TRANSACTIONS OF THE FOURTH ANNUAL MEETING
of the
VA-Armed Forces Coccidioidomycosis
Cooperative Study

I. ADMINISTRATIVE MEETING

December 3, 1959

The Administrative Meeting was held in the Los Angeles VA Outpatient Clinic (formerly the Los Angeles VA Regional Office) through the kind cooperation of Dr. A. Jerome Sparks, Manager.

The general Study Unit Meeting convened at 10:30 A.M., and was welcomed by Dr. Sparks. The following Study Units were represented as indicated:

VAH, Albuquerque, N. M.	Dr. William Hentel
VAH, Fresno, Calif.	Dr. Stephen Cheu
	Mr. Royal H. Sorensen
	Dr. Kean F. Westphal
VAH, Houston, Texas	Dr. Irving Chofnas
VAH, Kerrville, Texas	Dr. John A. Carswell
VAH, Long Beach, Calif.	Dr. Leroy Hyde
	Dr. Edwin A. Brosbe
VAC, Los Angeles, Calif.	Dr. Sydney Finegold
VAH, Oakland, Calif.	Dr. Selig Weinstein
VAH, Phoenix, Ariz.	Dr. Bernard M. Lipschultz
VAH, San Fernando, Calif.	Dr. David Salkin
	Dr. John D. Steele
	Dr. Milton Huppert
	Dr. Lawrence G. Wayne
VAH, Tucson, Ariz.	Dr. Solomon Netzer
VAC, Whipple, Ariz.	Dr. J. C. Soderstrom
US AFBH, Davis-Monthan, Ariz.	Dr. Peter R. Meis
US AFBH, Lackland, Texas	Col. Robert B. Stonehill
USNH, San Diego, Calif.	Capt. John F. Chace
WBAH, El Paso, Texas	Col. R. C. Hunter
Fitzsimons AGH, Denver, Colo.	Col. James A. Wier
	Major Dean F. Winn
VACO, Washington, D.C.	Dr. Edward Dunner
	Dr. J. W. Raleigh
	John Williams, Jr.
Walter Reed Army Institute of Research	Charlotte C. Campbell
Area Office, San Francisco, Calif.	Dr. Arthur L. Ringle
L. A. County Dept. of Charities	Dr. Roger O. Egeberg

COMMITTEE REPORTS

Reports were presented by the Executive, Laboratory, and Surgical Committees, which had met prior to the general administrative session.

Report of Executive Committee

The Executive Committee met on the morning of December 3, prior to the general Study Unit Meeting. The following subjects were discussed:

New Study Units: Oakland VAH is now a member of the Study. Dr. Weinstein is attending as their first representative. Nothing definite has been heard from the Air Force Surgeon General's Office concerning approval of Edwards AFB as a Study Unit.

Exhibit: The Exhibits Committee will continue to function; there have been requests for publications based on the exhibits, as well as for further showings of the display. Certain modifications will be made before it is taken to additional meetings. In the meantime, the exhibit may be set up at various VA hospitals which request it; intra-VA transportation costs will probably have to be borne by the hospitals involved. When it is desired to ship the exhibit for display at meetings, specific requests, detailing costs, should be sent to Mr. Florey at VACO.

The Registry: The retrospective study, covering the years 1955 through 1958, yielded about 900 cases of coccidioidomycosis. Some objections were raised to the maintenance of a protracted registry of all types of cases in the future. Specific interests to date have included chemotherapy, surgery, disseminated cocci and cavitary cocci.

In view of the fact that many cases will be lost to followup, and that new areas of interest may arise, it was decided to maintain a total registry indefinitely. In order for this to be practical, the new combined registry - followup form has been simplified and shortened. The registry will pro - vide data on numbers and types of cases available. Special interest followup forms will be prepared and distributed as needed for cases falling in one of the special study categories only.

Follow-up: Some old questions, few new answers. Mr. Williams at VACO said that he is studying setups for automated followup programs in VACO. Dr. Wier wanted to know if a reciprocal agreement for inter-VA-Armed Forces followup could be made.

Miscellaneous: VACO has agreed to support a statistician to consult locally with the Cocci Executive Committee. This person would advise in the preparation of report forms, protocols, etc., as well as perform preliminary analyses of Study data.

Some discussion was held over whether the Study should attempt to contact pharmaceutical companies for possible new drugs, or whether we should wait to be approached. Since, in the past, many companies showed little, if any, interest in coccidioidomycosis, the former course might bring to light some drugs which otherwise would never get out of the company laboratories. The subject was deferred for later consideration.

Report of Laboratory Committee

Administration

The Laboratory Committee functions as a subcommittee to the Executive Committee of the Cooperative Study and its responsibilities include the Control Laboratory located at Veterans Administration Hospital, San Fernando, California. The Control Laboratory is supervised by Dr. Milton Huppert and it is operated with funds allocated by the VA Central Office. The budget allows for three full-time personnel (in addition to Dr. Huppert); two laboratory associates and one secretary, who serves as secretary for the Cooperative Study group.

During the past year the Laboratory Committee met in two sessions. On October 30, 1959, the committee reviewed the operation of the Control Laboratory, reviewed and discussed the laboratory data collected on report forms, and prepared a new laboratory report form for future use. On December 3, 1959, the Laboratory Committee formulated the recommendations which were presented later in the day to the entire Cooperative Study Group.

Control Laboratory

The operations of the Control Laboratory have been divided into two sections. The Biological Section is conducted by Mrs. Leila Walker and the Immunological Section is under Mrs. Ruth Russell.

The Biological Section is responsible for the Culture Bank collection, for identification of cultures referred to the laboratory, for determining the antibiotic susceptibility of C. immitis strains, and for the development and testing of new procedures of potential value in laboratory diagnosis. The Culture Bank collection is being expanded to include strains of all fungi which may cause pulmonary disease.

The Immunological Section is responsible for dispensing Dr. C. E. Smith's Lot 64D4 coccidioidin for skin testing, for the manufacture of fungous antigens to be used in serological tests, and for performing serological studies on sera sent to the Control Laboratory. The coccidioidin is available to all units participating in the Cooperative Study, and they are urged to use this as a uniform supply of antigen. The complement-fixing and precipitating antigens are available also to all participating units desiring to do

their own serology. The procedure to be used must be the overnight fixation at 5° C technique described by Dr. C. E. Smith. This technique has been reproduced in the "Standards for the Laboratory Diagnosis of Coccidioidomycosis."

Standards for the Laboratory Diagnosis of Coccidioidomycosis

The standards have been revised for the minimum and recommended laboratory requirements in the diagnosis of coccidioidomycosis. The revisions include an expansion of the standards to encompass the isolation and identification of fungi other than C. immitis. The histopathological techniques employed by the Armed Forces Institute of Pathology have been adopted as the standard.

Revision of Laboratory Report Form

The Laboratory Committee has prepared a new report form which provides adequate space for listing all laboratory data by date, specimen, and results. This laboratory report form is to be used in conjunction with all other report forms. The information on the laboratory report form will be correlated with the course of the disease by the Control Laboratory. Models of this new laboratory report form have been tested against patients' charts and the new form has been found most satisfactory. It required but a few minutes to transfer to the report form the information on the clinical laboratory report slips in the patient's chart.

Review of Laboratory Data from Retrospective Studies

All of the case registry report forms for the 1957-58 series had not been received by the time of the meeting. A provisional summary of the laboratory data was prepared, however, and presented to the Cooperative Study group. This provisional summary was reported separately by Dr. Huppert.

Recommendations of the Laboratory Committee

1. The Culture Bank collection should be enlarged to include all fungi capable of producing pulmonary disease.
2. Coccidioidomycosis serology should be performed weekly during the first month of clinical disease, monthly during the first year after initial disease, and tri-monthly for the second year. Additional serology would be performed as requested by the reviewing physician in follow-up studies.
3. Greater use should be made of the Control Laboratory as a source of uniform serological results. This could be accomplished by:
   a. Setting up a training program for coccidioidomycosis serology at the Control Laboratory;
   b. Regular testing of split specimens from those laboratories performing their own serology;

c. Sending test specimens to laboratories doing their own serology;
d. Having the Control Laboratory do all serology on patients who are on protocol studies.

4. The Control Laboratory recommends:
   a. That media containing 0.4 - 0.5 mg/ml of cycloheximide (Actidione) be used as a screening procedure for the identification of C. immitis, since all strains of this fungus grow on such media while most saprophytic fungi are inhibited;
   b. That spinal fluid specimens be filtered through a membrane medium (such as the Millipore Filter), which can be cultured directly, and that the filtrate be used for serology and clinical chemistry.

Report of Surgical Committee

1. The Committee has reviewed the incomplete Preliminary Data on the pulmonary resections for coccidioidomycosis reported in the Cooperative Study for the four years from 1955 through 1958, compiled and analyzed by Dr. Salkin.

Because of the existing confusion in respect to complication rates and indications for resectional surgery for cocci, it is recommended that the surgical data in this study, when complete, be published without delay, as a preliminary report. It appears, on the basis of the preliminary data reviewed, that complication rates in resectional surgery for cocci are quite similar to those for tuberculosis.

2. The Committee recommends that, if a separate report form is required for surgical data, the items pertaining to surgery in the November, 1958, general form appear adequate with the following additions and corrections:
   a. Correct item 55 to delete the word "Additional";
   b. An item on chemotherapy used as surgical coverage;
   c. The preoperative diagnosis;
   d. Indication of recurrent disease, if any.
3. Pertinent follow-up data should be obtained on all surgical cases--possibly by the addition of several items to the regular follow-up form.
4. Consideration should be given to a study of routine scalene node and paratracheal node biopsies in all cases of pulmonary cocci.
5. Complement fixation titres should be obtained on all cocci resections at weekly intervals for the first month and thereafter at monthly intervals until static.

DISCUSSIONS OF COMMITTEE REPORTS

Laboratory; In addition to changes in Laboratory Standards mentioned in the Committee Report, the in vitro Amphotericin B sensitivity protocol has been dropped. Dr. Huppert's experiences indicate little if any correlation between in vitro results and mouse protection. In the future, the Control Laboratory will perform mouse protection tests. It was requested that pre- and post-chemotherapy cultures from patients on Amphotericin B protocol be sent to Dr. Huppert for mouse tests, to permit detection of changes in susceptibility to the drug.

Surgery: The serial complement fixation titers on all surgical patients are to be plotted on graphs. Preliminary studies indicated that the post-surgical response of the patient is reflected in the shape of the titer curve.

The recommendations for routine node biopsies triggered the familiar discussion "What is really and truly dissemination?" which raised the question as to the limits of the natural draining lymphatics of the lung. Some question was raised of the hazard of cutting into scalene nodes of hot primaries. We may confidently expect this whole discussion to show up at every meeting, at least until a biopsy survey has been made.

PRESENTATION OF STUDY DATA

Drs. Salkin and Huppert presented extensive analyses of the data accumulated in the 1955-1958 study period. Portions of these data were also presented at the Scientific Reports Session on the following day, and abstracts of these presentations are included with the December 4 portion of the transactions.

REPORT FORMS

Copies of the combined Case Registry-Case Followup Report Form and of the new Laboratory Report Form had been distributed to representatives prior to the meetings. Time did not permit discussion at this session. Comments by mail were invited with deadline set at January 30, 1960, and the committees concerned were authorized to prepare the final forms. Any final adoption of the new Registry form will be deferred until the Statistical Consultant has been hired, and can give us advice.

NEW PROTOCOLS

No new protocols were presented,, and the Administrative Sessions of the Meeting were concluded.

II SCIENTIFIC REPORTS SESSION

December 4, 1959

Because of the growing number of people wishing to attend our Scientific Sessions, this portion of the meeting was held in the auditorium of the Los Angeles County Medical Association Building. This also permitted us to assemble for a luncheon in the building. Abstracts of the papers presented at this session follow:

9:00 A.M. Session: Dr. A. L. Ringle, Moderator

CLINICAL DATA FROM THE VA-AF COCCIDIOIDOMYCOSIS COOPERATIVE STUDY

by
David Salkin, M.D., V.A. Hospital, San Fernando, California

GENERAL DATA

To date, we have received Report Forms on 674 patients.

Since we expect a total of 900 to 1000 cases for the 4-year (1955-58) study, detailed data has not been evaluated and only a few items will be mentioned:

1. Racial distribution - Total White 77%, Negro 14%, Latin American White 4%, Filipino 4%. (The Latin American White figures are inaccurate for a detailed history was not made in many cases.
2. Geographical History - 70% lived in the endemic area, 20% visited in endemic areas, 1% have not been in known endemic areas.
3. Pulmonary involvement:
   Side involved - right side in 52%, left in 36%, bilateral 12%.
   Type of lesion - Cavitary 35%, Nodular 42%, Pneumonic 13%, Other 10%.

DISSEMINATED CASES

To date, 93 patients showed disseminated disease out of 676 reported. (14%)

1. Racial distribution - Of the disseminated admissions, 9% of the patients were White, 34% were Negroes, 25% were Latin Americans, and 30% were Filipinos.
2. Duration from first illness to evidence of dissemination - In 31%, the time was unknown. In the rest, 16% disseminated in the first month, 93% in the first 6 months, and the rest up to 6 years.

3. Sites of dissemination - Almost everywhere in the body especially the skin, lymph nodes, bones, and central nervous system.
4. Prognosis -
   a. All cases - Inactive 22%, active 48%, dead 30%.
   b. Meningeal cases (Total 27) - dead 19, alive 8 (after 13 to 120 months duration of dissemination)
   c. Non-meningeal - (Apparently similar for the various types) - Inactive 30%, active 55%, dead 14%.
5. Pulmonary Involvement - 61 patients with cavities in 6 patients.
6. Treatment - The variability of the disease is best shown by the following:

   Treatment

   None or symptomatic - 33% improvement (moderate or marked)

   Various "specifics" (Omitting IV Amphotericin B) 25% improvement.

SURGICAL CASES

To obtain equitable figures, the following evaluation is made only upon those patients who were operated upon for the first time in 1955-8 and only pulmonary resections of an elective nature are included.

1. Number of cases - 177 patients (178 operations - 1 bilateral)

2. Indications for Surgery

Cavity - Known Cocci - Asymptomatic	16	
-Symptomatic	62	
Unknown Cocci - diagnostic	27	
		105
Nodules - diagnostic		71
Pneumonia - diagnostic		1
		177

3. Type of Resection - Subsegmental 32%, Segmental 37%, Lobectomy 31%.

4. Complications - major

None	152 patients	
Specific Cocci	9 -5%	(Study Unit 3%; Non-Study 11%)
Non-Cocci	16 -9%	(Study Unit 10%; Non-Study 6%)

5. Cocci Complications - 9 pts. - BP fistula - Empyema - Exacerbation of disease - New cavities.

   Factors - Palpable disease left in - 3 ex 5 - 60%.
   - Study vs Non-Study Units - (4 vs 5) or 3% vs 11%)
   - Type of disease - cavity 7% (8 ex 105)
   - nodules 1% (1 ex 71)
   - Type of resection - Subseg. 0; Seg. 5%; Lobes 11%

Fate of 9 cases - Well 3; Improved 2; Unimproved 4.

Comparison with TB Surgery: Empyema Index (Specific and others)

	TB	COCCI	
		All Units	Study Units
Subseg.	1%	2%	2%
Seg.	4	6	2
Lobes	4.5	11	4
	(Similar)		

AMPHOTERICIN (IV) RESULTS

To date (11-20-59), 49 cases treated.

	Total	Definite improvement	None, indifferent, inconclusive	% improvment
Meningitis	8	2	6	25%
Other dissem.	24	5	19	20
"Residual" cavity	9	3	6	33
Acute pneumonia	4	0	4	--
Chronic pneumonitis	2	0	2	--
Post. Op. Empyema	2	2	0	--
	49	12	37	24%

Comments:

Meningitis - In 3 additional cases, there was an immediate favorable response which did not persist. Only 1 case had intrathecal therapy. In 2 cases who improved generally, a cord bladder and a monoplegia developed.

Other disseminations - In the 5 improved patients, treatment was considered life-saving in two, excellent in two, and good in one. In 5 additional cases, there was an immediate favorable response which did not persist. Difficulties in assessment are shown in two cases who improved their original lesions but developed new ones while under therapy.

"Residual" cavities - In the 3 improved cases, one 2 cm cavity closed and two others became smaller.

Acute primary pneumonia - no effect.

Chronic pneumonitis - no effect.

Postoperative Cocci Empyema - considered helpful.

LOCAL TREATMENT WITH I. V. AMPHO. B. Done on 5 patients.

NO.	Tissue involved	Results: Excellent	Good	None
1.	Bone			x
2.	Bone	x		
3.	Knee joint		x	
4.	Sinus tract	x		
5.	Lymph nodes-6		x	
		2	2	1

IMPRESSIONS:

There is no doubt as to the beneficial effects of Ampho B in many human cases but the drug is a toxic one and the relapse rate is high. More time is needed to assess it properly. It appears that the most favorable results are associated with (1) long duration of treatment, for months and perhaps years, and (2) large total dosage, above the 2000 mg. range.

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LABORATORY DATA FROM THE VA-AF COCCIDIOIDOMYCOSIS STUDY

by
Milton Huppert, Ph.D., VAH, San Fernando
California

Not all of the report forms for cases admitted to hospital during 1957-58 had been received at the time the laboratory data was tabulated. This report is based upon data from 189 cases of the 1957-58 series. Since it is not known how much effect the unreported cases would have upon the final tabulation, the incomplete series for 1957-58 was compared with the complete series for 1955-56. The results of these two series correlated well with each other, and, therefore, it is believed that the additional 1957-58 cases will not affect the percentile figures or inferences recorded in the following, which represent the combined results for both series.

With non-surgical patients, the highest proportion of positive results was obtained with serological techniques (76%). Cultures (45% positive results) and direct microscopic examination (36% positive results) ranked second and third. With surgical patients, however, the results by culture and direct microscopic examination were equivalent to those obtained with serology.

Analysis of the data by type of specimen indicated that the best yields in terms of positive results were obtained with tissues and purulent material. By direct examination, 86% of these specimens were positive, and by culture

75% were positive. In contrast, the corresponding figures for sputum, bronchial, gastric, and body fluids specimens were 30% positive by direct examination and 48% positive by culture.

The data for intracutaneous hypersensitivity substantiated the conclusion that a negative coccidioidin skin test does not exclude a diagnosis of coccidioidomycosis. Twenty-seven percent of the patients tested had negative coccidioidin skin tests, and of these negatives, 89% were proven cases of coccidioidomycosis. There was no significant difference in this respect between the disseminated cases and the non-disseminated. A high proportion of these patients had been tested also with histoplasmin, and 50% of these were histoplasmin positive. From the data available, it was not possible to determine whether these positive cases represented a development of histoplasmin hypersensitivity, which was unrelated to their coccidioidomycosis, or whether there was some cross-reaction in skin hypersensitivity between the two diseases.

The results with complement-fixation serology paralleled those which have been reported by C. E. Smith and his colleagues, particularly with reference to the relationship between a "critical" titer and dissemination. It is important to consider these serological results not only in terms of disseminated disease versus non-disseminated, but also to separate the former into meningeal and non-meningeal cases. Of those patients who had a positive complement-fixation serology, 80% of the non-disseminated cases had titers of 1:16 or less. In the non-meningeal disseminated cases, 90% had titers greater than 1:16. The pattern for the meningitis cases fell between these two extremes; only about 50% had titers greater than 1:16.

One unexpected result appeared in the relation of negative skin test to positive serology. Of those patients who had a negative coccidioidin skin test almost 70% had a positive complement-fixation test, and there was no significant difference in this group between the disseminated and the non-disseminated cases. This is in contrast to the findings of C. E. Smith and his coworkers who reported, "Serological tests were positive only after allergy was established, unless dissemination was associated with anergy." It should be noted that Smith's conclusions were based on a much larger series of cases.

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FURTHER EXPERIENCES WITH AMPHOTERICIN B IN COCCIDIOIDOMYCOSIS

by
Robert C. Hunter, Jr., Col. MC, Wm. Beaumont Army Hospital,
El Paso, Texas

Introduction.

In the past 2 1/2 years we have used Amphotericin B in 7 patients with disseminated coccidioidomycosis and 1 patient with chronic pulmonary

coccidioidomycosis. Our data are inadequate statistically and inconclusive generally, so I shall present the statistics first and the conclusions later.

Oral Therapy.

Two patients with disseminated disease were treated with oral Amphotericin B. It was not apparent that the treatment had any effect on the course of their disease.

Local Injections.

In 3 patients with disseminated disease, 5 lymph node and 3 bone abscesses were injected a total of 17 times with Amphotericin, 25 mg. in 2.5 cc water per injection. There were no untoward systemic nor local reactions. All of the abscesses improved. Two of the patients were also on intravenous therapy at the time.

Intravenous Therapy.

We treated 5 patients with intravenous Amphotericin B. Four had disseminated, and one chronic pulmonary coccidioidomycosis.

Case 1. Chronic skin ulcers healed promptly with a total dose of 1125 mg Amphotericin B.

Case 2. Chronic fever ceased and sinus tracts over sternum stopped draining with a total dose of 1800 mg. Amphotericin B. She is now relapsing, 6 months later. She had been a failure on oral therapy.

Case 3. This patient was much improved after 2550 mg. Amphotericin B. He relapsed two months after cessation of therapy. He was given 1890 mg. more and sent to duty. I have intentionally lost track of him. His proclivity for fighting with the police made case difficult to manage. His total dose of Amphotericin B was 4440 mg.

Case 4. The next is my prize patient. He was admitted two years ago critically ill, with cough, chills, fever, (temperature of 103-104°F), weight loss of 25 lbs., skin lesions, generalized lymphadenopathy, hepatosplenomegaly, anemia (requiring 4 blood transfusions the first month), eosinophilia, pulmonary infiltration, hilar adenopathy, peritonitis, and ileus. We were able to recover C. immitis almost anywhere we looked. His complement-fixation titer was 1:16.

Many of the nodes in his parotid areas, neck, and groin broke down into abscesses which we injected locally with Amphotericin B. We also gave him 7000 mg. IV over a 10 month period. At the end of that time he appeared perfectly healthy. However, his complement-fixation was 1:256.,

Two months after cessation of therapy, he relapsed, with many new tiny skin lesions, a destructive lesion in a phalanx, and general toxicity. We have given him an additional 6500 mg. Amphotericin B intravenously.

We also injected the lesion in the phalanx. He again appears healthy, but his energy is somewhat diminished. His complement-fixation is now 1:512. I fully expect another relapse when we stop treatment this month. His total dose of intravenous Amphotericin B so far is 13,500 mg.

Case 5. This patient had hemoptysis for two years and a thin-walled pulmonary cavity for at least 19 months. In recent months he has developed considerable infiltration about the cavity. His sputa were almost 100% positive for C. immitis. Because of his symptoms, increasing size of the lesion, and our increasing experience with Amphotericin B, we have treated him with this drug intravenously despite the fact that his disease was not disseminated. He is doing well after 7 weeks therapy and a total of 1365 mg. It is too early yet to evaluate the results.

Topical Ointment.

We have treated one patient with Amphotericin B ointment. He had 6-7 granulomata of the skin and a complement-fixation of 1:8. We used a 0.67% of Amphotericin B in aquaphor. He was supposed to treat only half the lesions, but somehow he treated all of them. (NB: He had obviously never heard of the man who baptized one of a pair of twins and saved the other for a control.) In 3 months his skin had healed, and his titer was 1:2.

I suspect that Amphotericin B quickly becomes inactive in aquaphor, and that either we were observing the natural course of his disease or that aquaphor is good therapy for coccidioidomycosis of the skin.

In any event, this was what happened.

Conclusions:

1. Amphotericin B is useful in treating coccidioidomycosis.

2. The toxicity of the intravenous preparation is acceptable if the dose is increased gradually, if it is administered slowly, and if it does not exceed 65 mg. three times weekly in the average case.

3. Local injections into coccidioidal abscesses are highly effective. Local or systemic reactions are negligible.

4. Relapses are to be expected on cessation of therapy.

5. Prolonged therapy is essential. I have the distressing feeling that some patients may require indefinite maintenance therapy, much as in diabetes mellitus. I should like to leave this thought with you as my contribution to this meeting.

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CASE REPORT OF COCCI-MENINGITIS WITH SUDDEN DEATH AFTER AMPHOTERICIN B, AND UNUSUAL PATHOLOGICAL KIDNEY FINDINGS

by

S. Netzer, M.D. and I. A. Beeman, M.D., V A Hospital, Tucson, Arizona

A 28 year old male who had resided in the Southwest for two years became ill on October 31, 1957, complaining of headaches. He received antibiotics with no effect. His headaches persisted and he also developed double vision and vomiting. He was admitted to VA Hospital, Tucson, Arizona, on November 26, 1957, where a diagnosis of coccidioidal meningitis was confirmed by blood and spinal fluid serology for C. immitis.

He received three courses of intravenous Amphotericin B for a total of 5,331 mg. His symptoms subsided but his blood serology and spinal fluid titres remained at elevated levels. He was discharged on July 23, 1958, and returned to work as a mechanic.

He was rehospitalized on September 8, 1959, because of double vision and vomiting. He was again started on 60 mg. of Amphotericin B in 500 cc. of glucose, over a period of six hours. Patient was frequently warned to stop accelerating the flow of his infusion. On October 21, 1959, his I.V. Amphotericin was given in the usual way; however, when he was checked twenty minutes later, it was found that patient had accelerated the flow and but 20 cc. remained of the original 500 cc. Patient was unconscious and pulseless and expired ten minutes later.

Autopsy revealed coccidioidal involvement of the brain stem, meninges, lungs and kidneys.

It is believed that the acceleration of the I.V. therapy could have been the immediate result or have contributed to this patient's death.

We also feel that this patient should have had Amphotericin B therapy during the period from July 25, 1958, to September 8, 1959, although he was symptom free; however, his titres (blood and spinal fluid) continued to be elevated.

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10:30 A.M. Session: Dr. R. O. Egeberg, Moderator

EXPERIMENTAL RESPIRATORY INFECTION OF MONKEYS WITH COCCIDIOIDES IMMITIS

by

E. P. Lowe, Converse, J. L., Blundell, G. P., and Castleberry, M. W., Ft. Detrick, MD.

Macaca mulatta monkeys were exposed to calculated inhaled respiratory

dose levels of 10, 10^2, 10^3, and 10^4 Coccidioides immitis, Silveira arthrospores grown and harvested from solid medium and aerosolized from a dry powder. The pathogenesis of coccidioidomycosis was followed by means of serology, skin sensitivity reactions, x-rays, weight changes, body temperature recordings, mycological cultures, and gross and microscopic histopathological studies of autopsy material.

Clinical and laboratory observations of these monkeys indicated (1) infection of 100% of the animals, even with 7 to 8 arthrospores, (2) dose dependency of the incubation period and the severity of the disease, (3) deaths in 1 to 3 weeks resulted from primary acute pulmonary infection, with little or no evidence of extra-pulmonary dissemination, (4) in general, the immediate cause of death from primary acute pulmonary infections was probably anoxia, resulting from coccidioidal pneumonia in which 50 to approximately 90% of the alveolar lung space was inactivated, (5) substantial weight losses associated with acute infection, (6) good correlation between rising CF titer and disseminated infection, in animals surviving longer than 3 weeks, and (7) no evidence of communicable infection from infected animals to control animals housed in the same cages.

Microscopically visible lung lesions, containing spherules, were present in monkeys five days after respiratory exposure to approximately 1500 arthrospores, and body-temperature rises averaging 2.5°F, noted in the same group of animals on the 7th day, reached 5°F by the 9th day following exposure, thus suggesting an incubation period of approximately 4 days for a calculated inhaled respiratory infection with 1500 arthrospores.

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PATHOGENESIS OF COCCIDIOIDOMYCOSIS IN THE MACACA MULATTA FOLLOWING AEROSOL EXPOSURE WITH ARTHROSPORES

by

George P. Blundell, Merida W. Castleberry,
Edwin L. Lowe and John L. Converse,
Fort Detrick, Maryland

The development of coccidioidomycosis in the Macaca mulatta has been studied. The disease was induced by the dissemination of the arthrospores of Coccidioides immitis in graded doses in an aerosol. The pathological changes resulting from the disease were observed in tissues obtained by killing the monkeys at varying intervals for the analysis of the earliest stages of the disease, while additional later stages were observed in animals dying spontaneously of the disease.

Lesions were present in the lung on the fifth day post challenge. Microabscesses developed first and were followed by a pleomorphic cellular infiltration. Necrosis of the central portion of the lesion occurred by the 7th day.

Subsequently the discrete lesions increased in size and became confluent. Animals dying spontaneously between the 10th and 20th day showed the additional feature of marked pulmonary edema. Peribronchial lymph nodes were not involved until the eleventh day and lesions occurred in the liver and other organs after the fifteenth day. All tissues of the body were susceptible to involvement in the late stages of the disease. Endospores and spherules were commonly found within most of the lesions. Mycelial filaments were found only in cavitated lesions.

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1:30 P. M. Session: Dr. J. F. Chace, Moderator

PILOT STUDY OF HISTOCHEMISTRY OF TISSUE REACTIONS IN PULMONARY COCCIDIOIDOMYCOSIS

by
P. J. Melnick, M. D., Ph. D., and John D. Steele, M. D.
VAH, San Fernando, California

A pilot study was made of the enzyme histochemistry of the tissue reactions in several resected specimens of pulmonary coccidioidomycosis. Esterase, alkaline phosphatase, lipase, phosphamidase, and succinic dehydrogenase were found in the epithelioid cells and giant cells in the healing nodules. In the nodules that were not healing these enzymes were reduced or absent, and viable spherules were found containing enzymes and endospores. In the healing nodules the few remaining spherules were seen to be empty shells without enzymes or endospores. In the caseous centers of some nodules M-nadi oxidase and 5'-nucleotidase were found, which may be responsible for liquefaction and cavitation.

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SOME PROBLEMS IN IMMUNITY TO COCCIDIOIDOMYCOSIS

by
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We now have a constant supply of in vitro spherules of C. immitis for morphological and immunological studies. A series of spherule cultures grown without subculture is more than 270 days old.

Histochemically the in vitro spherule is similar to the in vivo spherule and therefore should be a satisfactory source of antigen. The major difference is the lack of the external phospholipid layer, which may be produced by the host. The walls contain neutral polysaccharide but the central vacuole of the immature spherule contains both neutral and acid mucopolysaccharide.

Electron microscopy verifies the similarity between in vitro and in vivo spherule with the exception of the lack of the external phospholipid layer. The central vacuole (polysaccharide) is formed in close association with what may be the Golgi Apparatus. The walls and septa are composed of layers of (polysaccharide) fibres embedded in a (protein) matrix. The mycelium has a simple wall which is less affected by trypsin. These structural differences may result in the superiority of the spherules as a source of antigen.

Killed in vitro spherules produce a positive skin test in hamsters but not in mice. Both the wall and contents are antigenic and fix complement. Many antigens and many "antibodies" may be involved in protection from coccidioidomycosis.

In the mouse, coccidioidin reduces ability to withstand the disease, possibly by binding some antibody. Killed mycelium gives some protection and killed in vitro spherules induce greatest protection. But in no method used is protection from coccidioidomycosis complete. Mice may be desirable for study of infectivity, but they may not be satisfactory for evaluating antigen because they, like patients who get disseminated coccidioidomycosis, may be genetically deficient in antibody mechanism.

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THE ACCURACY OF SEROLOGIC METHODS IN DIAGNOSIS

by
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Several different types of serologic tests are currently employed in the diagnosis and study of three of the potentially systemic mycotic infections, histoplasmosis, blastomycosis and coccidioidomycosis. In many cases, however, the serologic reactions appear to be more confusing than enlightening. This is because of extensive cross reactivity inherent in the crude antigenic preparations used in the serologic tests, and the extremely varied clinical patterns manifested by each of these diseases. Much of the confusion is due to failure to consider these clinical variables and to earlier claims of specificity for each of the antigens which experience revealed did not exist. In spite of these shortcomings that are both apparent and real, serologic evaluation is widely employed at the present time.

The present report is an attempt to summarize serologic reactions in different clinical types of the three infections with particular reference to time of onset, duration and the amount and type of tissue involved. The most frequently observed and troublesome cross reactions are emphasized,

and the types of infection in which the serologic tests are of little or no value are pointed out. Finally, the critical need for additional study of the antigenic mosaics of all of the potentially systemic mycotic agents is discussed. Experience in the serologic evaluation of the approximately 50,000 sera from which the findings in this summary are drawn reveals that accuracy in the serologic test is directly related to the purity and concentration of an appropriately reactive antigenic component. The degree of accuracy desired cannot be achieved with the antigenic preparations currently employed, as these contain mixtures of components which vary in number and concentration, neither of which is reproducible from lot to lot.

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THE DETERMINATION OF THE GEOGRAPHIC DISTRIBUTION OF COCCIDIOIDES IMMITIS IN ARIZONA BY COCCIDIOIDIN TESTING HOME RAISED CATTLE

by

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From various areas of each county of Arizona 11,643 home raised cattle 1-6 years of age were coccidioidin tested and 2,859 (24.6%) were found to be positive. Whereas previous human skin test surveys have given only indefinite indications of the extent of the endemic areas, this study revealed rather definite boundaries and the relative infectivity of various parts of the endemic area of the State. The endemic areas were found to be practically co-terminus with the Lower Sonoran Life Zone -- an area with high average daily temperatures for both summer and winter and with low annual rainfall rates.

The low altitude areas of Gila, Yavapai, and Mohave counties were established as endemic areas for the first time and several areas of the state of above 5500 feet altitude, previously in a suspect classification were found to be non-infective to cattle.

The areas of the state in the altitude range of 1000 to 2500 feet had high annual conversion rates (.24 and above). The rates became progressively lower with increases in altitudes above 2500 feet so that at 4500 feet the rate became negligible (.01) and at 5500 feet and above it was .00. The parts of the state with altitudes of less than 1000 feet had lower conversion rates than 1000 to 2500 feet area. The area with less than 1000 feet altitude is an area which averages less than 5 inches of rainfall per year -- thus it is the driest part of the state, and is apparently too dry to be an ideal area for Coccidioides immitis.

Although several herds of cattle at above 4500 feet had been fed sizable quantities of food raised in the parts of the state where cattle had high annual conversion rates, only a few animals in these herds were coccidioidin positive.

The annual conversion rates (Manos method) for cattle were found to be almost identical with the actual human infection rate in those counties where this relationship was studied.

Annual Conversion Rates to a Positive Coccidioidin Test in Home-raised Cattle in Arizona Counties

County	Annual Conversion Rates
Apache	.00
Cochise	.05
Coconino	.00
Gila (In Lower Sonoran Life Zone)	.25
Gila (Above Lower Sonoran Life Zone)	.00
Graham	.15
Greenlee	.13
Maricopa	.26
Mohave (In Lower Sonoran Life Zone)	.11
Mohave (Above Lower Sonoran Life Zone)	.01
Navajo	.01
Pima	.24
Pinal	.34
Santa Cruz	.12
Yavapai (In Lower Sonoran Life Zone)	.09
Yavapai (Above Lower Sonoran Life Zone)	.01
Yuma	.07

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NITROGEN MUSTARD THERAPY IN EXPERIMENTAL COCCIDIOIDOMYCOSIS IN RABBITS

by

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Clinical improvement in patients with disseminated coccidioidomycosis has been observed following nitrogen mustard therapy. The importance of the reticulo-endothelial system in resistance is well-documented, but since disseminated coccidioidomycosis is a granulomatous disease associated with hyperplasia of the reticulo-endothelial tissues, Kurnick postulated that the therapeutic effect of nitrogen mustard is the result of its action as a depressant of the reticulo-endothelial system, creating an unfavorable environment for Coccidioides immitis. A study of the treatment of experimental coccidioidomycosis in rabbits with nitrogen mustard was undertaken to determine whether there is experimental evidence to support this hypothesis. New Zealand albino rabbits were inoculated intravenously with 10,000, 16,800 and 6,000 viable units of C. immitis respectively, in the three experiments to be reported.

Animals whose serum showed a complement-fixation titer of at least 1:32 were divided into treated and non-treated groups. Nitrogen mustard was administered as a single, intravenous injection in doses of 1.75 or 1.5 mg per kg body weight. Treatment was repeated 2 or 3 times at six-week intervals, beginning 6 to 7 weeks following infection. The experiments were terminated at one year. There was no significant difference in the complement fixation titers of treated and non-treated animals. Depending on the infective dose, the maximum complement-fixation titer in the treated rabbits ranged from 1:64 to 1:4096 and was reached at the 5th to 14th week after infection as compared to a similar range of 1:64 to 1:4096 attained at the 4th to the 19th week in the non-treated group. Good correlation was observed between the complement-fixation titer at the time of death and the extent of disease found at autopsy. There was, however, no strict correlation between the level of maximum complement-fixation titer reached and the prognosis. Titers also appeared to fall whether or not the animals recovered. A higher mortality occurred in the nitrogen mustard-treated rabbits; even after exclusion of animals which died of acute drug toxicity, there were 11 of 13 deaths within one year as compared to 11 of 20 deaths in the controls. There was, therefore, no benefit from nitrogen mustard therapy under the conditions of these experiments.

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BENEFICIAL THERAPEUTIC EFFECTS OF SOLUBILIZED AMPHOTERICIN B FOLLOWING ORAL ADMINISTRATION IN EXPERIMENTAL COCCIDIOIDOMYCOSIS, HISTOPLASMOSIS AND CRYPTOCOCCOSIS IN MICE

by

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Amphotericin B (Fungizone) is accompanied by distressing side effects in many patients, even when the drug is administered by a slow intravenous drip procedure over a six to eight hour period. In a recent series of experiments the presolubilized preparation of Amphotericin B, designed for intravenous use in humans, was administered orally to mice with experimental histoplasmosis, cryptococcosis and coccidioidomycosis in their drinking water. Delayed as well as immediate therapy regimens were carried out.

It was found that total dosages of as little as 70 mg/kilo prolonged the survival of mice and produced negative cultures even when therapy was delayed for as long as four and in some instances ten days after infection. Serum levels of 1.2 mcg/ml were demonstrated after only four days of oral therapy with 25 mg/kilo, or a total dosage of 100 mg/kilo. Four consecutive daily doses of 2.5 mg/kilo, or a total dose of only 10 mg/kilo, produced a serum level of 0.32 mcg/ml.

The presolubilized preparation thus was readily absorbed from the gastrointestinal tract of mice, in contrast to the insoluble preparations designed for oral use. The results suggested that oral administration of the presolubilized preparation might warrant investigation in the treatment of these systemic mycoses in man.

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