

ABSTRACTS OF THE  
TENTH ANNUAL COCCIDIOIDOMYCOSIS  
CONFERENCE

SPONSORED BY

Tuberculosis and Health Association of California  
California Thoracic Society  
Tuberculosis and Health Association  
of Los Angeles County

Palm Springs, California

April 19, 1967

# INDEX

Program	Page 3
Planning Committee	Page 6
Foreword	Page 7
Mycelial Forms of <i>C. immitis</i> in Osteomyelitis of the Sternum - Stephen Cheu, M.D., Royal Sorensen, B.S.	Page 8
A Case of Disseminated Coccidioidomycosis Is Developing During the Administration of Prednisone - Dr. J. T. Duncan	Page 9
Virulence and Immunizing Capacity of Selected Auxotrophic Mutants of <i>Coccidioides immitis</i> - Henry A. Walch, Ph.D.	Page 10
Review on the Influence of Vaccination Against Coccidioido- mycosis in Experimental Animals - H. B. Levine, Yi-Chi M. Kong, and C. E. Smith	Page 11
The Phagocytic Activity of Leukocytes from <i>Coccidioides</i> Infected Dogs - Thomas Wegner, Ph.D.; R. J. Trautman, M.S.; R. E. Reed, D.V.M.; D. Beavers, B.S.	Page 13
An Outbreak of Coccidioidomycosis among Eight Children in the Pacific Beach Area of San Diego, California - J. B. Askew, M.D., M.P.H.; Donald G. Ramras, M.D., M.P.H.; Henry A. Walch, Ph.D.; John P. Murray, M.D.; Bernice H. Davidson, M.D.	Page 14
The Canoga Park Epidemic Revisited After Two Years - Dean W. Gilman, M.D., M.P.H.; and David Salkin, M.D.	Page 15
Studies on the Agar-Gel Precipitin-Inhibition Test for Coccidioidomycosis Serological Test Antigen - John G. Ray, Jr., Ph.D.	Page 16
Evaluation of a Latex Particle Agglutination Test for Coccidi- oidomycosis - M. Huppert, E. T. Peterson, S. H. Sun, P. Chitjian, and W. Derrevere	Page 17
Skin Reactivity and Serological Response to Coccidioidin Skin Tests - Evelyn B. Wallraff, Ph.D.	Page 19
Disc Electrophoretic Studies of Proteins from <i>Coccidioides</i> <i>immitis</i> - Yaakov Shechter, Ph.D.; J. W. Landau, M.A.; V. D. Newcomer, M.D.	Page 20

Continued

INDEX  
(Continued)

The Course of Coccidioidal Meningitis Following Inadvertent Administration of 25 mgs. of Amphotericin B Intracisternally - William L. Hewitt, M.D.	Page 21
Coccidioidal Meningitis 1961 - 1966; A progress Report - Charles W. Holeman, Jr., M.D.; Hans Einstein, M.D.; Joseph Anderson, M.D.	Page 22
Therapy of Pulmonary Coccidioidomycosis with Amphotericin-B Aerosol - W. H. Oatway, Jr., M.D.	Page 23
Testing of killed Vaccines of Coccidioides immitis in Humans - D. Pappagianis, M.D.; H. B. Levine, Ph.D.; C. E. Smith, M.D.	Page 24
Treatment of Coccidioidal Pulmonary Cavitation - Leroy Hyde, M.D.	Page 25
The Ommaya Valve in the VA - David Salkin, M.D.	Page 26

\* \* \* \* \*

TENTH ANNUAL COCCIDIOIDOMYCOSIS CONFERENCE

Wednesday, April 19, 1967

WELCOME

Samuel J. Sills, M.D., Chairman

- \* The Fungistatic Effect of Local Anesthetics on Bronchial Specimens for Culture

Royal H. Sorensen, B.S.  
Vincent S. O'Hara, M.D.

The Occurrence of Mycelial Forms of C. immitis in Pus of Sternal Abscess. What does it mean?

Stephen H. Cheu, M.D.  
Royal H. Sorensen, B.S.

A Case of Disseminated Coccidioidomycosis Developing During the Administration of Prednisone

James T. Duncan, M. D.

- \* Disseminated Coccy Involving Lung, Lymph Nodes, and Bones Presently in Inactive State

James D'Angelo, Capt., MC, USAF

Virulence and Immunizing Capacity of Induced Auxotrophic Mutants of Coccidioides immitis

Henry A. Walch, Ph.D.  
Anna Kalvoda, M.D.

Histopathological Response of Mice to Killed Vaccines of Coccidioides immitis

D. Pappagianis, M.D., Ph.D.

The Phagocytic Activity of Leukocytes from Coccidioides Infected Dogs

Thomas Wegner, Ph.D.

- \* The Lymphocyte Transformation Test in Coccidioidomycosis

Norman E. Levan, M.D.  
Carl Korn, M.D.

A Localized Epidemic of Coccidioidomycosis in Visiting College Students with Unusual Clinical and Laboratory Features

Hans E. Einstein, M.D.  
C. Ross Hampson, Ph.D.

- \* Cutaneous Manifestations in Uncomplicated Primary Cocci in Children of Tuscon, Arizona

Buchanan M. McKay, M.D.

- \* Coccidioidin Skin Test Conversion Rate in a Transit Air Force Pediatric Population

Jerrold L. Wheaton, Col., MC, USAF

An Outbreak of Cocci Among Eight Children in the Pacific Beach Area of San Diego; Clinical and Soil Studies

Donald Ramras, M.D.  
Henry A. Walch, Ph.D.  
John F. Murray, M.D.  
Bernice H. Davidson, M.D.

A Two Year Follow-up on the Canoga Park Epidemic

Dean W. Gilman, M.D., M.P.H.;  
David Salkin, M.D.

Coccy Conference Continued

Studies on the Agar-gel Precipitin-Inhibition Test for Cocci. Serological Test Antigen Studies in Agar-gel	John G. Ray, Jr., Ph.D.
Evaluation of Hyland Latex Particle Agglutination Test for Coccidioidomycosis	Milton Huppert, Ph.D. E. T. Peterson P. Chitjian S. H. Sun W. Derrevere
Skin Reactivity and Serological Response to Coccidioidin Skin Tests	Evelyn B. Wallraff, Ph.D. Ruth M. Van Liew, M.S. Susanne Waite, B.S.
Disc Electrophoretic Studies of Proteins from Coccidioides immitis	Yaakov Shechter, Ph.D. J. W. Landau V. D. Newcomer
The Course of Cocci Meningitis Following Inadvertent Administration of 25 mgs. of Amphotericin B. Intracisternally--case report with two-year follow-up	William L. Hewitt, M.D.
Follow-up of Coccidioidal Meningitis Patients Treated with Amphotericin B, 1957 to 1966	Charles W. Holeman, M.D. Hans E. Einstein, M.D. Joseph E. Anderson, Jr., M.D.
Therapy of Pulmonary Coccidioidomycosis with Amphotericin-B Aerosol	W. H. Oatway, Jr. M.D.
Vaccine Panel - Discussion	H. B. Levine, Ph.D. Milton Huppert, Ph.D.

Thursday, April 20, 1967

* Coccy in Pregnancy	Thomas R. Larwood, M.D.
* Panel Discussion on the Indications for Amphotericin	Thomas R. Larwood, M.D. Leroy E. Smale, M.D. Marjorie Biddle, Ph.D.
Coccidioidal Pulmonary Cavitation	Leroy Hyde, M.D.
* The Management of Ruptured Coccidioidal Cavities	Ralph T. Cunningham, M.D. Robert I. Katz, M.D. John D. Steele, M.D.

Coccy Conference Continued

Cavitary-Abscess Lesions of the Lungs of  
Residual Coccidioidal Type--a Study of  
300 Cases

William A. Winn, M.D.

Ommaya Valve Session - Movie  
and Discussion

A. K. Ommaya, F.R.C.S.

Panel

A. K. Ommaya, F.R.C.S.  
Annabel M. Irons, M.D.  
W. Gierson, M.D.  
William A. Winn, M.D.  
George Ablin, M.D.

\* No abstract received from author.

#### PLANNING COMMITTEE

Samuel J. Sills, M.D., Chairman  
Milton Huppert, Ph. D.  
Thomas R. Larwood, M.D.  
William B. Leftwich, M.D.  
Richard E. Osgood, M.D.  
Maxwell Rosenblatt, M.D.  
David Salkin, M. D.

#### STAFF

Mrs. Margaret Casey  
Tuberculosis and Health Association of California

Mrs. Rose Schlichter, R. N.  
Tuberculosis and Health Association of Los Angeles County

#### EDITOR FOR ABSTRACTS

Leroy Hyde, M. D.

These abstracts are published and distributed by the Tuberculosis and Health Association of Los Angeles County.

## FOREWORD

The Tenth Annual Coccidioidomycosis Conference opened on a sad note. We had lost a valued friend, a man who had been known as "Mr. Coccy", Dr. Charles C. Smith.

As we stated at the opening of the conference, "We had a robust child who suffered some of the stigma of illegitimacy." But through the good offices of the Tuberculosis and Health Association of California and the California Thoracic Society, the conference has now been legitimized. The California Thoracic Society assumed a paternal role for this worthwhile medical meeting.

I want, personally, to thank the committee and the participants who worked so diligently and gave of their time and enthusiasm.

This conference would never have taken place without the cooperation and help of the Tuberculosis and Health Association of California and the Tuberculosis and Health Association of Los Angeles County. Without the aid and time devoted to the conference by Mrs. Margaret Casey and Mrs. Rose Schlichter, this conference would never have got off the "pad". Behind us there was always the sinister figure of Dave Salkin prodding us on.

By resolution, the California Thoracic Society has adopted this conference and its program of work and in the future, it shall be known as the CHARLES C. SMITH COCCIDIOIDOMYCOSIS CONFERENCE.

Samuel J. Sills, M. D.  
Chairman



## MYCELIAL FORMS OF *C. IMMITIS* IN OSTEOMYELITIS OF THE STERNUM

Stephen Cheu, M. D.

Royal H. Sorensen, B. S.

This report presents an example of mycelial forms of *C. immitis* observed in a localized destructive lesion of the sternum of one year's duration. The finding of mycelia elements in an extrapulmonary lesion has been reported only once in the literature. It seems that the appearance of myceliae in a given lesion will depend on the various environmental factors affecting the organism. Mycelial forms of *C. immitis* will appear in the animal host under conditions which approximate those of the external environment.

The patient was a 31 year old white male who had a solitary mass in the anterior chest. He had no evidence of coccidioidomycosis in the lungs or other viscera. Coccidioidin skin test 1:100 was positive. The highest complement fixation titer was 4+ in 1:4. X-ray of the sternum disclosed a localized destructive lesion. Surgical excision of the osteomyelitic area was done. Examination of the fresh debris by wet mounts showed hyphae elements. Culture of the material grew *C. immitis* which was confirmed by animal inoculation.

A CASE OF DISSEMINATED COCCIDIOIDOMYCOSIS IS DEVELOPING DURING  
THE ADMINISTRATION OF PREDNISONE

Dr. James T. Duncan

A thirty-nine year old Mexican-American field worker was admitted for severe far advanced tuberculosis. He was treated with Prednisone as well as anti-tuberculosis drugs due to a life threatening situation. There was a positive gel diffusion test for coccidioidomycosis as an early incidental finding with negative complement fixation and precipitins. There was subsequently a rapid rise in coccidioidal complement fixation titer and precipitins progressing with dissemination of the coccidioidomycosis, coincidental with the use of Prednisone for the tuberculosis. The patient developed coccidioidal meningitis and probable pericarditis at the time dissemination was diagnosed. Treatment of the dissemination was accomplished by intravenous and intrathecal Amphoterecin to a total dosage of over 3000 mgm I.V. and 0.5 mgm once a week and later every two weeks intrathecally. More recently, the tuberculosis has been treated by a thoracoplasty for persistent cavitation with repeated hemorrhages. Patient has negative tuberculosis cultures at the present time and his coccidioidomycosis appears to be quiescent.

(EDITOR'S NOTE: It would be interesting to know the exact time relationships of exposure to *C. immitis*, admission to hospital, administration of prednisone, dates of coccidioidal serologies, and date of development of meningitis. Although two similar cases were reported from Tucson several years ago, many other patients with coccidioidomycosis have received corticosteroids without difficulty. More research and case reports may help clarify this problem.)

VIRULENCE AND IMMUNIZING CAPACITY OF SELECTED AUXOTROPHIC  
MUTANTS OF COCCIDIODES IMMITIS

Henry A. Walch, Ph. D.

Three induced diauxotrophic mutants derived from a common prototrophic strain were tested as viable immunogenic agents. All of these mutants had one of their two nutritional requirements in common which could be supplied by either cysteine, cystine, or methionine. The second requirement is listed as per the coded strain number: biotin (540-228), niacin (2-51), p-aminobenzoic acid (95-291). Strain 95-291 was temperature sensitive and would make only a limited amount of growth in the temperature range of 37° C. All three strains differed in their comparative virulence when different numbers of arthrospores were introduced by the intranasal route into an inbred strain of brown mice. In accordance with their comparative virulence, they also varied in their ability to cause infection of the lungs, liver, and spleen that was dose related. Other routes of infection as the intraperitoneal and shallow-subcutaneous (accompanied by the formation of a bleb) were also used at various dose levels. Based on these findings, studies were conducted to compare the immunizing capability of the three strains when given by the intranasal, intraperitoneal and shallow-subcutaneous routes. Challenge by intranasal instillation at various arthrospore levels, at various time intervals postimmunization gave a pattern of survival and clearance of the internal organs that indicated a good immune response.

(EDITOR'S NOTE: diauxotrophic = requiring extra growth factors, related to nutritional genotype.)

REVIEW ON THE INFLUENCE OF VACCINATION AGAINST  
COCCIDIOIDOMYCOSIS IN EXPERIMENTAL ANIMALS

H. B. Levine, Yi-Chi M. Kong, and C. E. Smith

SUMMARY

Induced immunity to Coccidioides immitis in mice challenged intranasally was enhanced greatly by the injection of a booster dose of 20 to 200 µg of killed spherules; fungal multiplication was suppressed up to 5 million-fold and 60 to 75% of boosted mice harbored no organisms in their lungs at 10 to 90 days post-challenge with arthrospore doses as high as 100 LD<sub>50</sub>. Enhancement of immunity was most pronounced when the booster injection was given at >2 months post-vaccination and 6 to 7 days before challenge and was not induced by injecting another fungus, Cryptococcus neoformans.

Low numbers of C. immitis in the lungs of boosted mice reflected marked clearance activity during the first 3 days postchallenge. In contrast, clearance activity during this period was less in nonboosted mice and was observed only in those vaccinated >2 months before challenge. Additionally, enhanced clearance in the lungs of boosted mice was paralleled by an early rise in numbers of inflammatory cells obtained by tracheobronchial wash; total cell numbers reached a maximum 2 to 3 days earlier in boosted than in nonboosted mice.

Despite the demonstration of precipitins, attempts to transfer immunity passively with serum were unsuccessful. However, less fungal multiplication occurred after challenge in nonvaccinated recipients of live splenic cells from boosted mice than in recipients of live cells from normal mice or killed cells from boosted mice. The role of cells or serum in immunity was difficult to assess in passive

transfer studies, because injection of normal splenic cells, their carrier fluid, or normal serum into intranasally infected mice often potentiated their infection.

Five of ten Cynomolgous monkeys (Macaca irus) died and two were moribund within 9 months after respiratory infection with 200 coccidioidal arthrospores. None of seven animals previously vaccinated with formalinized spherule-endospore elements of the fungus succumbed to the above dose but one vaccinated animal challenged with approximately 400 arthrospores died within a 7-week period. It was demonstrated that both early and late roentgenographic changes in the lungs were less extensive in the vaccinated group than in the control group and these observations were in accord with pathologic findings at necopsy. The etiologic agent was recovered from the lungs of all surviving animals but histopathologic studies suggested that the parasite was contained more effectively within lesions in the vaccinated monkeys.

## THE PHAGOCYtic ACTIVITY OF LEUKOCYTES FROM COCCIDIOIDES INFECTED DOGS

Thomas Wegner, Ph.D., R. J. Trautman, M.S.,  
R. E. Reed, D.V.M., D. Beavers, B.S.

An in vitro phagocytic system was developed containing a buffered suspension of stained arthrospores of Coccidioides immitis and leukocytes from Coccidioides infected and noninfected beagle dogs. This suspension was placed on a vasoline-ringed slide, incubated for fifteen minutes at 37° C., and the percentage arthrospores phagocytized was noted. Five dogs were used in this twenty-week study: one had been infected with 100,000 particles six months prior to the study, two were infected with 50,000 particles on the first day of the trial, and two were noninfected controls. At weekly intervals complement fixation test for C. immitis antibody, a complete blood count, sedimentation rate, and a phagocytic index was performed for each dog. The results show that dogs infected with Coccidioides have a significantly higher ( $P < .05$ ) phagocytic index ( $71.4 \pm 14.7$ ) than control dogs ( $24.0 \pm 15.0$ ). Leukocytes from infected dogs showed some strain specificity, since arthrospores of a different strain of C. immitis than was used to infect the dogs significantly lowered ( $P < .05$ ) the phagocytic index ( $24.2 \pm 11.1$ ). Arthrospores of Geotrichum candidum also produced a significantly lower index. There was no strain or species shown with leukocytes from control dogs. There was a strong correlation between complement fixation, sedimentation rate, and the phagocytic index in the infected dogs. Four dogs vaccinated with an irradiated spherule vaccine showed a significantly higher ( $P < .05$ ) phagocytic index ( $55.7 \pm 18.9$ ) than nonvaccinated controls ( $17.8 \pm 14.4$ ). A phagocytic index using the leukocytes from eight human subjects, one with a disseminated case of Coccidioidomycosis, showed some correlation with the coccidioidin skin tests on these individuals.

(EDITOR'S NOTE: More details would be of great interest to clinicians.)

AN OUTBREAK OF COCCIDIOIDOMYCOSIS AMONG EIGHT CHILDREN  
IN THE PACIFIC BEACH AREA OF SAN DIEGO, CALIFORNIA  
Clinical, Epidemiological, and Soil Studies

J. B. Askew, M.D., M.P.H.; Donald G. Ramras, M.D., M.P.H.;  
Henry A. Walch, Ph.D.; John P. Murray, M.D.; Bernice H. Davidson, M.D.

This outbreak of Coccidioidomycosis occurred among eight young boys residing in the same area of San Diego City. The children showed a fairly consistent pattern of low grade fever, cough, chest pain, skin rashes, infiltrates on X-ray, but no chest signs on physical examination. The appearance of erythema nodosum in one of the children alerted the physicians involved to the possibility of Coccidioidomycosis. All children had positive coccidioidin skin tests. Complement fixation tests were positive in five children tested.

Epidemiological study revealed that none of the children had left the city in the two months prior to the onset of illness. All had been playing in the same vacant lot, bombarding each other with dirt from this area.

Coccidioidomycosis immitis was recovered from this soil in 14 of 38 soil samples that were processed. Isolation was made by the direct culture method of Omieczynski and Swatek and by intraperitoneal injection of mice.

This was the first recovery of Coccidioidomycosis immitis within the City of San Diego. Although the presence of the fungus is of minimal importance to the population because of the specific conditions normally needed for transmission, it should be considered in the differential diagnosis of acute chest conditions.

(EDITOR'S NOTE: A similar community epidemic was recently reported from El Paso, Texas. Am. Rev. Resp. Dis., 96:766, [1967])

## THE CANOGA PARK EPIDEMIC REVISITED AFTER TWO YEARS

Dean W. Gilman, M.D., M.P.H. and David Salkin, M.D.

On February 22, 1965, a number of children dug a hole in a *Coccy* infested area and all became ill with acute *Coccy* including a father who refilled the hole and a mother who shook the dust out of her children's clothes. In general, the *Coccy* was a typical primary, having the usual clinical and laboratory features. All twenty-five patients recovered and none disseminated. They were restudied two years later with the following results:

Radiology: Out of fifteen patients originally showing parenchymal infiltrates, nine (or 60%) showed residual nodules, some with calcification.

Skin Tests: Repeated on twenty patients. No change in size from two years ago in seven patients, significantly larger now in eight patients, significantly smaller in three patients, and now entirely negative in two patients who were positive previously.

CF Tests: The previous 1:256 titer was now 1:16; the two who had a 1:128 titer were now 1:16 and 1:8; the three who had 1:64 were now 1:8, 1:4, and 1:4; the two who had 1:32 were now 1:8, and 1:4; the four with 1:16 now showed 1:8, 1:4, 1:4, and 0; the three with 1:8 now showed 1:8, 1:8, and 0; the two with 1:4 titer now showed 1:4 and 0; and three cases with a previously equivocal titer now showed zero.

It was felt that even such a small series showed the need for long-term observation to clarify the relationships of the clinical and laboratory phenomena and the lifetime fate of the so-called benign residuals. In the *Coccy* iceberg, much seems to go on below the symptomatic surface.



STUDIES ON THE AGAR-GEL PRECIPITIN-INHIBITION  
TEST FOR COCCIDIOIDOMYCOSIS SEROLOGICAL TEST ANTIGEN  
STUDIES IN AGAR-GEL

Ten Minutes

John G. Ray, Jr. Ph. D.

The presence and persistence of spherule antibodies in serologically negative suspect Coccidioidomycosis human sera, and also in sera from positively established cases, indicates that further study and use of this antigen-antibody reaction is necessary for accurate serodiagnosis of Coccidioidomycosis. This antigen-antibody reaction needs further study in its relationship to the disease prognosis, skin test reactivity, and possibly in vaccine evaluations. The sensitivity and specificity of this antigen-antibody reaction in relation to the other systemic mycotic diseases should also be investigated.

EVALUATION OF A LATEX PARTICLE AGGLUTINATION  
TEST FOR COCCIDIOIDOMYCOSIS

M. Huppert, E. T. Peterson, S. H. Sun,  
P. Chitjian, and W. Derrevere

A new serological test for Coccidioidomycosis has been evaluated by the VA Coccidioidomycosis Reference Laboratory and the Mycology Research Laboratory, both located in the VA Hospital, San Fernando, California. This new procedure is a latex particle agglutination (LPA) test, and it has been compared with four other serological tests for Coccidioidomycosis currently in use. The complete set of tests was performed independently by two individuals. One of the two people had prior knowledge of the patient source and the results of some tests completed earlier than other tests. The second person had no information available until after all tests were completed. A total of 436 specimens were tested and diagnoses were obtained for 328 of them.

No single test was adequate for detecting all positive specimens from cases diagnosed as probable or definite Coccidioidomycosis. A combination of the LPA test with the immunodiffusion test (IDCF) which correlates with results of the complement fixation test did detect 93% (95% Confidence Interval, 87-97%) of the specimens from diagnosed cases. These two tests (LPA and IDCF) make an excellent combination for use as a screening procedure to detect reactive specimens. Both techniques require only seconds per specimen to set up the test. The LPA test is completed in four minutes while the IDCF test, if positive, is usually readable within one day. Both tests are highly reproducible between individuals performing the tests under different conditions. The combination of these two tests may produce a small number of false positives, and these must be evaluated by additional criteria.

It is recommended that all specimens submitted for Coccidioidomycosis serologic testing should be tested first by the LPA and IDCF tests. Any positive reaction in either test should be confirmed with the tube precipitin and complement fixation procedures, the two tests which have been employed routinely for many years and for which there is a firm foundation upon which to base interpretations with clinical significance.

(EDITOR'S NOTE: These LPA and IDCF tests should be most helpful for screening because of simplicity and rapidity of performance.)

## SKIN REACTIVITY AND SEROLOGICAL RESPONSE TO COCCIDIOIDIN SKIN TESTS

EVELYN B. WALLRAFF, Ph. D

The contribution of diagnostic coccidioidin skin testing to altered skin reactivity and positive serology was investigated in positive and negative skin reactors using Smith's Lot 64D4 skin test coccidioidin and Huppert's XVB52F coccidioidal antigen.

Data on skin test positive individuals indicates a reduced reaction area when the antigen is administered in precisely the same site three times at two week intervals. Results with Huppert's coccidioidal antigen were variable and can be attributed to a loss of potency upon storage.

Three of twelve skin test negative individuals had positive skin reactions when tested with Smith's 1:10 or Huppert's 1:4 coccidioidin following two same site negative tests with 1:100 or 1:40 of the respective antigens. These positive skin reactions have been interpreted as due to preexisting low grade skin hypersensitivity which was enhanced by the testing procedure. Attempts to demonstrate humoral antibody in serial serum specimens by several sensitive immunodiffusion techniques were unsuccessful. Repeated skin testing per se with standard strength coccidioidin does not produce a humoral antibody response as is the case with histoplasmin.

## DISC ELECTROPHORETIC STUDIES OF PROTEINS FROM COCCIDIODES IMMITIS

Yaakov Shechter, Ph. D.; J. W. Landau, M. A.; V. D. Newcomer, M. D.;  
Department of Medicine, Division of Dermatology, U.C.L.A. School of  
Medicine, Los Angeles, California 90024

Disc electrophoresis, a highly sensitive method for protein analysis, was applied to studies of nondialyzable proteins of culture filtrates of Coccidioides immitis strain Silveira. The fungus was cultured in duplicate at 30°C. on a rotary shaker in asparagine, Roessler's, and Sabouraud media for 15 and 30 days. Filtrates of cultures were saturated with ammonium sulfate, dialyzed against distilled water, and lyophilized. The resulting preparations were fractionated by disc electrophoresis using 7.5% polyacrylamide gel, 300 µg of protein per gel, tris-glycine buffer (pH 8.3, ionic strength 0.01) and a regulated current of 5 ma per gel. The gels were analyzed with a recording electrophoresis densitometer. Each of the preparations yielded a distinct reproducible electrophoretic protein pattern. The culture filtrate from asparagine medium yielded 10, from Roessler's eight, and from Sabouraud six fractions after 30 days of incubation. Significant differences in electrophoretic mobility were found and only two fractions were common to all culture filtrates. Quantitative differences in the concentrations of proteins per electrophoretic fraction were observed between the 15 and 30 day cultures in each medium. These data indicate that the culture medium is an important determinant of the electrophoretic protein patterns of C. immitis.

THE COURSE OF COCCIDIOIDAL MENINGITIS FOLLOWING  
INADVERTENT ADMINISTRATION OF 25 MGS. OF AMPHOTERICIN B INTRACISTERNALLY  
CASE REPORT WITH TWO-YEAR FOLLOW-UP

William L. Hewitt, M. D.

The immediate results after the administration of this high dose were about what one might anticipate but the patient who had very abnormal spinal fluid findings at the time of the accident has shown gradual clinical and laboratory improvement over the two-year period with no subsequent amphotericin treatment of any sort.

## COCCIDIOIDAL MENINGITIS 1961-1966; A PROGRESS REPORT

Charles W. Holeman, Jr., M. D.; Hans Einstein, M. D.;  
and Joseph Anderson, M. D.; Bakersfield, California

Since 1960, twenty-one cases of Coccidioidal Meningitis have been treated with Amphotericin B.

Eighteen were continued on suppressive therapy after initial treatment. Two treated chiefly by the IV route, died of active Meningitis. Of the sixteen receiving Amphotericin intrathecally, one expired with a subarachnoid hemorrhage after 0.5 mgm. of Amphotericin B intrathecally. The other fifteen are living. Most are clinically well.

Of the three patients who received no suppressive therapy, one is dead and two are well, three years after onset.

Adverse reactions to Amphotericin intracisternally include subarachnoid hemorrhage, convulsions, cranial nerve palsies, spastic paresis of the lower extremities and urinary frequency as well as headache, nausea and vomiting.

Convulsions and subarachnoid bleeding did not occur when intrathecal hydrocortisone was employed. Cranial nerve palsies and UMN signs have not occurred when 0.1 mgm. of Amphotericin is given at six week intervals. This regimen has prevented recurrence in most cases. The immediate unpleasant sequelae of cisternal therapy can be minimized by over-night hospitalization with adequate pre-medication and sedation.

(EDITOR'S NOTE: A most impressive series, and results are quite different from ten years ago.)

THERAPY OF PULMONARY COCCIDIOIDOMYCOSIS  
WITH AMPHOTERICIN-B AEROSOL

W. H. Oatway, Jr., M. D.

Pulmonary "Coccy" has occasionally been treated by inhalation, but notable effects have not been reported. Amphotericin-B is helpful for coccy disease, but the toxicity is so great that it can be used only when there is a great hazard with obvious dissemination for a high and rising complement fixation titer.

Inhalation of an Amphotericin-B Aerosol in three cases has been done. This is admittedly a small number, but sputum, serum, and x-ray checkups were made. The results have been quite good as far as tolerance and results are concerned, especially when pressure (IPPB) was used with the Aerosol in the last two cases.



TESTING OF KILLED VACCINES OF  
COCCIDIOIDES IMMITIS IN HUMANS

D. Pappagianis, M. D.; H. B. Levine, Ph. D.; C. E. Smith, M. D.

The killed Coccidioides Immitis spherule vaccine of Levine and coworkers has now been tested in 79 human volunteers. In a variety of regimens, doses in the range of 0.15 to 20 mg have been administered intramuscularly. The uniform development of cutaneous sensitivity to coccidioidin observed in the original group of recipients studied by Levine and Smith has not been matched in the later studies. In one group studied which included 4 subjects with prior reactivity to coccidioidin, 2 of the 4 developed mild illness, enhanced reactivity to coccidioidin, and antibody responses persisting for 3 months. No skin test or serologic changes were induced in the other 2 prior reactors. Thus the immunologic irregularity of spherule vaccine was evident even in prior reactors. The use of the smaller dose (0.15 mg) of spherule vaccine in 4 subjects also failed to evoke an immunologic response. On the basis that killed C. immitis mycelium has induced hypersensitivity and serologic responses in animals, a mycelial vaccine was tested in 18 individuals. Mycelial vaccine was also noted to be irregular in inducing reactivity to coccidioidin. It is doubtful at present that mycelial antigen could be added to provide an immunologic "marker" to spherule vaccine.

## TREATMENT OF COCCIDIOIDAL PULMONARY CAVITATION

LEROY HYDE, M. D.

Chief, Pulmonary Disease Service, Veterans Administration  
Hospital, Long Beach, California, and Associate Clinical  
Professor of Medicine, University of California at Los  
Angeles School of Medicine

Nonsurgical and surgical management of 211 patients with coccidioidal pulmonary cavities has been compared. In the former group, half of the cavities closed spontaneously. Most patients were asymptomatic, but 23% had bleeding, usually slight, at some time. One new cavity developed while the patient was being followed with serial roentgenograms.

In the surgical group, most were asymptomatic, although 29% have a history of bleeding, usually minimal. 30% of resection patients developed bronchopleural fistulae with empyema, and more than 20 additional surgical procedures were required. Furthermore, new cavities appeared, postoperatively, in 18% of resection patients.

On the basis of this study, a conservative, nonsurgical regimen appears desirable for coccidioidal pulmonary cavity, unless (1) the cavity is over 5 cm. in diameter, or (2) the cavity is enlarging significantly, or (3) there are recurrent, severe hemoptyses.

## THE OMMAYA VALVE IN THE VA

David Salkin, M. D.

All VA hospitals were canvassed and nine\* had used the Ommaya valve on fourteen patients. The cause of the meningitis was Coccy in eight patients, Crypto in three, and one each for Pseudomonas, Blasto, and Actino. The indications included difficulty with intrathecal therapy in eight patients, to intensify local therapy in three, a block of the foramina in two, and a lumbar arachnoiditis in one patient after 185 lumbar punctures. The valves were left in place from six months to two years.

In general, it was satisfactory in eleven cases and otherwise in three. However, the complication rate was very high - seven out of the fourteen cases presented problems, three with plugging necessitating new valves and five with superimposed infections such as micrococcus, streptococcus, and staph epidermis (one had both plugging and infection).

Despite the fact that the procedure is a remarkable advance in therapy, infection and plugging may occur. Cases for the procedure must be selected properly, the surgery done correctly, and the subsequent needle therapy done carefully.

\* VAH: Jackson, Long Beach, Memphis, New Orleans, Oklahoma, Pittsburgh, Salt Lake City, San Fernando, Tucson.