Yucatán.[21]

In 1959,[22] the primary acute pulmonary histoplasmosis is addressed, which records the data of 9 epidemics and two isolated cases, highlighting the clinical aspects, diagnostic procedures, the association of infections with bat guano, the wide distribution in the country and the severity of the infection that reaches a 28.4 of lethality in 74 cases.

In the period from 1961 to 1964 the great importance of severe epidemic histoplasmosis and the use of amphotericin B in treatment with good results is confirmed.[23-26] In the Colima outbreak of the 59 subjects exposed, in 35 of them it was possible to apply intradermal reaction with histoplasmin, in 28 of these patients the test was negative, while in 7 the test was positive and the subjects did not become ill, this fact allowed to establish as a preventive measure of histoplasmosis, which is only contracted to work histoplasmin positive individuals. This observation was converted into a preventive measure and established by presidential decree, in order to avoid outbreaks and to continue to exploit guano as fertilizer. The decree required that workers entering caves be recruited among positive histoplasmin individuals.[24]

These investigations radically changed the knowledge of acute pulmonary primary histoplasmosis during the period from 1958 to 1964.[26] In chronic secondary histoplasmosis, important data were also obtained, by studying 1132 sera from patients from 5 institutions for tuberculosis, 30% from samples, 221 with positive serology by complement fixation for histoplasmosis.[27]

A final work in 1977[28] on histoplasmosis includes the communication of the epidemic outbreak in the State of Hidalgo, where 15 workers were infected and 6 were fatal cases. In one of the cases studied, it was proposed that it was due to reinfection due to the presence of already calcified lesions suggestive of previous histoplasmosis, an aspect that was confirmed by the histopathological examination of the lesions. Currently these observations would be confirmed in histoplasmosis. This disease has become a major obstacle to the exploration of old abandoned mines, hindering the country's mining development.[29]

3.1.2. Sporotrichosis

In 1963[30-31] he proposed a clinical classification that explained the different clinical modalities that the disease presents and that other clinical classifications did not consider. The classification divided the mycosis into tegumentary and internal. The first would group three clinical forms: the lymphangitic, the fixed type and the hematogen. The lymphangitic would include the ascendant of the extremities and the rubbery of other regions. The fixed type would present 5 clinical variants including the forms: ulcerative, verrucous, acneiform, infiltrated plaque and erythematous-squamous, while the hematogen would be observed as the gummy disseminated throughout the integument. The internal cases would include the primitive pulmonary, secondary to the lymphangitic and fixed tegumentary types, and the concomitant with the cutaneous hematogenous type. This classification was published in the Handbook of Tropical Dermatology edited by Simons in 1953 and was also adopted in some classic Dermatology treatises.

3.1.3. Paracoccidioidomycosis

In this mycosis, the South American mycologists expert in the disease had proposed that the primary lesion occurred in the oral, nasal or skin mucosa and hence the fungus would be disseminated hematogenously to other regions. Gonzalez-Ochoa in 1972 proposed for the first time the airway studying three Mexican cases.[32] This proposal had been made since 1956, studying 2 Mexican cases, the picture was pulmonary and the oropharyngeal and cutaneous lesions were presented secondarily.[33] It is now known with certainty that the respiratory route is the entry route for *Paracoccidioides brasiliensis*.

3.1.4. Candidosis

It is conditioned to multiple factors and it is essential to understand the increase in the population of *Candida*, a biological phenomenon caused by various circumstances, of the pathological disease phenomenon. He proposed the division of candidosis into tegumentary and deep to clarify this concept. The tegumentary candidiasis would comprise the cutaneous and mucosal clinical forms producing a lesion in the superficial layers, without going beyond the dermis and the chorion. In the external tegument, candidiasis would originate intertrigos, eczematiform lesions, onyxis, perionixis and affection of the external auditory canal; in the mucous tegument it would produce oral, esophageal, bronchial, intestinal, urinary and genital candidiasis.

Deep candidosis would include those lesions where the yeasts invade structures beyond the dermis and the chorion, attacking all kinds of tissues or systems, visceral locations, the nervous system, serous, etc. The diagnosis of candidosis in both forms, would imply that in the tegumentary candidosis the microscopic examination would be the diagnostic test of choice, the culture would be only for determinative purposes of the species, while in the deep candidosis the microscopic observation of the species was not indispensable. yeast, isolation from blood, cerebrospinal fluid, ascites, empyema, closed abscesses, would establish the diagnosis. The other resource would be histopathology, although there was no characteristic tissue reaction, the presence of yeast, pseudohyphae and hyphae would be conclusive for the diagnosis.[34]

4. EPIDEMIOLOGY

4.1. Histoplasmosis

The exploration of the cutaneous reactivity with histoplasmin, during the period from 1948 to 1971, was carried out in 33 549 individuals grouped in 34 localities belonging to 21 States of the Mexican Republic. The percentages of positives ranged between 5 and 50%. The results allowed to know its distribution in Mexico and what was the magnitude of the problem in some endemic areas (center, south and southeast of the country), as well as knowing that histoplasmosis should be considered in the differential diagnosis of respiratory diseases in any area of our country.[35]

This disease has now been confirmed to be the most important secondary tegumentary or systemic mycosis in the country. In the period from 1956 to 1998 there were 102 epidemics and 1444 patients.[36] During the period from 1999 to 2016, there were 15 more outbreaks with 2563 patients, of whom 620 had positive serology (24.1%) for IgM antibodies against *Histoplasma capsulatum*. 10 outbreaks occurred in the open field, 4 in confined spaces (caves or caves) and 1 in a sinkhole. No fatal cases were recorded, but a significant percentage of patients required hospitalization. It stands out in these outbreaks, the histoplasmosis acquired in the open field.

Figure 1 shows the records of epidemic outbreaks in the ISET-InDRE, excluding epidemics from 1948 to 1955, for incomplete data. The State of Guerrero ranks first with 23 outbreaks, followed by Puebla with 13, Chiapas with 7, Morelos and Colima with 6, Querétaro with 5 and Tamaulipas with 4. The other States have a lower frequency between 1 and 3 outbreaks. Two outbreaks of open field epidemics occurred in 2001 in a hotel in Acapulco, Guerrero. It

is estimated that about 30,000 people were exposed in each event. 1050 people were studied, 694 in the first outbreak and 356 in the second. The percentages of positive symptoms were 34.2 and 21.3%, respectively.[37]

4.2. Coccidioidomycosis

The first coccidioidin survey was conducted in Hermosillo, Sonora, in 1944.[38] From 1961 a systematic program was established in most of the States, applying nearly one million tests. The percentages of positive cases were distributed in four groups of positive reactors: from 5 to 9.9; from 10 to 29.9; from 30 to 49.9; and from 50 to older.[39] The geographical distribution extends through three large areas: the northern, the Pacific and the central, plus two tropical micro-areas, a surface that covers more than half of the national territory. The information allowed to know in which States of the Republic the disease exists, which ones are free, as well as the magnitude of the problem in some endemic areas.

Current data on epidemiology reveal that in the In DRE during the period from 1957 to 2017, there were 93 cases of disseminated coccidioidomycosis.[37] The State of Michoacán is the one that has registered the highest number of cases, followed by Coahuila, Sonora, Chihuahua, Sinaloa, Baja California and Tamaulipas. Less frequently are San Luis Potosí, Durango, Zacatecas, Aguascalientes and Estado de México. Predominantly cases with skin and lymph node involvement, followed by the pulmonary and central nervous system (meningitis and meningoencephalitis). Occasional isolations have occurred in bone marrow, peripheral blood, bronchial lavage and pleural fluid.

Regarding primary pulmonary coccidioidomycosis, during the period from 1960 to 2016, there were 435 positive cases (21.6%) of a total of 2011 serological samples. The states that have sent the most samples are Chihuahua, Sonora, Coahuila and Tamaulipas. The distribution in the other States behaves in a similar way to the cases registered in the disseminated forms. In 2011, the State of Sonora, Arizona, Chihuahua and New Mexico, initiated a collaboration with several institutions, among them; The Center for Diseases Control and Prevention, USA, Dirección General de Epidemiología and the InDRE to study the importance of this mycosis. Two commercial tests (Meridian) were used, an enzyme-linked immunosorbent assay (ELISA) as a screening test and immunodiffusion as a confirmatory test. The results revealed a higher prevalence in Sonora. In both States, 25 cases of coccidioidomycosis were confirmed in 169 positive samples by ELISA.

Recently the InDRE collaborated in a study of molecular biology, where the results obtained refer current data on the prevalent species of the genus Coccidioides,[40] the *Coccidioides* species predominating widely *Coccidioides posadasii*. In a previous work on the description of the new species *Coccidioides posadasii* in 2002, 9 strains of the InDRE were studied with code 52, seven strains were reclassified as *Coccidioides posadasii* and 2 were kept as *Coccidioides immitis.*[41]

Figure 1: Number of outbreaks of acute pulmonary primary histoplasmosis, by State, ISET- InDRE: 1955-2016.

4.3. Dermatophytosis

The first studies in 1947 revealed the high incidence of tinea capitis caused by *Trichophyton tonsurans* and a low percentage by *Microsporum canis.*[42] Subsequently, the agents involved in tinea corporis, tinea pedis, tinea cruris and tinea favosa were studied. The study of 65 cases with 52 positive cultures identified the following species in order of frequency: *Trichophyton mentagrophytes* 30.9%; *Microsporum canis* 25%; *Trichophyton rubrum* 21.2%; *Trichophyton tonsurans* 15.3%; and *Epidermophyton floccosum* 3.8%. Tinea corporis (22 cases) and tinea pedis (21 cases) were the most frequent cases, while in tinea cruris there were 6 cases and in tinea favosa 1 case, this one caused by *Trichophyton tonsurans.*[43]

In 1955 he found that tinea pedis is one of the five most frequent dermatoses in the Mexican population.[44] The isolated species were *Trichophyton mentagrophytes* 50%, *Epidermophyton*

floccosum 20% and *Trichophyton rubrum* 10%. In tinea corporis the agents were *Trichophyton rubrum* 35.5%, *Trichophyton tonsurans* 30%, *Microsporum canis* 20% and *Trichophyton mentagrophytes* 10%; while in tinea cruris the isolated species were *Trichophyton rubrum* 50%, *Trichophyton mentagrophytes* 20% and *Epidermophyton floccosum* 20%.

In 1957 with the study of 100 cases of tinea unguis[45], the panorama of the distribution of dermatophytes was completed. *Trichophyton rubrum* was isolated in 50%, *Trichophyton tonsurans* in 37% and *Trichophyton mentagrophytes* in 13%. In this study the culture exhibited a low sensitivity and was only positive in 32 cases.

Finally, in the period from 1960 to 1970[46], a total of 785 cases with positive culture were analyzed. Tinea capitis (234 cases) and tinea pedis (227 cases) were the most frequent, while tinea corporis (154 cases) was very similar to tinea unguis (153 cases) and tinea cruris occupied the least frequency with 23 cases. The isolated dermatophytes in order of frequency were *Trichophyton rubrum* 253, *Trichophyton tonsurans* 239, *Trichophyton mentagrophytes* 165, *Microsporum canis* 108 and *Epidermophyton floccosum* 20. The comparison of the frequency of the different clinical forms between the period from 1960 to 1970, with the period from 1940 to 1950, revealed insignificant differences.

4.4. Candidosis

There are numerous observations that show an imbalance in the normal intestinal microbiota, from the use of sulfonamides and antibiotics. The study in 1947 of 100 stool samples, before the use of antibiotics, compared with two studies in 1951 and 1956, clearly showed a marked increase in the carriers of *Candida albicans*, which was 44 to 77%. A modification was also observed in the composition of the intestinal microbiota with the isolation of *Candida tropicalis*, as well as a decrease in *Candida parakrusei* from 38 to 15%.[47]

The development of candidiasis as a disease seems to be related to the constitutional modification of the host, rather than to the pathogenic factor of the yeasts. The study of 65 cases of oral candidosis in 1955, allowed to know some epidemiological factors on the pathogenesis of the disease. Oral candidiasis affected 4.6% of children who lived directly with the mother (frequency of 4.4% in full-term and 9.6% in premature infants) and who were breast-fed, while children who were separated from their mothers and that were bottle-fed, candidosis had a frequency of 0.17%. In relation to age, candidiasis showed a marked

increase between 5 and 9 days (44.9%) and fell sharply between 15 and 19 days (5%). No contacts were found with candidosis in mothers or staff.[48]

The yeasts of the genus *Candida* and particularly *albicans* penetrate the human body during the first days after birth, are installed in the intestinal tube and less frequently, in decreasing order, in the mouth, skin and mucous membranes open to the outside. Isolates from exogenous sources have been exceptional. The sampling during 1 year of anemophilic fungi did not allow the isolation of *Candida albicans*.[49] The most important source of colonization is the mothers' vagina. This hypothesis was proposed by González-Ochoa since 1955.[48] and was confirmed by performing cultures of oral and rectal mucosa in 100 newborn children. Colonization by *Candida* occurred in 90% of children 30 days after birth.[50]

4.5. Cryptococcosis

In cryptococcosis until 1960, no cases had been recorded. During the period from 1961 to 2000 there were 31 cases, of which 6 were HIV^+. During 2001 to 2016, 37 more cases were registered, of which 6 were HIV^+. In both periods there were 8 patients in treatment, with positive microscopic examination and negative culture. The tendency of cryptococcosis in recent years is to increase, considering the increase of clinical samples.

In 2002, 25 strains of *Cryptococcus* sp were identified, 14 as var. *neoformans* and 11 as var. *gattii*.[51] The strains were sent to the Ibero-American Group. There was a 100% agreement in the two varieties, but the strains considered as var. *neoformans* were reclassified in the new var. *grubii*.[52] Currently the genus *Cryptococcus* includes two species of medical importance. In isolates from the InDRE, the predominant species is *Cryptococcus* neoformans var. *grubii* with 57.6%, followed by *Cryptococcus gattii* with 42.4%. The Ibero-American Group has focused its research on the VGIII biotype of the species *Cryptococcus gattii*. In a recent study,[53] 10 strains of the InDRE were included. The group identified that the VGIII biotype is an emerging pathogen of disease in humans and animals throughout the world.

4.6. Actinomycetic mycetoma

During the period from 1942 to 1982, 272 cases of mycetoma were studied. The predominant species was *Nocardia brasiliensis* with 231 cases and with a much lower frequency of *Actinomadura madurae* in 18 cases; *Nocardia asteroides* in 8; *Nocardia otitidis caviarum* in 2 and *Streptomyces* sp in 1 case. In 2005, 12 isolates of *Nocardia asteroides* complex were studied by PCR / RFLP / Hsp 65. Nine strains were identified *as Nocardia asteroides* with

several subtypes (I, II and VI), one as *Nocardia farcinica* and two as *Nocardia* sp. The cultures came from different patients of a single Hospital. The data ruled out the possibility of a common source of contamination.[54]

In the period from 1983 to 2010, 23 strains of *Nocardia brasiliensis* and 4 of the *Nocardia asteroides* complex were selected to amplify a fragment of 606 nucleotides of the gene coding for the fraction 16S rRNA, with the oligodeoxynucleotides Noc1 and Noc2. Most strains were sequenced by the universal 16S rRNA gene with a size of approximately 1500 bp.[37]

The results of genotypic identification correlated in 20 strains of *Nocardia brasiliensis*, but 2 strains were identified as *Nocardia wallacei* and *Pseudonocardia* sp. Of the 4 strains of *Nocardia asteroides* complex, these were identified as *N. nova*, *Nocardia seriolae*, *Micromonospora echinospora* and *Nocardia otitidiscaviarum.* The species *Nocardia seriolae* and *Micromonospora echinospora* are considered nonpathogenic; The first strain was isolated from pleural fluid and pustules in the forearm of a malnourished 15-year-old patient with a severe prognosis. The second species was isolated by blood culture in a patient with a diagnosis of severe thrombocytopenia and pancytopenia (unpublished data).

The identification of pathogenic actinomycetes by biochemical tests showed an excellent correlation in urea, casein and gelatin, but the tests with tyrosine, hypoxanthine and xanthine were highly variable and did not correlate with the positivity percentages reported in the literature.[15,16] The disadvantage observed are the delayed reactions that occur between 15 and 45 days. Twenty strains of *Nocardia brasiliensis* casein positive, only 13 of them hydrolyzed tyrosine and only 7 gave a weak reaction to hypoxanthine. In 3 reference strains of *Nocardia otitidiscaviarum*, in two of them the xanthine test was positive at 50 days and occurred as a weak positive reaction. The strain identified as *Nocardia otitidiscaviarum* by molecular biology had given the negative xanthine test, the test was repeated and considered negative at 45 days.

5. DIAGNOSIS (SKIN TESTS AND SEROLOGY)

5.1. Coccidioidomycosis

Laboratory tests for the diagnosis of primary pulmonary coccidioidomycosis were established in the United States of America by Smith et al. in 1956. The tests were the intradermal reaction with coccidiodine, the tube precipitation that detected Ig M antiserum and the

complement fixation for IgG antibodies. The scope, significant importance and limitation of these tests, was related to the phase or stage of the disease, time of evolution, clinical form, intensity of the lesions and their location.

These well-managed and interpreted tests have a high diagnostic and prognostic value, so that it is not possible to manage a case without the use of these resources. Its practical application in patients studied at the Institute of Health and Tropical Diseases, contributed to the study of residual, disseminated and late forms of primary infection.[55]

5.2. Histoplasmosis

The immunological resources were widely studied. The skin test with histoplasmin, unlike coccidioidin, has very variable results and in acute primary pulmonary histoplasmosis the conversion from negative to positive occurs late. The reaction of precipitation in tube and fixation of complement, are an important support when they are correctly evaluated. Tube precipitation from the beginning has been a fundamental test for the diagnosis of outbreaks of primary pulmonary histoplasmosis, to date only exceeded in sensitivity (10-12%) by the ELISA.[36] The fixation of complement has diagnostic and prognostic value, and is very useful in the chronic type.[24]

5.3. Sporotrichosis

In patients with negative culture, it was necessary to develop other tests to confirm the presumptive clinical diagnosis. In this part, the intradermal test of sporotricin was contributed. The polysaccharide antigen *of Sporothrix schenckii* was obtained in 1947.[56] The initial evaluation revealed great practical utility, the test was applied in 12 cases of sporotrichosis and was positive in 11 of them. A case of generalized sporotrichosis was negative. The antigen showed great specificity and no cross-reactions were observed with other diseases, including other mycoses.

The test with sporotricin was widely accepted in medical practice and the specificity has been confirmed in several hundred patients. The results with sporotricin also allowed us to confirm the great importance of packing grass in the acquisition of sporoticosis as a disease.[57]

The other test used was indirect immunofluorescence, with an anti-*Sporothrix schenckii* antibody labeled with fluorescein isothiocyanate.[58] The IFI was standardized in an animal model and applied in three human cases. The test was evaluated in 34 patients with positive

culture.[59-60] Thirty-one cases were positive with a sensitivity of 89%. In 12 cases with negative culture, one of the patients was positive by the indirect immunofluorescence test, this patient was also positive to the intradermal test with sporotricin. The advantage of indirect immunofluorescence was obtaining results in 2 hours, detecting non-viable cells, while the culture was positive between 72 and 96 hours. These results were in agreement with those obtained in previous investigations by Kaplan in 1960.[61]

5.4. Actinomycetic mycetoma

In 1953, a polysaccharide was obtained from *Nocardia brasiliensis*, which, when applied intradermally, showed excellent clinical correlation. The test was positive in 16 confirmed cases and negative in 30 cases of various mycoses and negative in 10 healthy subjects.[62] Subsequent results confirmed the specificity. The test was negative in 27 patients with other mycoses and negative in 20 healthy individuals. In 4 cases of mycetoma by *Actinomadura madurae* the test had variable results. In this work three serological tests were evaluated for the search of antibodies, using two antigens, the supernatant and sediment of a cellular suspension of *Nocardia brasiliensis* prepared in a sonicator. Only complement fixation was useful, being positive in 10 of 18 confirmed cases. There were no cross reactions in cryptococcosis and sporotrichosis, but in a low percentage with histoplasmosis and coccidioidomycosis.[63]

The test has continued to be used with excellent results, in 40 more cases that were positive with diameters of induration in a range of 8 to 25 mm. The close taxonomic relationship of *Nocardiae* with *Micobacteriae* led to the investigation of cross-reactions in patients with tuberculosis. In 1987, 50 patients with tuberculosis confirmed by culture or pathology were studied and also gave a positive reaction to tuberculin: 41 cases of pulmonary tuberculosis, 6 cases of tuberculous meningitis, 2 cases of tuberculous granulomatous hepatitis and 1 case of renal tuberculosis. The intradermal reaction with nocardin was negative in the 50 patients, two of them gave an induration reaction of less than 5 mm, reason why they were considered negative. These results allowed us to conclude that there are no cross-reactions of the *Nocardia brasiliensis* polysaccharide with tuberculosis patients.[64]

5.5.Dermatophytosis

In 1955 some mycologists suggested that the fundamental pathogenic factor of the dermatophytosis of the hairless skin was allergic in nature. The direct inoculation of *Trichophyton mentagrophytes*, *Trichophyton concentricum* and *Microsporum canis*, on the

flexure surface of the forearm, produced a scaly erythematous plaque from the fourth day. The addition of fluorocortisone to the inocula on the other forearm produced no injury. The histopathological response in the lesions: spongiosis, intracellular edema and vesicles allowed us to conclude that the lesions in the hairless skin due to dermatophytes are of the type of allergic eczematous dermatitis to an allergen produced by the fungus.[65]

6. PATHOLOGY

6.1. Acute pulmonary primary histoplasmosis

Acute pulmonary primary histoplasmosis. One of the important contributions in the necropsies of the deceased patients, was that there was no spread of the fungus to other organs, the process was limited to lung, the process was self-limiting with growth of tracheobronchial lymph nodes. The pathology of the lungs revealed alveolar destruction and blockage, due to the accumulation of inflammatory cells and necrosis material.[24]

The histopathological description, in one of the cases was as follows: Deep alteration of the structure of the organ due to congestion, edema, cellular changes, necrosis and invasion by parasites. The congestion is diffuse and intense: the vessels are dilated and engorged with erythrocytes and there are some little extensive hemorrhagic foci. The edema is equally diffuse, but becomes more evident when it invades the alveolar cavities.

The cellular changes are seen in the lining elements of the alveolar cavities that are turgid, detached and sometimes filling, by its multiplication, the alveolar cavity, and in the diffuse invasion in some places and with tendency to the granulomatous organization, in others, of inflammatory cells, predominantly macrophages, lymphocytes and, in some places, forming accumulations, neutrophil polymorphonuclear leukocytes. Frequently there are masses of elements in necrosis, most commonly filling alveolar cavities, sometimes with a completely amorphous, granular appearance, in other cases nuclear remains are still recognized and what appears to be remnants of parasites. Occasionally macrophages are found in necrosis in which they still recognize traces of their nuclei and cytoplasm loaded with compact masses of parasite remains. Finally, in free form in some cases and more generally included within macrophage cells, parasites are found with the characteristics of *Histoplasma capsulatum*.

In 1987, F. Vargas collaborator of González-Ochoa extended the histopathology of histoplasmosis, describing four histological patterns: 1. Granulomatous reaction of monocytes and histiocytes that destroys *Histoplasma capsulatum* and ends with fibrosis

without evidence of the fungus; 2. Circumscribed tuberculoid granulomatous reaction of epithelioid cells, giant polynucleated cells, caseous necrosis, fibrosis and calcification, with moderate amount of yeast; 3. Tuberculoid granulomatous reaction of epithelioid cells, polynucleated giant cells, plasma cells, lymphocytes, neutrophils, eosinophils, large areas of necrosis and abundant microorganisms; and 4. Macrophage proliferative reaction with a tendency to granulomatous organization; the fungus multiplies in the cytoplasm of the macrophage, so there is an abundant amount of intracellular and free yeasts.[66]

7. EXPERIMENTAL AND THERAPEUTIC MODELS

7.1. Histoplasmosis

The first isolation of *Histoplasma caspulatum* in Mexico was carried out in 1957, inoculating hamsters via *i. p.* a sample of pulmonary secretion. The patient was part of a group of 5 students from the Technological Institute of Monterrey and had entered a cave located between Monterrey and Ciudad Victoria, which contained abundant bat guano, checking for the first time the diagnosis of histoplasmosis and resulting in a excellent susceptibility model for this mycosis.[21]

7.2. Actinomycetic mycetoma

An important contribution in pathogenic actinomycetes was the isolation of *Nocardia brasiliensis* and *Nocardia asteroides* from soil samples.[67] Samples were collected in the State of Morelos, considered one of the endemic areas worldwide. The inoculation *i. p.* to mice showed that 5 of 6 strains identified as *Nocardia brasiliensis* were pathogenic, observing the formation of abscesses with grains, while 2 of 4 strains considered *Nocardia asteroides* produced mild lesions without grain formation, observing acid resistant filaments and bacillary fragments.

The investigations on pathogenicity and virulence of the *Nocardia* species produced important results. Inoculation of *Nocadia brasiliensis i. p., i. v., i. m., s. c.*, or intracutaneously produced transient lesions with a tendency to spontaneous healing or death of animals in the first two pathways, whereas inoculation in the footpad of the hind legs of the mouse produced for the first time, a clinical picture similar to the behavior of human actinomycetoma. The implication of this experimental model was the possibility of using it for drug research in the treatment of actinomycetoma, and given the relationship between *Nocardia brasiliensis* and *Mycobacterium leprae*, it could also be used in the search for leprosy medications.[68]

The results also allowed us to know that *Nocardia brasiliensis* is the most virulent species, behaving as the most frequent primary pathogen in the actinomycetoma, while *Nocardia asteroides* and *Nocardia otitidiscaviarum (Nocardia caviae*) are rare agents that adopt an opportunistic character.[69]

7.3. Chromomycosis

Until 1972 it had not been possible to develop an experimental model in animals that reproduced the clinical characteristics of the disease. Based on the success obtained in the experimental reproduction of mycetoma by *Nocardia brasiliensis* in the mouse footpad, this model was used to inoculate cultures of *Fonsecaea pedrosoi*, the most frequent etiological agent in this mycosis. The results showed that three newly isolated strains were pathogenic with observations between 2 and 8 weeks. In some animals lesions were observed after one week of inoculation, these lesions increased with time and by the tenth week, all the inoculated animals presented lesions. The animals were kept under observation for 24 weeks. Some of these animals were left under observation for a period of 12 months. The results showed that the lesions only appeared in the leg that had been inoculated. Histopathological studies showed a dense inflammatory response, consisting of plasma cells, lymphoid cells and foamy histiocytes. Large quantities of fumagoid cells were observed in the center of nodular lesions with central necrosis and the presence of foamy histiocytes. This experimental model can be used to evaluate drugs for the treatment of this mycosis.[70]

7.4. Actinomycetic mycetoma

Since 1947, several clinical studies were conducted on the treatment of actinomycetoma. The results with 4, 4'-Diaminodiphenylsulfone (DDS) were encouraging from the start.[71] These observations were confirmed, combining the oral route and direct injection in the lesions,[72] but the results were not satisfactory in cases with extensive lesions and bone disease.

The use of 4-amino-N-(5,6-dimethoxypyrimidin-4-yl)benzene-1-sulfonamide (Fanasil) in 1969, combining oral and intra-arterial administration, also offered promising results in 60 cases of mycetoma, of which in 18 cases there was clinical cure, in 36 cases improvement and in 6 cases it fails treatment.[73] The results forced us to continue experimenting with other drugs, first with in vitro studies and then in vivo, either in the experimental model of the mouse or in human cases. Studies with [(2,4-diamino-5- (3,4,5-trimethoxy-benzyl)] (Trimethoprim) have been started since 1966. The results were better when combining

trimethoprim with various sulphonamides, particularly [N^1]-(5-methyl-3-isoxazolyl) sulfanilamide] (Sulfamethoxazole).

In 1969, the first therapeutic evaluation with trimethoprim and sulfamethoxazole was performed in 14 patients.[74] The results showed a great efficiency, obtaining 43% of healing and 57% with clear improvement. In October 1968, a female patient with a bone condition was treated with sulfamethoxazole. In April 1969, the patient became pregnant and, as there were no reports of toxicity, treatment was continued until August 23. During that time the clinical cure was reached and the dose was reduced by half, with suspension on December 9 of that same year. In January of 1970 the child was born healthy without congenital abnormalities, the child grew healthy and the mother did not show recurrence of the mycetoma.[75] By 1976, the patients treated with sulfamethoxazole had 39 and the results were similar; in 26 cases (67%) there was clinical cure, in 2 cases there was marked improvement and in 2 cases there was failure. Two cases with relapses healed and 7 abandoned treatment (18%). There was one case of liver toxicity and another case of leukopenia, in which the treatment was suspended.[76] With these results, there were two active drugs for the treatment of actinomycetamic mycetoma, diamino dimethyl sulfone and TS, but in both drugs, the prognosis depended mainly on the extension and chronicity of the lesions and, fundamentally, on the existence or absence of bone invasion.

7.5. Candidosis

In 1955 the clinical experimentation of nystatin had been extensive, but the number of cases studied in 37 publications amounted to about 118 cases, in some of them, laboratory results called into question the diagnosis. The study of 75 children with oral candidosis, aged between 3 days and 5 years, included a uniform criterion for diagnosis and treatment with nystatin.[77] The laboratory diagnosis was established by the direct microscopic examination of the material of the lesions and the culture was only performed when the previous resource was positive and was used for the identification of the species. The clinical cure was obtained on average of 2.4 days, the topical application of nystatin showed remarkable healing results.

7.6. Cryptococcosis and chromomycosis

The severity of mycosis, as well as the limitations of amphotericin B due to its toxicity, the only treatment available and that only cured about 60% of cases, led to the therapeutic assessment of 5-fluorocytosine. The clinical cure of 2 cases of cryptococcosis and 2 of chromomycosis in the Institute of Health and Tropical Diseases, confirmed the great activity

of 5-fluorocytosine and its lack of toxic effects.[78] The encouraging results in chromomycosis seemed to change the outlook in this mycosis, since until 1970 there was no effective treatment, and the reports of cures with several drugs in early cases, had failed in other patients. The new drug however had some disadvantages, required high doses of 5 grams and prolonged times to reach the cure of cases of chromomycosis (220 days and 88 days), while in cases of cryptococcosis the administration time had been shorter (30 and 61 days).

The 1972 publication summarizes the advances in the treatment of fungal infections and refers to the experiences of fungal diseases treated at the Institute of Health and Tropical Diseases.[79] Some fungal diseases continue to be practically incurable, in other cases the prognosis depends on a reliable and timely diagnosis.

8. DIFERENTIAL DIAGNOSIS

8.1. Cutaneous tuberculosis

One of the most important diseases in the differential diagnosis with initially tegumentary mycosis (subcutaneous mycosis) and secondarily tegumentary (systemic mycosis) is pulmonary and cutaneous tuberculosis. Given the lesional polymorphism that the disease presents, the traditional clinical classifications are mostly incomplete. González-Ochoa in 1965 proposed a practical classification with immunological bases considering the Koch phenomenon, which would clearly explain the response to primary infection and subsequent reinfections.[80]

The tuberculous tegumentary primary infection shows a great similarity with the lymphangitic type sporotrichosis, the presence of regional adenopathy is a constant indicator in tuberculosis. The verrucous tuberculosis, frequent clinical form tends to ulcerate and its differential diagnosis with fixed sporotrichosis and especially with chromomycosis is quite difficult, given that the histopathology is very similar in the two diseases. Ulcerative tuberculosis is an extremely chronic condition and must be separated from sporotrichosis with an ulcerous form. The colicuativa tuberculosis can originate from a ganglionar center, the location in the neck would facilitate its diagnosis, but in other locations it should be differentiated from the *Nocardia* infections.

9. CONCLUSIONS

In conclusion, the research carried out by Dr. González-Ochoa contributed not only to the knowledge and classification of fungi, but also to the understanding of development of fungal

diseases using experimental models. Advances in the treatment of fungal infections were important, some mycoses continue to be practically incurable, in others, the prognosis depends on a reliable and timely diagnosis.

10. REFERENCES

1. Cortes Tamayo R. Perfiles de México No. 511. Antonio González Ochoa. Periódico El Día vocero del pueblo mexicano, 1982; XXI(7355): 12.
2. González-Ochoa A. El conocimiento de la micología médica en el lapso de 1924 a nuestros días. Prensa Médica Mexicana, 1974; 39: 153-9.
3. González-Ochoa A. Las enfermedades por hongos en México. Rev Inst Salubr Trop Mex, 1955; 15: 133-47.
4. González-Ochoa A. Micosis superficiales más frecuentes en México. I. Introducción. Gac Med Mex, 1966; 96: 1043-8.
5. González-Ochoa A. Las micosis pulmonares en México y Centroamérica. Aspectos epidemiológicos. Rev Invest Sal Pub Mex, 1969; 29: 179-96.
6. González-Ochoa A, González-Mendoza A. La micología médica en México. Revisión de la bibliografía aparecida durante el periodo de 1946 a 1958. Mycopath et Mycol Applic, 1960; 18: 48-71.
7. González-Ochoa A. Micología y Dermatología Tropical. Trigésimo aniversario del Instituto de Salubridad y Enfermedades Tropicales. Rev Inst Salubr Enferm Trop Mex, 1970; 100: 119-25.
8. González-Ochoa A. Panorama de las micosis en México. Rev Salud Pub Mex, 1981; 23: 213-6.
9. González-Ochoa A. Hallazgo del Fonsecaea pedrosoi var. cladosporioides en México. Rev Inst Salubr Enferm Trop Mex, 1941; 2: 187-91.
10. Martínez Baéz M. Un caso de probable dermatitis verrucosa. Rev Inst Trop Mex, 1941; 1: 323-38.
11. González-Ochoa A, Lavalle P. Dermatofitos causantes de las diversas tiñas de la piel lampiña observadas en nuestro medio. Rev Inst Salubr Enferm Trop Mex, 1947; 8: 265-72.
12. González-Ochoa A. El micetoma por Actinomyces mexicanus Boyd y Crutshfield, 1921, en México. Rev Inst Salubr Enferm Trop Mex., 1942; 3: 303-17.
13. González-Ochoa A, Vázquez-Hoyos, A. Relaciones serológicas de los principales actinomicetes patógenos. Rev Inst Salubr Enferm Trop Mex., 1953; 13: 177-87.
14. González-Ochoa A, Sandoval, M. A. Características de los actinomicetes patógenos más comúnes. Rev Inst Salubr Enferm Trop Mex, 1955; 15: 149-61.

15. Serrano, JA, Sandoval AH, Beaman BL. Actinomicetoma. México: Editorial Plaza y Valdéz, S. A. de C. V., 2007.
16. Rippon, JW. Medical Mycology. The Pathogenic Fungi and The Pathogenic Actinomycetes. USA: W. B. Saunders Company, 1974.
17. González-Ochoa A, Sandoval, M. A. Revisión determinativa de algunas especies de actinomicetes patógenos descritos como diferentes. Rev Inst Salubr Enferm Trop Mex, 1956; 16: 17-25.
18. González-Ochoa A, Sandoval MA. Estudios sobre cinco especies del género Candida Berkhout 1923, causante de lesiones humanas. Rev Inst Salubr Enferm Trop Mex, 1943; 4: 149-61.
19. González-Ochoa A. Clasificación clínica de las micosis. Rev Inst Salubr Enferm Trop Mex, 1956; 16: 1-8.
20. González-Ochoa A. Histoplasmosis. Rev Med Sec Mar Mex, 1957; 3: 5-14.
21. González-Ochoa A. Histoplasmosis pulmonar aguda primaria. Gac Med Mex, 1957; 87: 733-44.
22. González-Ochoa A. Histoplasmosis primaria pulmonar aguda en la República Mexicana. Rev Inst Salubr Enferm Trop Mex., 1959; 19: 341-50.
23. González-Ochoa A. Peculiaridades de la histoplasmosis pulmonar primaria grave en el país. Gac Med Mex, 1961; 91: 5-14.
24. González-Ochoa A, Cervantes O A. Histoplasmosis epidémica y su prevención. Con especial referencia al brote observado en Colima durante los meses de agosto y septiembre de 1960. Rev Inst Salubr Enferm Trop Mex, 1960; 20: 129-45.
25. González-Ochoa A. Epidemiología de la histoplasmosis primaria en México. Rev Inst Salubr Enferm Trop Mex, 1963; 23: 65-80.
26. González-Ochoa A. Realización de la investigación científica en México para la Salud Pública. III Histoplasmosis. Gac Med Mex, 1964; 94: 981-6.
27. González-Ochoa A. Symposium sobre histoplasmosis pulmonar primaria. I.- Generalidades. Aspectos del problema en México. Gac Med Mex, 1964; 94: 501-8.
28. Velasco CO, González-Ochoa A. Primary pulmonary epidemic histoplasmosis in an abandoned mine. Mykosen, 1977; 20: 393-9.
29. Valdespino GJL, Velasco CO, Escobar GA, del Río ZA, Ibáñez BS, Magos LC, Editores. Enfermedades tropicales en México. Diagnóstico, tratamiento y distribución Geográfica. D.F., México, 1994.
30. González-Ochoa A. Contribuciones recientes al conocimiento de la esporotricosis. Actas finales 5º. Congreso Ibero Latino Americano de Dermatología, 1963; 309-12.

31. González-Ochoa A. Contribuciones al conocimiento de la esporotricosis. Gac Med Mex., 1964; 95: 463-74.
32. González-Ochoa A. Theories regarding the portal of entry of Paracoccidioides brasiliensis: A brief review. Paracoccidioidomycosis. Proceedings of the first Pan American Symposium. OPS. Scientific Publication, 1972; 254: 278-0.
33. González-Ochoa A, Domínguez L. Blastomicosis sudamericana. Casos mexicanos. Rev Inst Salubr Enferm Trop Mex, 1957; 17: 97-104.
34. González-Ochoa A. Monilias y moniliasis. Rev Inst Salubr Enferm Trop Mex, 1957; 17: 13-21.
35. González-Ochoa A, Félix D. Distribución geográfica de la reactividad cutánea a la histoplasmina en México. Rev Invest Sal Pub Mex, 1971; 31: 74-7.
36. Contreras-Pérez C, Shibayama-Hernández H, Gutiérrez-García P. Aportaciones del INDRE a la Histoplasmosis. Rev Inst Nal Enf Resp Mex, 1998; 11: 216-220.
37. Contreras Pérez C. La micología médica en el Instituto de Salubridad y Enfermedades Tropicales (ISET). En: López Martínez R editor. Historia de la Micología Médica en México. López Martínez R editor. Academia Mexicana de Dermatología, A.C., México, 2015; 93-124.
38. González-Ochoa A, García F. Coccidioidiomicosis. Prensa Med Mex, 1944; 14: 245-52.
39. González-Ochoa A. Epidemiología de la coccidioidiomicosis en México. Gac Med Mex, 1967; 97: 1383-92.
40. Luna-Isaac JA, Muñiz-Salazar R, Baptista-Rosas RC, Enríquez-Paredes LM, Castañón-Olivares LR, Contreras-Pérez C, Bazán-Mora E, González GM and González-Martínez MR (2014). Genetic analysis of the endemic fungal pathogens Coccidioides posadasii and Coccidioides immitis in Mexico. ISHAM Medical Micology, 2014; 52: 156-66.
41. Fisher MC, Koenig GL, White TJ, Taylor JW. Molecular and phenotypic description of Coccidioides posadasii sp. Nov., previously recognized as the non-California population of Coccidioides immitis. Mycologia, 2002; 94(1): 73-84.
42. González-Ochoa A, Romo V B. Dermatofitos causantes de tiña de la piel cabelluda en la ciudad de México. Rev Inst Salubr Enferm Trop Mex, 1945; 6: 145-8.
43. González-Ochoa A, Lavalle P. Dermatofitos causantes de las diversas tiñas de la piel lampiña observadas en nuestro medio. Rev Inst Salubr Enferm Trop Mex, 1947; 8: 265-72.
44. González-Ochoa A. El problema de las dermatomicosis en México. I.- Las tiñas de la piel lampiña. Rev Med Sec Mar Mex. 1955; 1: 15-31.
45. González-Ochoa A, Orozco V C. Dermatofitos causantes de la tinea unguis en México. Rev Inst Salubr Enferm Trop Mex, 1957; 17: 93-5.

46. González-Ochoa A, Orozco V C. Frecuency of occurence of principal dermatophytoses observed in Mexico City. J Int Derm, 1974; 3: 303-9.
47. González-Ochoa A, Sandoval M A. Levaduras en padecimientos intestinales no tratados con antibióticos. Rev Inst Salubr Enferm Trop Mex, 1956; 16: 15-9.
48. González-Ochoa A, Domínguez L. Algunas observaciones epidemiológicas y patogénicas sobre la moniliasis oral del recién nacido. Rev Inst Salubr Enferm Trop Mex, 1956; 17: 1-12.
49. González-Ochoa A, Orozco V C. Los hongos del aire en la ciudad de México. Sus relaciones con los factores atmosféricos. Rev Inst Salubr Enferm Trop Mex, 1956; 4: 259-64.
50. González-Ochoa A, Alvarez M H. Aislamiento de Candida en el recién nacido. Rev Invest Sal Pub Mex, 1968; 28: 248-54.
51. Romero Maya C, Contreras Pérez C. 2002. Identificación de 25 cepas de Cryptococcus sp aisladas de pacientes en el Instituto de Diagnóstico y Referencia Epidemiológicos (InDRE). Tesis de Licenciatura. Facultad de Estudios Superiores Cuautitlán/UNAM.
52. Meyer W, Castañeda A, Jackson S, Huynh M, Castañeda E, Iberoamerican Cryptococcal Study Group. Molecular typing of Iberoamerican Cryptococcus neoformans isolates. Emerg Infect Dis., 2003; 9: 189-95.
53. Firacative C, Chandler C R, Malik R, Ferreira-Paim K, Escandón P, Sykes JE, Castañón-Olivares LR, Contreras-Pérez C, Samoyoa B, Sorrell TC, Castañeda E, Lockart SR, Engelthaler DM and Mayer W. MLST and Whole-Genome-Based Population Analysis of Cryptococcus gattii VGIII Links Clinical, Veterinary and Enviromental Strains, and Reveals Divergent Serotype Specific-Subpopulations and Distant Ancestors. PLoS Negl Trop Dis., 2016; 10(8): 2-31.
54. Valenzuela TJF, Contreras PC, Shibayama HH, Chávez GL, Vázquez- Chacón C, Olivera DH. Biochemical identification and molecular characterization (PCR-RFLP) of Nocardia isolates from sputum. Archives of Medical Research, 2005; 36: 356-61.
55. González-Ochoa A. Conceptos fundamentales en inmunología de la coccidioidomicosis. Rev Fac Med Mex, 1965; 7: 571-75.
56. González-Ochoa A, Figueroa S E. Polisacáridos de Sporothrix schenckii. Datos inmunológicos. Intradermoreacción en el diagnóstico de la esporotricosis Monilias y moniliasis. Rev Inst Salubr Enferm Trop Mex, 1947; 8: 143-53.
57. González-Ochoa A, Ricoy E, López R, Navarrete. Valoración comparativa de los antígenos polisacáridos y celular de Sporothrix schenckii. Rev Invest Sal Pub Mex, 1971; 30: 303-15.
58. Kaplan W, González-Ochoa A. Application of the fluorescent antibody techinique to the rapid diagnosis of sporotrichosis. J Lab Clin Med., 1963; 62: 835-41.

59. González-Ochoa A, Kaplan W. El uso de anticuerpos fluorescentes en el estudio de algunas enfermedades infecciosas. II Diagnóstico rápido de la esporotricosis. Gac Med Mex, 1964; 94: 309-13.
60. González-Ochoa A, Félix D, Anaya M. Inmunofluorescence in sporotrichosis. Dermat Ib Lat Am Engl, 1967; II: 77-82.
61. Kaplan W, Ivens S. Fluorescent antibody staining of Sporotrichum schenckii in cultures and clinical materials. J Invest Dermatol, 1960; 35: 151-9.
62. González-Ochoa A, Baranda F. Una prueba cutánea para el diagnóstico del micetoma actinomicósico por Nocardia brasiliensis. Rev Inst Salubr Enferm Trop Mex, 1953; 13: 189-97.
63. González-Ochoa A, Shibayama H, Félix D, Anaya M. Inmunological aspects of actinomycotic mycetoma and nocardiosis. Proceedings of XII International Congress of Dermatology, 542-51.
64. Sosa R A. Especificidad de la intradermorreacción con polisacárido de Nocardia brasiliensis en el diagnóstico diferencial con tuberculosis [Tesis de Especialidad]. [CDMX, México]: Hospital de Infectología Centro Médico Nacional "La Raza", IMSS, 1987.
65. González-Ochoa A, Córdova J. El factor sensibilización en las dermatofitosis de la piel lampiña. Rev Inst Salubr Enferm Trop Mex, 1957; 17: 107-13.
66. Vargas O F. Histoplasmosis. Sal Pub Mex, 1987; 29: 201-5.
67. González-Ochoa A. Aislamiento de Nocardia brasiliensis y asteroides a partir de suelos. Rev Inst Salubr Enferm Trop Mex, 1960; 20: 147-51.
68. González-Ochoa A. Producción experimental del micetoma por Nocardia brasiliensis en el ratón. Gac Med Mex, 1969; 99: 773-81.
69. González-Ochoa A. Virulence of Nocardiae. Can J Microbiol, 1973; 19: 901- 4.
70. García E G, González-Ochoa A, Vargas O F. Experimental reproduction of chromoblastomycosi with some features of the human disease, in white mice. Fifth International Conference on the Mycoses superficial, cutaneous and subcutaneous infections. OPS. Scientific Publication, 1980; 396: 265-8.
71. González-Ochoa A, Shiels J, Vázquez P. Acción de la 4, 4 diamino difenil sulfona frente a Nocardia brasiliensis. Gac Med Mex, 1952; 82: 345-53.
72. González-Ochoa A, Ahumada M. Tratamiento del micetoma actinomicósico por la inyección local de diamino difenil sulfona. Acción de la 4, 4 diamino difenil sulfona. Rev Inst Salubr Enferm Trop Mex., 1958; 18: 41-4.
73. González-Ochoa A, Stark B, Vázquez IR. Fanasil in the actinomycetic mycetoma caused by Nocardia brasiliensis. Fifth Intl Congress of Chemotherapy, 1967.

74. González-Ochoa A. Trimethoprim and sulfamethoxazol in pregnancy. JAMA, 1971; 217: 1244.
75. González-Ochoa A, Tamayo L. Tratamiento del micetoma actinomicético por N. brasiliensis con Ro6-2580/11. Comunicación preliminar. Rev Med Mex, 1969; 49: 473-6.
76. González-Ochoa A. Nocardiae and chemotherapy. In: Goodfellow M, Brownell GH, Serrano JA, editors.The Biology of the Nocardiae. Academic Press, New York, 1976; 429-50.
77. González-Ochoa A. Tratamiento de la moniliasis oral con nystatin. Rev Inst Salubr Enferm Trop Mex, 1955; 15: 195-202.
78. González-Ochoa A. Curación de la criptococosis y de la cromomicosis con 5-fluorocitosina. Rev Invest Salud Pub Mex, 1970; 30: 63-76.
79. González-Ochoa A. Avances en el tratamiento de las infecciones por hongos. Gac Med Mex. 1972; 104: 450-6.
80. González-Ochoa A. Tuberculosis cutánea. Rev Inst Salubr Enferm Trop Mex, 1963; 17: 13-21. Actas finales 5º. Congreso Ibero Latino Americano de Dermatología, 1963; 931-7.