

TRANSACTIONS OF THE
SEVENTH ANNUAL MEETING OF THE VETERANS ADMINISTRATION -
ARMED FORCES COCCIDIOIDOMYCOSIS STUDY GROUP

November 29 and 30, 1962

Held at the Los Angeles County Medical Association Building
1925 Wilshire Boulevard
Los Angeles, Calif.

Edited by

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INTRODUCTION

The seventh annual meeting has again been noteworthy. The attendance included 123 students of the disease. The meeting was considered by all present to be one of the most instructive to date.

The basic reports included a series of immunological papers and a description of a new serological screening test; experimental production of the disease in monkeys, dogs, mice and guinea pigs; and a group of reports on epidemiology.

The clinical sessions were equally exciting and dealt with nephrotoxicity due to amphotericin B, results of treatment with the same drug, a classification of the clinical activity of the disease, and the management of ruptured coccidioidal cavities.

We are again indebted to our colleagues in Central Office for the success of the meeting, and especially those who have been very closely associated with us - Doctors James H. Matthews, Edward Dunner and William B. Tucker.

A special note of commendation is due Dr. Lawrence G. Wayne who has served so ably as the Secretary to the Study since its inception. His work was invaluable to the development of the study and in the compiling and editing of the Transactions. His expanding researches on the Mycobacteria have so curtailed his time that he was forced to relinquish the secretaryship. We will nevertheless call upon him for advice. Thank you, Larry!

DAVID SALKIN, M.D.

Chairman

General Study Group Session
Thursday Morning, Nov. 29, 1962

Official Representatives Attending

David Salkin, M.D., San Fernando, Calif. (Chairman)
Lawrence G. Wayne, Ph.D., San Fernando, Calif. (Secretary)
Milton Huppert, Ph.D., San Fernando, Calif. (Director, Coccidioidomycosis Central Laboratory)
James H. Matthews, M.D., VACO, (Chief, Pulmonary Research in Pulmonary Diseases)
Arthur L. Ringle, M.D., San Francisco, Calif. (Area Director, Professional Services)
Wilfrid J. Dixon, Ph.D., Los Angeles, Calif. (Statistical Consultant)
Roger O. Egeberg, M.D., Los Angeles, Calif. (Consultant)
Lyle A. Baker, M.D., VA Hospital, Tucson, Ariz.
Stephen H. Cheu, M.D., VA Hospital, Fresno, Calif.
John L. Converse, U.S. Army Research & Development, Ft. Detrick, Md.
Ray Cowley, M.D., Fitzsimons Army Hospital, Denver, Colo.
Sydney Finegold, M.D., VA Center, Los Angeles, Calif.
A. Gerson Hollander, M.D., VA Hospital, Oakland, Calif.
Leroy Hyde, M.D., VA Hospital, Long Beach, Calif.
Donald C. Kent, M.D., US Naval Hospital, San Diego, Calif.
Howard E. Liston, M.D., VA Hospital, Phoenix, Ariz.
Peter R. Meis, M.D., Davis-Monthan AFB, Ariz.
James R. Rasch, M.D., Lackland AFB, Tex.
J. C. Soderstrom, M.D., VA Center, Whipple, Ariz.

Dr. Salkin opened the Administrative Session with brief mention of current literature appropriate to the coccidioidomycosis study. These included Dr. Greer's recent book on fungus diseases, Dr. Cheu's up-to-date bibliography on coccidioidomycosis and the transactions of the recent meetings of this study group. The 1960 transactions were printed through the courtesy of the Los Angeles TB and Health Association and 250 copies were prepared. The California Tuberculosis and Health Association prepared 800 copies of the 1961 transactions. The question was raised as to whether this year's transactions should be prepared and distributed in the same manner as before, or whether a condensed version should be submitted to the American Review of Respiratory Diseases for publication there. The consensus was that the transactions should be printed as in prior years, with a brief summary of the meeting to be submitted to the American Review of Respiratory Diseases.

The exhibit prepared several years ago has been updated somewhat and presented at the A.M.A. meeting in Los Angeles in December of 1962, and will be presented at the American Industrial Health Conference in Washington, D.C. in March, 1963.

Dr. Salkin next presented the following summary review of the data from the four-year Retrospective study:

REPORT TO THE COCCIDIOIDOMYCOSIS STUDY GROUP

David Salkin, M.D.

Introduction

The figures presented are only approximate. I had hoped that, at this session, I would have been able to report exact figures on the Retrospective 4-year study. Indeed, I received a fair amount of statistical aid from the System Development Corporation. Unfortunately, however, when I checked the various forms against the original charts I found many discrepancies and I have therefore undertaken the task of reviewing every chart and x-ray film in the study to develop a correct set of forms. I am being assisted in this by Mrs. Cleo McCubbin, the secretary to the study.

I am sure that the final figures, in many of our phases, will be different from those I will present today, but I think the overall percentages in many aspects will not be too different. In addition, with several years more experience behind us, we will be able to better evaluate and classify the natural course and type of disease.

The figures presented to the Group were sizable in numbers to show not only the findings but also the problems involved. Those to be presented in these transactions will be a smaller number. The final figures, to be presented at the next meeting, will be quite voluminous.

Interpretations of the following data should be made with regard to the fact that these are patients from a selected segment of the population, and may not be representative of the information which would be obtained from a similar study of the general population.

General statistics

1. Number of patients for the 4-year (1955-58) study:

Total	765
Study Units	661
Non-Study Units	104

It is felt that this number forms about 80% of the possible total, since there was a uniform lack of reporting by the Armed Forces. No more cases of the 4-year period will be included from now on or the study would never be completed.

2. Sex:

Male	745	(97.4%)
Female	20	(2.6%)

A review of the female charts shows a picture similar to that of the males.

3. Race:

	Pts.	% (approx.)
White	593	77
Negro	99	13
Latin American	35	5
Filipino	30	4
Japanese	4	1
American Indian	2	
Chinese	1	
Malayan	<u>1</u>	<u> </u>
	765	100

The Latin American figures should be higher; the lack of a breakdown of the "White" race in most charts will preclude exact figures.

4. Circumstances leading to present hospitalization:

Symptoms:

Present	524 pts.	69%
Absent	<u>241 "</u>	<u>31%</u>
	765 pts.	100%

Signs:

Physical signs present (Inc. x-ray)	642 pts.	84%
No signs	<u>123 "</u>	<u>16%</u>
	765 pts.	100%

5. Type of Pulmonary Lesion by X-ray:

Incidence:

Lesion present	717 pts.	94%
No lesion	<u>48 "</u>	<u>6%</u>
	765 pts.	100%

Predominant character

Cavity	246 times	33%
Infiltration/pneumonia	244 "	33%
Nodular	212 "	29%
Other	<u>39 "</u>	<u>5%</u>
	741 pts.	100%

These figures should prove more meaningful when considered in terms of the type of disease viz. acute or chronic.

6. Coexisting Tuberculosis

TB present in 69 patients or 9.0%.

In 8 patients, the two diseases occurred in the same lobe; in three patients in the same segment; in 5 patients in the same cavity.

Disseminated Cases:

1. Number: 104 patients or 13% of the Study.

2. Incidence by Race:

White	46 pts.	7.7% of 595 in Study
Negro	39 "	39 % of 99 in Study
Latin American	8 "	22 % of 35 in Study
Filipino	10 "	33 % of 30 in Study
Indian	<u>1 "</u>	-
	104 pts.	

3. Onset of Dissemination after Primary (75 known cases):

In first 6 mos.	67 pts.	90%
In 7-12 mos.	4 "	5%
Over 12 mos.	<u>4 "</u>	<u>5%</u>
	75 pts.	100%

4. Main Systems Involved (lung excluded):

Bones	44 times	15 times	alone
Skin	43 "	11 "	"
C.N.S.	32 "	14 "	"
Lymph nodes	26 "	10 "	"
Other	19 "		

Surgical Cases

1. Non-pulmonary surgery 7 cases
2. Pulmonary resection 277 cases
Done prior to 1955 35 pts.
During 1955-58 242 pts.

Control Group 1955-58: 242 operations on 240 patients.
Lesion: Cavitory 118; Nodular 117; Pneumonia 7.
Resections: Subsegmental 101; Segmental 86; Lobar 55.

REPORT OF THE CENTRAL LABORATORY

Milton Huppert

The Mycology Research Laboratory of the VA Hospital, San Fernando, has continued serving as the Central Laboratory for the VA-AF Coccidioidomycosis Study Group. At the request of the Study Group, the Central Laboratory conducts three activities: 1/ distribution of coccidioidin; 2/ a serology program; 3/ a culture bank.

The coccidioidin in use was furnished by Dr. C. E. Smith and designated by him as Lot 64D4. The Central Laboratory maintains a supply of the original concentrate and distributes the 1:100 and 1:10 dilutions to participating units on request. Up to the present time, sufficient coccidioidin has been sent out for 22,750 test doses of the 1:100 strength and for 3,900 test doses of the 1:10 dilution. Ten VA units and five Armed Forces units have been receiving the coccidioidin.

For the serology program the Central Laboratory has manufactured and standardized C. immitis antigens for use in the complement fixation and precipitin tests. These antigens have been available to Study Group members for either experimental investigations or serology testing. The Central Laboratory has established a program of periodic checking of split specimens with those units which perform coccidioidomycosis serology. During the past year, the U.S. Naval Hospital at San Diego was set up to do their own

serological testing. The comparative serology program now includes direct comparisons with the Navy Hospital and with the VA Center, Los Angeles. An indirect comparison is made with Dr. C. E. Smith through the VA Hospital at Phoenix which does not do its own testing but sends split specimens to Dr. Smith and to the Central Laboratory. The agreement between the results of these three units and those of the Central Laboratory continues to be better than 90%.

The Central Laboratory also performs complement fixation and precipitin tests for the Study Group members not having their own serology program. In addition to the tests for coccidioidomycosis, the Central Laboratory does a gel immunodiffusion test for histoplasmosis. Any specimens which are positive in this test are followed with complement fixation using Histoplasma yeast phase antigen and Histoplasma mycelial phase antigen. Serology for blastomycosis is available only for proven cases; routine testing for this fungus infection has been discontinued.

The Culture Bank collection now exceeds 700 cultures of fungi, of which 300 are strains of C. immitis. Other cultures in the collection include strains of H. capsulatum, B. dermatitidis, Crypto. neoformans, and various species of Candida, of the dermatophytes, and Nocardia. The Central Laboratory is prepared to: (1) identify submitted fungus cultures; (2) perform amphotericin B susceptibility tests on strains of C. immitis; (3) assay the virulence for mice of strains of C. immitis.

During the past year, the Central Laboratory has initiated two new investigations: (1) the use of serial complement fixation studies as an objective measurement of the response of patients to therapy for coccidioidomycosis; (2) a gel immunodiffusion test as a screening test to select those serum specimens which should be followed with complement fixation studies. Dr. C. E. Smith's group has established that the titer of the complement fixation test for coccidioidomycosis tends to parallel the severity of the infection. Studies by the Central Laboratory of selected cases undergoing therapy for this disease would seem to indicate that the titer also parallels the response to therapy; as the patient responds successfully to therapy the complement fixation titer decreases, whereas, the titer remains unchanged or actually increases when therapy has not been effective. Since the number of cases which have been studied is small, the above statements are tentative only. Participating members of the Study Group have been invited to cooperate in this investigation.

The gel immunodiffusion technique proved to be highly accurate for screening serum specimens to select out those which would yield positive complement fixation tests associated with a relevant diagnosis of coccidioidomycosis. The results of this investigation are reported in a presentation during the Scientific Session part of this program. Participating members

of the Study Group have been invited to cooperate in an extension of this investigation to determine whether individual laboratories can use the immunodiffusion procedure to screen their own specimens for those which would yield positive complement fixation results. If this should prove successful, it would provide an early report of a positive result at the local laboratory level and overcome the serious drawback of a delay in obtaining the results of complement fixation tests.

(Ed. The proposed collaborative studies on (1) serial complement fixation tests as objective measurement of response to chemotherapy and (2) gel immunodiffusion test as a screen to select specimens requiring complement fixation tests aroused considerable interest, and a number of study members contacted Dr. Huppert individually to arrange to participate.)

PROJECTS UNDER WAY

Dr. Matthews next asked for a roll-call of Study Group Representatives, with a brief statement of the actual projects which are underway at each of their stations.

1. Tucson, Arizona VAH: Dr. Baker stated that Dr. Wallrath's study on the nature of the immune response in coccidioidomycosis is the only pertinent study at his station. There are no organized clinical studies of this disease.

2. Fresno, California, VAH: Dr. Cheu is continuing clinical studies with correlation between serological, clinical and laboratory findings. Mr. Sorensen is continuing his studies on ecology of the fungus, the role of soil humidity and temperature, and drug studies.

3. Los Angeles, California, VA Center: Dr. Finegold stated that the clinical studies were continuing. Mr. Abraham Breslau, who contributed so well to this study from its inception, has passed away recently. Mr. Breslau's work on histochemistry and immunology of *Coccidioides* and coccidioidomycosis have contributed much to our understanding of the organism and the disease which it produces. His contributions will be missed a great deal by all who have benefitted from them in the past. Dr. Guze, at the LA Center, will attempt to assemble the most recent information from Mr. Breslau's laboratory and publish his material, and perhaps find someone to carry on the work.

4. San Fernando, California, VAH: Dr. Salkin is continuing to analyze the data from the Cooperative Study and is developing practical

classifications on the pathogenesis, clinical aspects and clinical activity of the disease. The Reference Laboratory is continuing as reported and the Mycology Research Laboratory is carrying on a program of screening compounds for chemotherapeutic activity against Coccidioides and other fungi.

5. Long Beach, California, VAH: Dr. Brosbe is continuing studies on experimental coccidioidomycosis in rabbits and in tissue culture, and correlations between nitrogen mustard chemotherapy and complement fixation. A program of screening of new drugs is also under way. Dr. Locks is reviewing literature on the use of ventriculoatriostomy in meningitis. Dr. Hyde is continuing to review clinical data on cavitory cases of coccidioidomycosis.

6. Phoenix, Arizona, VAH: Dr. Liston states that there is no laboratory activity in coccidioidomycosis there now, but he is interested in participating in the gel diffusion study.

7. Whipple, Arizona, VA Center: Dr. Soderstrom says there is no organized study of coccidioidomycosis at Whipple.

8. Fort Detrick, Maryland: Mr. Converse says this group is continuing its study of the course of disease as well as immunization of animals.

9. Fitzsimons Army Hospital, Denver, Colorado: Dr. Cowley states there is no organized study of coccidioidomycosis there.

10. Beaumont Army Hospital, El Paso, Texas: Dr. Hunter has no organized study but is reviewing clinical cases and hopes to start a research program soon.

11. Lackland AFB, Texas: Dr. Stonehill is continuing studies on renal toxicity with amphotericin B, emphasizing function and pathology.

12. Davis-Monthan AFB, Arizona: Dr. Meis is interested in participating in the gel immunodiffusion study. He is also collaborating with Dr. Cheu and Mr. Sorensen in the study of chemical fungicides.

13. U.S. Naval Hospital, San Diego, California: Dr. Kent is carrying out several studies including study of the relationship of the clinical course of the disease, serial complement fixation titers and sedimentation rate in followup on the fate of cavitory coccidioidomycosis. He also would like to participate in the gel immunodiffusion study.

14. Naval Biological Hospital, Oakland, California: Dr. Kong, along with Dr. Levine, is continuing the study of immunology in animals and the development of a vaccine for use in man.

Dr. Matthews will look into the prospects of the NIH and Public Health Service participating or joining with us in some of these studies.

PANEL I

NEPHROTOXICITY ASSOCIATED WITH AMPHOTERICIN B
ADMINISTRATION

Moderator: R. B. Stonehill, Lackland AFB, Texas

Panelists: S. H. Cheu, Fresno, California
G. Iovine, Los Angeles, California
W. A. Winn, Springville, California

Dr. Stonehill: In the early days of amphotericin B usage, the renal function damage observed was considered to be transient. With further experience, investigators are now recognizing non-transient changes. Last year Dr. Rasch reported on nephrocalcinosis in patients treated with amphotericin B. This observation led to studies of all parameters of renal function in ten patients treated with this drug. In 5 of 6 patients biopsied, nephrocalcinosis due to the drug was observed, but the sixth patient had had these lesions before use of the drug. In a series of coccidioidomycosis patients who had not received amphotericin B, no nephrocalcinosis was observed, and one patient had coccidioidal lesions in the kidney.

Dr. Cheu: Nephrotoxicity due to amphotericin B is now well documented and is a serious complication of use of this drug; impaired kidney function in the patient is a contraindication for its use. The impairments of function and structure of the kidney as well as other toxic side effects are presented in the following table.

Effects of Amphotericin B on Renal Function

1. Fall in renal concentration ability
2. Decrease in P.S.P. excretion
3. Elevation of BUN and creatinine
4. Renal tubular loss of potassium resulting in hypokalemia
5. Reduction of G.F.A. and renal blood flow
(Amphotericin affects all functions in nephron)
6. Urine abnormalities - low fixed specific gravity,
cellular and granular casts, tubular epithelium,
RBC, WBC and albumin

Effects of Amphotericin B on Renal Parenchyma

1. Degeneration of tubular epithelium
2. Nephrocalcinosis

Other Side Effects from Amphotericin B

1. Chills, fever, headache, tightness in chest, flushing and perspiration
2. Anorexia, nausea and vomiting
3. Anemia (hemolysis and marrow depression)
4. Thrombophlebitis

Dr. Winn: Amphotericin B does not produce a true solution and this is probably a cause of the toxic effects of the drug. BUN tests are not adequate for following kidney damage due to this drug; the serum creatinine clearance test is now considered more useful for following kidney damage. When the coccidioidomycosis is severe enough, the clinician must be prepared to sacrifice some renal function in order to be able to treat the patient. The degree of nephrotoxicity correlates somewhat with the amount of chemotherapy received. One case was mentioned of a patient who received high dosage of amphotericin B along with steroids, and minimum damage to the kidney was observed.

Dr. Iovine: Described the study in progress at the L.A. County General Hospital. Renal function studies and biopsies were made on six patients with disseminated disease receiving amphotericin B and compared with a group of 26 patients who died of dissemination prior to the days of amphotericin B. He exhibited a number of striking slides showing both light and electron microscopy studies. Apart from specific granulomata, all untreated and pre-treated patients showed no significant renal abnormalities except for a high number of arteriosclerotic changes which were apparently not related to the age of the patient. The changes resulting from the use of amphotericin B included glomerular capillary thickening, calcification in the proximal tubules, and islets of tubular atrophy in the cortex with some regeneration. These changes occurred even after 700 mg. and 1500 mg. of the drug.

(Ed.: We need more such studies.)

Dr. Stonehill: There are multiple manifestations of nephrotoxicity due to amphotericin B. The damage may be suspected by routine tests and then well defined with a series of more sophisticated tests. Early changes in the urinary sediment generally precede elevated BUN level. The phenolsulfonphthalein test is better than the BUN.

Dr. Winn: Feels that the creatinine clearance test is both simple and sensitive and that the BUN is not reliable. At Springville, they are minimizing the use of intravenous chemotherapy, particularly in meningitis, where they are using intracisternal route, and thus

are minimizing the renal damage due to this drug. One patient gave a poor creatinine clearance test, one year and more after stopping intravenous amphotericin B.

Dr. Iovine: Does the serum potassium level drop parallel the impaired renal clearance, and does supplementary potassium feeding help to relieve this?

Dr. Stonehill: Is now investigating this question. Rough data to date suggests that the persistent change in the kidney is not actually due to the hypokalemia.

Dr. Rasch: In one case, forced feeding of potassium has improved the kidney function.

Winn: Similar observation to that of Dr. Rasch. All intravenous usage of amphotericin B leads to some loss of kidney function. When less than 2 gms. total dosage has been used, the kidney damage is probably not significant. As the total amount of the drug administered reaches from 2 to 5 gms., the damage does become important.

Dr. Stonehill: In patients who have received over 5 gms. of amphotericin B, has not seen a relationship between the amount of dosage and nephrocalcinosis. He has seen significant kidney damage in a patient who received only 1 1/2 gms. of amphotericin B.

Dr. Cheu: Believes that the kidney damage is more a function of individual susceptibility than total dosage of drug received by a patient. It seems that the rapidity of onset of renal toxicity may not be related to the total dose administered nor how many days one has been on amphotericin B therapy. Dr. Cheu encountered a Filipino male who was treated with 10-20 mg. amphotericin B a day for a week and developed all the side effects of amphotericin B named.

Dr. Winn: Agrees that kidney damage is a function of both individual susceptibility and total dosage of the drug. In patients on whom he now uses intravenous amphotericin B therapy, he gives daily treatment only while building up the level, and then drops to every other day. In general, he attempts to avoid daily amphotericin B treatment and also is inclined to give vacations from treatment; does not yet know if this change in regimen will help prevent kidney damage.

Dr. Reed: In dogs treated intramuscularly with amphotericin B, no serum level of the drug was observed. In dogs treated daily with the drug, intravenously, for ten days, at a one mg. per kg. per day dosage

schedule, the dogs died. The same total dosage, given on alternate days, produced no significant BUN changes in his dogs.

Dr. Winn: Intrathecal and oral therapy, and local administration of 4% suspension of amphotericin B do not lead to kidney damage.

Indications for use of amphotericin B

Dr. Winn: Stresses the fact that the indications for surgery presented in his table last year takes all combinations of the various indications into consideration. A single indication is not sufficient to justify use of amphotericin B. Is more conservative in use of the drug than he was earlier, because of the new information on toxicity. Still uses it in "impending" dissemination and in some ruptured cavities. As a prophylactic drug for surgery, gives it 3 to 4 weeks preoperatively and about 2 weeks postoperatively.

Dr. Stonehill: Uses amphotericin B only in progressive disseminated disease, but not on a disseminated patient who seems to be doing well, without chemotherapy. Does not use the drug for surgical prophylaxis, but most of the Air Force cases are surgeries for nodules, and so, less likely to need coverage.

All agree that amphotericin B should be used on meningitis cases.

Dr. Locks: Batches of amphotericin B vary in composition. Can a particular batch of drug employed be correlated with the degree of renal damage?

No answer was forthcoming from the Panel.

(Reference was made to a publication by Bell which noted no necropsy evidence of nephrotoxicity on patients who had received amphotericin B. It must be noted that the patients reported in this article were all started on chemotherapy terminally and had received low total dosage at time of death.)

(Editorial note: Please remember that if amphotericin B is dissolved in water the water must not contain a preservative or it will precipitate the drug.)

1. SKIN REACTION FACTORS IN COCCIDIOIDIN SENSITIVE INDIVIDUALS

Evelyn B. Wallraff
Tucson, Ariz.

Strauss and Stetson's Warburg Studies on the effect of in vitro addition of macromolecular substances to lightly heparinized human blood have indicated a stimulation of oxygen uptake upon addition of bacterial endotoxins and soluble antigen-antibody complexes. The effect has been attributed to stimulation of respiratory activity of blood leucocytes. (J. Exp. Med. 112/4, 653-669, 1960). Since skin sensitivity to coccidioidin has been transferred passively in humans with leucocytes from sensitive donors (Rapaport, et al, J. Immunol. 84, 358, 1960), it seemed worthwhile to investigate the addition of coccidioidin to whole blood samples from coccidioidin negative and positive reactors. Lightly heparinized whole blood samples (3 units heparin per ml. final concentration) from healthy normal coccidioidin positive and negative skin reactors were compared by the direct Warburg technique. Smith's lot 64D4 coccidioidin was used, both as the skin test antigen and as the test reagent for in vitro addition to whole blood. After equilibration of duplicate 2.8 ml. samples at 36°C, readings were taken at four 20 minute intervals before, and five 20 minute intervals after tipping in 0.2 ml. of 1:100 coccidioidin from the Warburg vessels. Duplicate conventional blood smears were made before, and from the Warburg flasks upon completion of, the manometric measurements. One set was stained by Wright's method and examined for evidence of leucocyte degeneration. The second set was examined by fluorescent microscopy using the fluorochrome acridine orange, in the hope of demonstrating attachment of the coccidioidin to the surface of the lymphocytes.

Results of the Warburg studies indicate that whole blood samples from positive skin reactors show a transient small, but statistically significant, increase in oxygen consumption upon in vitro addition of coccidioidin. Further studies are in progress. Under the conditions of the experiments, no evidence of alteration of mononuclear morphology was demonstrable by fluorescent microscopy. This approach, using the leucocytes of peripheral blood under more physiological in vitro conditions, has exciting possibilities for the study of the role of leucocytes in delayed hypersensitivity in general, including, in addition to coccidioidin sensitivity, tuberculin sensitivity, virus immunity and autoimmunity mechanisms.

Discussion from the floor:

Brosbe: Did the intensity of skin test reaction correlate with the degree of increased oxygen uptake of the cells? Answer: No data on this.

Question: What were the complement fixation titers of the patients? Answer: Don't know.

Locks: Would it be useful to obtain lymph from infected animals, since this contains practically pure lymphocytes? Answer: Different animals would react in a different manner.

* * * * *

2. IMMUNODIFFUSION AS A SCREENING TEST FOR COCCIDIOIDOMYCOSIS SEROLOGY

M. Huppert and J. W. Bailey
San Fernando, Calif.

An agar gel immunodiffusion test was developed as a technique for screening serum specimens for those which would yield positive results in the complement fixation and precipitin tests for coccidioidomycosis. The three tests were performed on serum specimens obtained as part of the routine procedure on admission to this hospital. After the results were obtained they were correlated with the final established diagnosis for each patient.

Specimens from a total of 433 patients were tested. Thirty-one patients had positive complement fixation tests and 23 of these patients also had positive immunodiffusion tests. In at least 7 of the 8 patients who had positive complement fixation and negative immunodiffusion results, a clinical diagnosis of coccidioidomycosis was eliminated as not being relevant to the current illness. All 7 patients were residents of areas known to be endemic for *C. immitis* infection. It was concluded, therefore, that the immunodiffusion test had detected 96% (23 of 24) of the patients yielding significant positive complement fixation results. Of the 402 patients whose specimens were complement fixation negative, 401 were negative also in the immunodiffusion test. For those patients from whom multiple specimens were obtained, the complement fixation and immunodiffusion results on each specimen were in perfect agreement.

It was found, therefore, that the immunodiffusion results correlated well with those of the complement fixation test. Both tests were positive on serum specimens from patients with a diagnosis of coccidioidomycosis relevant to the current illness. In those patients with negative complement

fixation tests, the immunodiffusion result was also negative, and in those patients from whom multiple specimens were obtained, the two tests were consistently in agreement. It was noted, however, that both tests failed to detect 6 cases with solitary pulmonary nodules, 3 of which were proven to be coccidioidal, and 1 case of proven coccidioidal cavitary disease. The two tests had been positive in 3 other cases with solitary pulmonary nodules and 4 other cases with cavitary disease.

A similar correlation did not exist between the precipitins and immunodiffusion tests. It was concluded that routine testing of serum specimens for coccidioidomycosis could be performed with the immunodiffusion and precipitin tests. A positive result with either procedure should be followed with complement fixation studies.

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Discussion from the floor:

Marcus: A variety of antigens can be used in the center well. Has used a ten-fold concentrated antigen, but, in guinea pigs, even this was found to miss some known infected cases. Answer: This study used diluted antigen rather than concentrated one and found a high degree of activity.

Question: A correlation has been noted between the complement fixation titer and number of lines in the immunodiffusion technique, using a glycoprotein antigen extract. Have you seen anything like this? Answer: Yes.

Newcomer: Were any cross reactions with other fungus diseases seen? Answer: None yet.

Hampson: Is this the same complement fixation antigen as is used elsewhere? Answer: Yes.

Locks: The clinician is interested in the low complement fixation case and questions discarding "non-relevant" low complement fixation cases.

Question: Were there any non-disseminated cases with multiple lines on the gel? Answer: Yes, one, a severe pulmonary case.

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3. PRIMARY LOCUS OF IMMUNOGENS IN COCCIDIOIDAL SPHERULES

Y. M. Kong, H. B. Levine, C. E. Smith
Oakland, Calif.

The immunogenicity for mice of the spherule-endospore phase of Coccidioides immitis was investigated in respect of the duration of immune response and the stability, locus, and specificity of the antigens. Formalized vaccine retained potency during 20 months' storage at 4°C in the presence of formalin, or after treatment with chloroform. Induced immunity did not decline during a 4 1/2 month interval between vaccination and intranasal challenge, and was effective against, lethal challenge with an heterologous strain (46) of C. immitis. Nonspecific resistance to Cryptococcus neoformans or Pseudomonas (Malleomyces) pseudomallei was not induced by vaccination. Immunogenicity resided primarily, but not exclusively, in the wall structure of disrupted spherules; some activity was demonstrable in the soluble cytoplasmic moiety by use of an adjuvant. Induced immunity appeared to be associated with an augmented capacity of the immunized host to suppress fungal multiplication by 50- to 500-fold in the lungs, and to delimit the loci of infection in these organs.

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Discussion from the floor:

Question: Have noted strain differences with respect to protection. Were only two strains tried here? Answer: Only two were tried here, and a third is now in process.

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4. A COMPARATIVE STUDY OF COCCIDIOIDAL ANTIBODY TITERS IN VARIOUS LABORATORIES

C. Ross Hampson
Bakersfield, Calif.

Eight pooled sera were sent to seven laboratories for coccidioidal antibody complement fixation tests. Accompanying each set of sera was a questionnaire, concerning some of the techniques employed in performing the tests.

Six questionnaires were returned and the results revealed that no two laboratories used identical techniques. The greatest variation appeared in the incubation period of the hemolysin and complement titrations and the total volume of the reagents in the test. There were four different antigens used.

One laboratory's results were consistently one dilution lower than the average of the results of the other laboratories participating in the survey. However, when these results were compared with the results obtained by the 2 hour fixation technique there was a 100% agreement. This presents a problem of possibly having two different "critical titers" (1:16 and 1:32), depending upon the laboratory performing the tests and techniques employed.

There were no false positives nor any false negative reports encountered in this survey.

In spite of the many different techniques and antigens employed in performing the complement fixation tests there was a surprisingly close agreement in the results.

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Discussion from the floor:

Marcus: The correlations in these studies look as good as those that have been reported in similar studies with syphilis, where the tests and the antigen are more standardized. Answer: Standardization is important here with respect to critical titer.

Huppert: There is probably a role of selection of the laboratories doing the test, showing up in this study. Answer: These are all labs that have been doing much of this work.

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FURTHER SEROLOGICAL STUDIES UTILIZING CULTURE SPHERULES OF C. IMMITIS

A. M. Breslau*

(Read by title)

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Discussion from the floor:

Dr. Huppert took this opportunity to pay tribute to the memory of Abraham Breslau, who contributed so much to the study of histochemistry, electron microscopy and immunology of coccidioidomycosis.

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*Deceased

6. CLASSIFICATION OF THE CLINICAL ACTIVITY
OF COCCIDIOIDOMYCOSIS

D. Salkin
San Fernando, Calif.

There is a great need for a uniform classification of the activity of the disease. Every clinician has his own concept of the significance of a cavity, a specific x-ray picture, or a positive serological test. We have, at the present time, enough knowledge of the natural history of the disease to enunciate certain broad principles which can be used as criteria in judging activity. It is proposed that the classification be subdivided into Active, Quiescent, and Inactive phases.

Principles Used as the Basis for the Classification

- (1) Symptoms and signs - their presence is important but their absence may be compatible with active disease.
- (2) X-ray - Any recent change signifies activity. All cavities mean active disease based on the actual pathology known to be present and the potential danger, regardless of even prolonged periods of asymptomaticity.
- (3) Mycology - A positive mycology means activity; a negative mycology does not exclude active disease.
- (4) Immunology:
 - (a) Skin test: A positive test means allergy to C. immitis antigen and may or may not signify active disease. A negative test may occur with active disease.
 - (b) Precipitin test: A positive test means active disease.
 - (c) Complement fixation test: A negative test may occur with active disease. A positive test of 1:8 titer or less may occur with active or inactive disease. Titers of 1:16 or over should always be regarded seriously. A positive test (any titer) with spinal fluid is diagnostic of CNS disease.

(5) Pathology: If available, it is an excellent index of the clinical status. However, small, well-defined stable nodules may be regarded as clinically inactive, even though many of them show pathological activity and viable C. immitis.

(6) A period of 6 months is adopted arbitrarily, although it is known that some lesions may be "inactive" for even years, and then reactivate.

STATUS OF CLINICAL ACTIVITY
(coccidioidomycosis)

	ACTIVE	QUIESCENT	INACTIVE
Symptoms & Signs	Present or absent	None	None
X-ray	Progressive, regressive, stable, cavity present.	Stable; recently blocked & filled cavities; no air containing cavities.	Stable and healed. No cavity.
Mycology	Frequently positive	Negative	Negative
Pathology (if available).	Active process.	Healed or healing.	Healed.
Immunology			
Skin test	Usually +.	+ or neg.	+ or neg.
Precipitins	+ or neg.	Neg.	Neg.
CFT	Usually +	Usually +.	Titer under 1:16
Duration		Above present for 6 mos.	Above present 6 mos. or more.

- (1) Although this classification is primarily one for pulmonary lesions, it is adaptable to extrapulmonary lesions.
- (2) Negative Mycology: Negative by smear, culture, animal inoculation, and tissue sections (if available). The secretions must be negative on at least 6 specimens taken 2 weeks apart during a 3-month period. This requirement is minimum.
- (3) A quiescent state may be present if all criteria of inactivity are present, but the complement fixation titer persists at 1:16 or higher.
- (4) The presence of specific pulmonary complications, such as coccidioidal empyema, classifies the disease as active.

(5) Active disease may be further described as *unimproved, progressive, stationary or improved.*

(6) Terms such as "activity *undetermined*" ~~*probably active*~~, ~~*probably inactive*~~ *should be used only temporarily*, and changed as soon as adequate studies are completed.

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Discussion from the floor:

Hollander: With reference to distinction between active and quiescent disease, how would you classify a cavitary case with 1:16 positive complement fixation titer, with all other tests negative. Answer: Active.

Hyde: This might be pathologically active but clinically inactive. Answer: Have not yet seen any perfectly healed pathology on any cavity, so feel that the cavities are active. Cavitary disease means activity. If a drug comparable to INH becomes available, then maybe really inactive cavities could exist.

Question: Does quiescent or inactive apply only to those previously known active, in the interest of diagnosis. Answer: Not necessarily.

Biddle: What tests are necessary to specify negative mycology? Answer: All specimens must be negative by smear, culture and animal.

Stonehill: What is the value of this classification? It should have a prognostic or therapeutic implication. It might be best to wait for a drug and then fit a classification to it. Answer: It does have prognostic and therapeutic implications.

Matthews: In TB, classification is important because of the communicability of the disease. Where would this classification, or any classification, apply for coccidioidomycosis? Should it be directed toward compensation? One must realize the implications of any tags that are applied. Answer: The practical applications include compensation also. A diagnosis of active disease does not mean a person cannot work; it depends upon the stage of the disease and other factors. For example, a mild emphysema case can do even heavy work. All cases would have to be evaluated individually.

Birsner: This classification will serve as a language for communication among investigators.

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7. CLINICAL OBSERVATIONS OF THE EFFECT OF AMPHOTERICIN B ON PULMONARY RESIDUALS OF COCCIDIOIDOMYCOSIS

D. C. Kent and H. O. Kendall
San Diego, Calif.

In the short span of time from the early 1900's until the present, a vast amount of information has accumulated about coccidioidomycosis. As with many other disease processes, our knowledge of many facts of this disease has preceded our therapeutic capabilities in adequately treating this disease. We have been faced with many problems in the evaluation of the various modalities suggesting themselves as effective in this disease; however, we finally have a drug with proven effectiveness in such treatment. Suitable information has now reached the literature regarding indications for the use of amphotericin B, and now, with the known nephrotoxicity of this drug, its use is being more limited. There does appear, however, to be a lack of information available regarding the effect of this therapy on the pulmonary residual lesion in the absence of dissemination.

This paper deals with a clinical report of four cases treated with short term, low dosage amphotericin B therapy, the total doses being limited to a total of two grams or less, with rapid clearing of the pulmonary lesions, and with no residual renal toxicity noted. In each case so treated there was either clearing of the infiltrate, closure of cavity, or decrease in size of cavity, the residual either disappearing, continuing to progress, or remaining stable after completion of the therapeutic course. In each case the change appeared to be more than would be expected in the natural history of the lesion, and even in spite of no controls, was felt to be an effect of the administered amphotericin B.

It thus appeared that this drug may have certain potentialities, some of which had as yet been incompletely explored. With further use of this drug in the low dosage, short term manner, it is wondered if one could decrease the dissemination rate in cases of severe pulmonary infiltrates by early treatment, while these remain in the acute, exudative phase. It was further postulated that it was also a possibility that one could decrease the number of, and extent of, chronic pulmonary residuals by early treatment of such infiltrates. The question thus remains unanswered as to whether one could reap benefits of value in pulmonary coccidioidomycosis by treating more cases of pulmonary coccidioidomycosis, by treating them earlier, and by using less drug in this low dose, short span treatment program.

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Discussion from the floor:

Hyde: How were these cases selected? Answer: Four serial cases, covered before planned surgery, which then became unnecessary. Originally it was planned for 3 to 4 weeks preoperatively, but the changes were so great in 3 to 4 weeks in the first case, that the chemotherapy was continued in 3 more serial cases.

Question: What were the precipitin reactions? Answer: All negative.

Salkin: This case adds to the need for a classification of the disease, which we hope we have provided.

Stonehill: Some of the cases looked like slowly resolving primaries. Most of the Air Forces nodule surgery is for differential diagnosis. Were these really residual lesions? Is the effect observed actually an amphotericin B effect? Answer: Resolution seemed faster than in untreated cases. This presentation was made to raise the question, rather than answer it, regarding possible use of early low-dose amphotericin B.

Einstein: Believes that these are not true, "old lesions". In acute exudative disease, low-dose therapy has value as he and Holeman mentioned in last years meeting.

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Case Presentations

a. AMPHOTERICIN B TREATMENT OF COCCIDIOIDAL OSTEOMYELITIS

H. E. Einstein
Bakersfield, Calif.

Presented was a 36-year old dentist who was first seen with a coccidioidal pneumonia, skin abscesses on the back, revealing positive cultures of C. immitis, with a high titer and marked clinical toxicity. He was treated with intravenous amphotericin B, with approximately 2 1/2 grams being given for his disseminated disease, with good response and return to work. The skin lesions cleared up and the lungs cleared.

Six months later he developed a coccidioidal osteomyelitis of the right wrist, with extensive destruction of the navicular bone and the terminal end of the third metacarpal bone. This was accompanied by a coccidioidal tenosynovitis. Extensive surgical removal of all infected and necrotic tissue was done and injection of amphotericin B through a catheter, 10 mgm. daily, was carried out for 10 days, while a so-called intravenous systemic cover of the drug was given also. On this therapy the patient made an uneventful recovery, his titer of complement fixation antibodies returned to normal, his wrist healed up completely, clinically and radiographically, a functional result was obtained, and he is now once again working full time without any limitation of motion.

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Discussion from the floor:

Balkin: Stressed the value of local amphotericin B.

Einstein: Surgery helped in this case by permitting better placement of the amphotericin B catheter. 100 mgms. of drug was used.

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h. REINFECTION COCCIDIOIDOMYCOSIS IN AN 82-YEAR OLD FEMALE

J. Anderson
Bakersfield, California

This is a report of a case of massive and fatal disseminated coccidioidomycosis from a primary spherule-containing calcified lesion.

This 82-year old white female was admitted to the Kern County General Hospital for the first time on July 28, 1962, with a chief complaint of fever. She had been febrile for 2 days prior to admission and had complained of generalized weakness and inability to walk for 3 to 4 weeks prior to admission. She had a two year history of marked rheumatoid arthritis, but had not been treated with steroids for this. She was a native of Arkansas, but had lived in the San Joaquin Valley continuously for approximately seven years.

Physical examination on admission - Temp. 102°F, BP 110/70, P.96. She presented as a thin, emaciated somewhat dehydrated, poorly nourished female. Physical examination was otherwise unremarkable, except for the findings of rather severe rheumatoid arthritis and a cellulitis of the 5th left toe.

Chest x-ray revealed a small calcified lesion in the right lateral mid-lung field. X-rays taken 2 years previously demonstrated the same lesion which was unchanged in size. Blood cultures were taken and she was placed on Terramycin, streptomycin and intravenous penicillin. Coccidioidin and tuberculin skin tests were negative. She remained febrile and her condition continued progressively downhill. She expired on the 6th hospital day.

At autopsy there was noted thickening of the cusps of the mitral, tricuspid and aortic valves. A small, soft vegetation was adherent to the mitral valve. Fungi could not be demonstrated within this vegetation. A small granuloma of the right middle lobe was preserved and found to contain a considerable amount of calcium and coccidioidal spherules. Spherules were also found in the spleen, liver and kidney.

Blood cultures which had been taken during her hospitalization subsequently grew out Coccidioides.

It is felt that her dissemination was the result of breakdown of the primary pulmonary lesion. Perhaps her debilitated state was partly responsible for this breakdown.

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Discussion from the floor:

Question: What was the serology on the patient? Answer: None was done.
The diagnosis was unsuspected until autopsy.

Question: Were steroids used? Answer: Don't know.

Meis: Has seen one similar case where steroids caused the dissemination.

Salkin: This is an interesting case of late dissemination and endogenous reinfection.

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c. ACCIDENTAL CUTANEOUS COCCIDIOIDAL ARTHROSPORE INFECTION IN AN IMMUNE INDIVIDUAL

R. Sorensen and S. Cheu
Fresno, California

Because primary cutaneous coccidioidomycosis is an unusual occurrence, and since there are no other reported cases of super-infected percutaneously challenged subjects who demonstrate previous immunity, it appeared that this case would be of general interest to the group.

On September 21, 1962, a Caucasian male, 12-year resident of the San Joaquin Valley, accidentally inoculated his left index finger at the middle phalanx while he was challenging a group of mice with a suspension containing approximately 4,000 arthrospores of *C. immitis* per ml. as determined by plate count. On September 25 (4 days later) an induration could be felt over a slightly tender area at the site of inoculation. A few days later a small firm nodule had developed but no increase in tenderness.

On September 26 (the 5th post-inoculation date) serum for complement fixation and precipitin tests were started and drawn at weekly intervals for the next 6 weeks and monthly thereafter.

By October 7 (the 17th day) a small white centered pustule had formed 2 mm. in diameter from which a very few spherules were seen microscopically and which grew colonies typical of *C. immitis*. This growth was inoculated into a mouse which died 10 days later, producing typical pathology and spherules.

By October 8th, an irritating pruritus had developed around the lesion but no lymphadenopathy was recognized.

By October 10th urticaria developed in the palms of both hands, which then spread to all parts of the body and persisted in spite of antihistamine treatment.

On October 22nd, the small remaining granuloma was biopsied. One-half was submitted for sectioning and the other half ground and cultured. The culture plates grew 33 colonies of *C. immitis* and the histology report revealed granulation tissue compatible with granuloma of the skin. PAS stained sections showed a few spherule shells and fragments at the margin of a tiny necrotic abscess.

The complement fixation results became positive 2 plus at 1:2 in 5 days, reached a 3 plus at 1:16 dilution in 4 weeks, and dropped to a 1:2 dilution by ten weeks. All precipitin tests were negative.

It seems most likely that the failure to produce precipitins, the absence of lymphadenopathy and the persistent urticaria can be explained on the basis of pre-existing immunity from an earlier respiratory infection; however, there are no other cases reported to compare it with.

Test No. in weeks	Date	Results			
		1:2	1:4	1:8	1:16
1.	9-26-62	++			
2.	10- 1-62	+			
3.	10- 8-62	++++	++++		
4.	10-15-62	++++	++++	++++	+++
5.	10-25-62	++++	++++	++++	
6.	11- 1-62	++++	++++		
7.	11- 5-62	++++	++++		
10.	12- 3-62	++++			

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Discussion from the floor:

Winn: Has had similar experience in Kern County with a lip lesion. The complement fixation test didn't become positive.

Meis: Has seen a primary nasal lesion persist for 7 years.

(Ed.: Chas. Smith stated in 1960 that Tiggert had such a patient of exogenous infection. See 1961 Coccy Transactions - Salkins Pathogenetic classification, p. 32. Salkin would appreciate hearing about other examples of Exogenous Reinfection).

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d. MATERNAL-FETAL TRANSMISSION OF COCCIDIOIDOMYCOSIS

Thomas R. Larwood
Bakersfield, California

Although several papers in the last decade have concluded that our favorite fungus is not transmitted from mother to child, at least three reported cases strongly suggested it and the one here reported seems to establish its occurrence.

A premature infant, born in August 1962, began nursing slowly on his ninth day of life, developed respiratory distress on the twelfth day, and died after twenty-five days of life with a progressive downhill course. It had never left the nursery, which is air conditioned. Autopsy revealed massive involvement of the lungs with coccidioidomycosis, with much less in the liver and spleen.

gave rise to the strong suspicion that the amniotic fluid or birth passages were the source of infection.

The 22-year old Mexican mother knows of no Filipino or Negro blood in her family, had one previous child and an uneventful pregnancy with this one. She had puerperal fever of undetermined cause for 3 or 4 days but was then well until 4 weeks later, when she was found to have disseminated coccidioidomycosis with meningitis, for which she is still under treatment. A dilatation and curettage done soon after this showed coccidioidal involvement of the decidua, endometrium and cervix. The placenta was reported as normal on merely gross inspection.

There seems no reasonable doubt that this represents maternal-fetal transmission of C. immitis, likely not from the placenta. We have since had another case of overwhelming maternal coccidioidomycosis with death at the eighth month of pregnancy. The placenta contained large coccidioidal granulomata but the cord and fetus were not involved. This adds another example following the usual rule that C. immitis does not cross this barrier.

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Discussion from the floor:

Wyderney: One such died after 42 days at San Diego, with no apparent external source. A plea was made for collection of such data by this Study Group.

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DISSEMINATED COCCIDIOIDOMYCOSIS COMPLICATED BY PREGNANCY

John R. Rasch
Lackland AFB, Texas

The problem of disseminated coccidioidomycosis complicated by pregnancy creates a difficult problem therapeutically, particularly in the deeply pigmented races. The reported mortality rate is extremely high in this situation and is reported to be 100% in a series of eight negro women. Of the cases reported to date, there were 14 cases resulting in maternal death.

A young negro woman was treated at USAF Hospital Lackland with disseminated disease, and after treatment for several months, it became evident that she was pregnant. After considerable debate, it was decided to continue her

treatment with amphotericin B without interruption of pregnancy. Her course was smooth and there was a progressive drop in her complement fixation titer. At term she was delivered of a viable infant. Followup studies of the infant were normal. After two years, the infant has shown no adverse effects to amphotericin B. Our observation would suggest that it may be justified to allow patients in this circumstance to continue their gestation.

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Discussion from the floor:

Winn: One of this morning's cases presented in Panel I, was pregnant and did well. The baby died at 48 hours, but not of coccidioidomycosis.

Anderson: Has seen one case like Rasch's, but without amphotericin B treatment, and the patient did well. A similar case was mentioned from the floor which was treated with amphotericin B and INH and did well.

Perkins: Has seen one case treated with amphotericin B that did well, but the titer was over 1:1000.

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f. CO-EXISTING HODGKINS DISEASE AND DISSEMINATED
COCCIDIOIDOMYCOSIS

John J. Isabel
Bakersfield, California

Patient is a 64 year old Caucasian farm laborer, who had been working and well until he had a minor accident in August of 1961. Routine chest x-ray at this time showed a cavity in the right upper lung. Skin test for coccidioidomycosis, PPD, and histoplasmosis were all negative. Coccidioides serology showed a rising complement fixation titer from 2 plus in 1:4 on 10/9/61 to 1 plus in 1:64 by 3/15/62. In November of 1961 Coccidioides immitis was isolated from sputum. Only conservative treatment was employed at this time, as the patient was doing well and was clinically well and he was sent home in November 1961 with a diagnosis of pulmonary coccidioidomycosis with cavitation in right upper lung. However, in January of 1962, he began to suffer from anorexia, nausea, weakness, night sweats, and weight loss. These grew progressively worse until his admission to Kern County General Hospital on June 22, 1962 for further workup and treatment.

On admission his complement fixation for coccidioidomycosis was 4 plus (1:32). His coccidioidin skin test was still negative, never having ever become positive. Since January of 1962, his course had been quite steadily and rapidly downhill. He had lost 20 pounds, was extremely weak, constantly anorectic, and anorexic. Chest x-ray showed enlargement of the cavity in the right infraclavicular area and additional small cavities present in the right upper lung, and a rather dense infiltrate just inferior to the large cavitation. Sputum tap was negative at this time.

Physical examination on this admission showed wasting and quite marked emaciation. A new finding, however, was rather large lymph nodes in the right supraclavicular area and along the posterior border of the right sternomastoid muscle. It was assumed that these nodes represented lymphadenopathy due to coccidioidal involvement. A lymph node biopsy was done and the pathologist report came back unequivocally Hodgkins Disease.

And there is where the rather strange part comes in. After two weeks of treatment with amphotericin B, it was noted that the cervical lymphadenopathy had greatly receded. After four weeks on amphotericin, the lymphadenopathy was practically completely gone, only one tiny node remaining. This node was biopsied and the pathologist report was again classical Hodgkins Disease and the outer borders of the node showed fibrosis and necrosis. Nothing could be found anywhere in the literature to explain this phenomenon and we could welcome any comment as to some plausible explanation for it. The patient's condition improved after about 2 grams of amphotericin B followed by a course of 16 mgms. of nitrogen mustard, and he was discharged on 1/20/62.

Other cases of co-existing coccidioidomycosis and Hodgkins have been reported in the literature, but this observation was never made; however, the only ones I found were reported prior to the use of amphotericin B.

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Discussion from the floor:

Dr. [unclear]: Had seen a case of Hodgkins Disease treated with x-ray and patient turned out to have coccidioidomycosis.

Dr. [unclear]: A study group in his area has used amphotericin B in Hodgkins. (Dr. [unclear]: What results?)

Dr. [unclear]: Could the shock induced by amphotericin B have stimulated the adrenals and have an effect on the Hodgkins Disease?

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C. Experimental Pathogenesis Papers

8. HIGH AMBIENT TEMPERATURE AND MORTALITY OF
COCCIDIOIDES INFECTED MICE

R. H. Diven
Tucson, Arizona

Twenty male namru mice were administered four hundred particles of *Coccidioides immitis* (strain 76-D) intranasally. Half of the mice were then housed at room temperature while the remaining mice were housed at 36.5°C. The room temperature mice began dying on the eleventh day post-infection and continued to die until 80 per cent were dead on the twenty-third day. Meanwhile, none of the mice that were housed at 36.5°C were dead when the study was concluded at thirty-three days.

In a second study, forty mice (twenty males and twenty females) were inoculated with eighty particles, while a similar group of forty mice were treated with one hundred sixty particles of *C. immitis*. Half of the males and half of the females from each group were housed at 36.5°C, and the remaining mice were held at room temperature. The lungs of the mice were taken at twenty-five days after inoculation. The lungs of the male mice held at room temperature, in contrast to the males housed at 36.5°C, appeared larger because they failed to collapse when removed from the thorax. This characteristic of the room temperature mice can be accounted for by greater induration, hence less elasticity. Also, individual granulomas were several volumes larger in the lungs of this group. While a similar hot and cold contrast was found in the lungs of the female mice, the beneficial effect from the elevated ambient temperature was not as great as it was for the male mice.

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Discussion from the floor:

Huppert: What were the body temperatures of the animals, and temperature sensitivities of the coccy strain employed in this study? Answer: Don't know, but other studies have shown elevation of the body temperature at higher ambient temperatures. Strain that was used was grown routinely at 37°, but this was not tissue phase growth.

gberg: How did the high temperature affect the comfort of the mice? In his experiments the higher temperatures caused much distress. Answer: The animals were very inactive and did not gain weight.

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4. EXPERIMENTAL PRIMARY CUTANEOUS COCCIDIOIDOMYCOSIS IN THE MONKEY

John L. Converse, Steven P. Pakes,
Ernest M. Snyder and Merida W. Castleberry
Fort Detrick, Maryland

Primary cutaneous coccidioidomycosis was studied in the Macaca mulatta, and a suitable strain of Coccidioides immitis for use as a viable vaccine.

Rhesus monkeys, in groups of four, were inoculated either subcutaneously or intradermally in the medial surface of the right forearm, with either 10 or 100 viable arthrospores of C. immitis, strains Silveira, Cash, M-11, D-76, or W-1 (isolate of Cash with reduced virulence and altered colonial morphology).

The monkeys were observed for a period of ten months for clinical signs of disease and for gross evidence of tissue reaction to the injections. At ten months, all animals were sacrificed (pentobarbital sodium) for complete autopsies. Precipitin titers were obtained on sera obtained at terminal bleeding, and cultures were made routinely from the inoculation site, the right axillary lymph node, and the lung. Any suspected lesions in other organs or tissues were also cultured.

Histopathological studies were made on sections of the inoculation site, the right axillary lymph node, and all the body organs, using the Giemsa, as well as specific fungus stains. Lung sections were also checked with fluorescent antibody techniques.

Intradermal inoculation produced more severe vaccination reactions (swelling at vaccination site and axillary lymph node hypertrophy) than subcutaneous injection. A subcutaneous vaccine dose of 10 arthrospores resulted in a less reaction than did a 100 spore dose. Moreover, dissemination beyond the regional lymph nodes did not occur, following injection of 10 spores of even the most virulent strains of C. immitis.

Two of the 5 strains tested exhibited very mild vaccination reactions, and appeared to have been cleared from the tissues, upon autopsy at 10 months

postchallenge. These two strains (Cash and CW 1) appear promising for further immunological studies with a viable vaccine.

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Discussion from the floor:

Huppert: Were the reisolates of the Cash strain from the monkeys changed in virulence? Answer: Don't know.

Kong: Will the Cash vaccine protect? Answer: This is to be determined.

Kong: In experiments with intranasal vaccination of mice with spherules, followed by arthrospore challenge, a degree of protection was noted but fungi persisted in the animals.

Marcus: How can you extrapolate the relative virulence of a vaccine in animals to relative virulence for man? Answer: Can't do that yet as the virulence varies from animal to animal.

Kravetz: What happens with vaccine in previously infected animals? Also who would get this vaccine? Answer: There is no significant amount of data as to the fate of the vaccine in previously infected animals. We don't yet know who will get the vaccine. It may be possible that a killed vaccine will be given, followed by a viable vaccine. It will be quite some time before the vaccine is ready for human use.

Egeberg: What was the mouse virulence of a Cash strain? Answer: Ten viable units killed the mouse. It is more virulent in mice and less virulent in monkeys. The M-11 strain is reversed, that is, it gives a severe vaccination reaction in monkeys and has a low virulence for mice.

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10. CLINICAL RESPONSE OF DOGS TO PERIPHERALLY INOCULATED COCCIDIOIDES

R. E. Reed
Tucson, Arizona

Three 8-month old male beagles were inoculated, subcutaneously, with 100 viable Coccidioides immitis particles, and observed for 18 weeks. The resulting lesions formed, abscessed and ruptured in 16 days, after which inflammation and edema extended to the axillae and prescapular lymph nodes,

also abscessed. By the end of the 4th week, body temperature had reached its maximum, as had the erythrocyte sedimentation rate and total leukocyte count. Hemoglobin and packed cell volume were lowest between 4 and 5 weeks. Serum amylase increased through the first 5 weeks. Peaks occurred in alpha-2 and beta serum proteins at 4 weeks and in gamma globulin at 9 weeks. Albumin decreased during the first 5 weeks, then gradually increased. Total serum protein increased gradually through the 12th week. Complement-fixing antibody titers reached 1:32 dilution at 8 weeks. Dogs had precipitins at 12 weeks. Coccidioidin sensitivity was first positive at 3 weeks. Weekly urinalysis, serum glutamic-oxaloacetic and glutamic-pyruvic transaminases, urea nitrogen, lipase, alkaline phosphatase and blood glucose tests either remained normal or fluctuated in an uninterpretable manner.

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Discussion from the floor:

Q: Has this type of work been done in tuberculosis? Answer: There has been some similar work.

Q: Were there any changes in the feed? Answer: There was uncontrolled variation in total feed intake.

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II. COMPARATIVE PATHOGENESIS OF CANINE AND SIMIAN COCCIDIOIDOMYCOSIS

M.W. Castleberry, E.P. Lowe, J.T. Sinski,
J.L. Converse, J.E. Del Favero, and S.P. Pakes
Fort Detrick, Maryland

Both man and dog live in the endemic areas of coccidioidomycosis and similarly respond to the disease in a similar manner. In consideration of the greater susceptibility of the M. mulatta to the disease, it was decided to study the aerosol-induced disease in the canine for comparative purposes.

Twenty-five dogs were exposed to aerosols of C. immitis arthrospores and partially sacrificed over a period of nineteen days. The remaining fifteen were divided into three groups of five each to determine the pathological effects of various doses of aerosol challenge. Each group was challenged with average inhaled dose of 300, 2000, or 10,000 dry arthrospores of the same

strain and sacrificed at 22 weeks post-exposure. The development of the disease was followed by gross and microscopic pathological studies. The results of these studies were then compared with those previously established in the M. mulatta.

The dog was found to be as susceptible to infection as the monkey but was better able to contain the disease. This was manifested by the ability to maintain a blood supply within the lesion for a longer period of time and by a faster and more prolific collagen response to the disease presence. The canine lesion was generally more proliferative and less necrotic than that of the M. mulatta.

The dog's response to challenge was practically the same, regardless of dose level.

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Discussion from the floor:

Question: What was the ambient temperature in this experiment? Answer: Ranged from 72° to 76° F.

Salkin: Was there any histologic data on the first 4 days after infection of these animals? Answer: There were inapparent changes in the studies performed between the first hour and the third day post-infection. Unable to find fungi or lesions in the tissue. There were possibly occasional arthrospores.

Salkin: Is there a difference in tissue reaction to whole spherules and rupturing spherules? Answer: Intact spherules are in the macrophages. Rupturing spherules exhibit a leukotaxic effect and the polys swarm in.

Salkin: Were any hyphae or arthrospores seen in older lesions? Answer: Only in cavitated lesions in monkeys. They were looked for carefully and none were seen in the dog. Dogs may not have been held long enough for cavitation. The hyphae and arthrospores were not seen in the nodules.

Biddle: Is there any predilection for a particular area within the dog lung? Answer: No. The disease is seen throughout.

Biddle: Has had a similar experience in monkeys. Has seen hyphae very early in lung, and also one spherule. Does the early appearance of exudate in monkey bronchioles contribute to cavitation? What about dogs? Answer: Dog lesions appear more proliferative.

Have you considered the use of radio-labeled arthrospores to help find the lesions in lung early after infection? Answer: Had thought about it but have not been able to work out the details.

In experiments with 200 spore per lung infection dose in monkeys, obtaining 90% retention and one spore yielding one lesion, this group counted 80 lesions by x-ray. What were your experiences along these lines? Answer: Haven't observed anything like this.

Were there any lesions in the abdominal cavity after aerosol infection? Answer: No. Nor have they been seen in the GI tract. Dissemination goes to liver, spleen and kidney.

Are the pleural lesions primary or secondary? Answer: They are considered primary.

One of the healthy control cagemates became infected; doesn't this suggest communicability? Answer: No. The whole head is in a chamber for the infection portion of the experiment so it is possible that this monkey became infected from arthrospores on the fur of the cagemate, even though the fur was washed.

Madley has reported spores in the feces of animals. Fomites should be examined.

12. COCCIDIOIDOMYCOSIS: COMPARISON OF EXPERIMENTAL AEROSOL INFECTION IN DOGS AND MONKEYS WITH EMPHASIS ON SEROLOGIC REACTIONS

J. T. Hinkki, E. P. Lowe, M. W. Castleberry, L. F. Maire III,
J. E. Del Favero, S. P. Pakes, and J. L. Converse

Fort Detrick, Maryland

Comparative study of respiratory coccidioidomycosis was made, using dogs and monkeys. The serologic findings are presented here. Three groups of monkeys and three groups of dogs were each exposed by the respiratory route to graded doses of approximately 9500, 2400 and 325 dry arthrospores of *Coccidioides immitis* strain Silveira. Blood for serological tests was drawn

from these animals prior to exposure and at 2, 5, 9, 14, 17 and 20 weeks post-exposure. Precipitins were detected using agar gel. Sera for C.F. antibodies was pretreated with complement before testing.

Infections in monkeys ranged from lethal to severe, whereas infections produced in dogs were severe to mild. All monkeys in the high dose group died between the 11th and 61st day of pulmonary coccidioidomycosis. No dogs died from the disease.

In sera from infected dogs, the highest median precipitin titers occurred at five and nine weeks post-exposure. Titers fell after these time periods in all dose groups. Precipitin titers in the medium and low dose groups of monkeys reached their highest median values at 14 weeks post-exposure and then declined slightly. Precipitin titers in infected monkeys were consistently higher than those in infected dogs.

Highest median titers for C.F. antibodies occurred at the five week test period in dogs of all dose groups. After this time period, the median titers in the sera of these dogs declined. In infected monkeys, maximum median titers of C.F. antibodies were observed at nine and 17 weeks post-exposure. The median titers forming this plateau were higher than the highest median titers found in sera of infected dogs for C.F. antibodies.

The serologic reactions in the two animal species appeared consistent with the pathologic findings.

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Discussion from the floor:

Kong: Doesn't man, in nature, get a lower dose of infection than animals in these experiments, and doesn't the titer thus reflect the dose? Answer: Yes. The human dose is usually small.

Winn: Considering biological gradience, is there a breed correlation in dogs with respect to susceptibility to coccidioidomycosis? Answer: In these experiments they purposely selected mongrels.

Winn: It would be of interest to select breeds of different susceptibility.

Marcus: What were the relative hypersensitivity conditions in the animals reported in these systems? Is impressed by the observations that dogs generally give less inflammatory response than monkeys. Is there a role for this in the greater degree of resistance of the dogs? Answer: Don't know.

Has previously believed that the dog was more susceptible than man. The dose size is important here. A higher incidence of the disease was inferred from the fact that 50% of the Arizona coccidioidal dogs are boxers but the relative boxer population is much less than 50% of the total dog population.

In her work with monkeys it required 2 to 3 weeks after infection for a positive skin reaction to appear. In this paper 5 weeks was required. Why? Answer: There were difficulties encountered in performing the test prior to 5 weeks so he has no answer.

Q: Any anticomplementary problems in complement fixation tests with dog serum? Answer: Some, but few.

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PANEL II

MANAGEMENT OF RUPTURED COCCIDIOIDAL CAVITIES

Moderator: J. E. Steele, San Fernando, Calif.

Panelists: B. H. Cotton, Los Angeles, Calif.
R. T. Cunningham, Bakersfield, Calif.
B. H. Evans, Fresno, Calif.
G. A. Paulsen, Bakersfield, Calif.
D. Salkin, San Fernando, Calif.

Moderator: What is your experience with ruptured coccidioidal cavities?

Dr. Paulsen: The incidence is low. Has had three cases; all have done well. Two of them were treated by decortication and one was treated as a spontaneous pneumothorax by tube.

Dr. Cotton: Of 130 operations, 8 were for ruptured coccidioidal cavities. All did well after resection with decortication. (A series of slides were presented. These included several cases which received medical management only, and these resulted in the development of new lesions.) One case of imminent rupture of cavity was presented and this was considered a surgical emergency.

Dr. Evans: Cannot always tell from the film, immediately, that it is a ruptured coccidioidal cavity. When this condition exists, surgery is necessary. The youngest case he has seen has been an eighteen month old child, although most are in the 20 to 30 year age group. Believes this condition is usually the result of rupture of a residual, rather than a primary, cavity. Most of the cases he has seen have done well.

Dr. Cunningham: Has had the same experience as previous speakers, but has followed one primary cavity which ruptured while being watched and the disease spread to the contralateral lung. Removal of the lobe resulted in satisfactory course.

Dr. Salkin: Presented 14 cases, 8 of them from the Cooperative Study. In 2 of the cases, the cavity enlarged under observation and then ruptured; 7 of them were asymptomatic and then suddenly ruptured; one was asymptomatic and then developed hemoptysis and then ruptured; one nodule developed into a cavity and then ruptured; and 2 developed spontaneous pneumothorax; in one the rupture was discovered at surgery. Of the 8 Cooperative Study cases, 5 did well and 3 remained active. Of the 3

which remained active, a bronchopleural fistula with empyema in one disseminated to the spine several years after the rupture of the cavity. Serology of these 8 cases ranged from negative complement fixation test to positive at 1:128 dilution. Most had negative precipitin tests, although one patient with a 1:2 CF titer and a negative precipitin, developed a positive precipitin after rupture of the cavity, and this fluctuated for several months, and finally reverted to negative.

Dr. Hyde: Has managed 3 cases with ruptured cavity and all have done well. However, since only 3% of cavities in his experience do rupture, and when it does happen it can be easily managed, why try to anticipate this phenomenon surgically, rather than waiting till it does happen, then treating surgically?

Dr. Einstein: Questions the 3% figure for incidence of ruptured cavities. Had no figures with him, but believes the incidence is much higher than 3%.

Dr. Salkin: In the Cooperative Study, of about 250 cavities during the control period 1955-1958, only 6 ruptured.

Dr. Cunningham: Believes the incidence is higher, including those cases which erode through but do not result in pneumothorax.

Dr. Winn: Has seen 8 ruptures in 250 residual cavities.

Dr. Salkin: Predominance of ruptured cavities in this series were in the right upper lobe. Is this significant?

Dr. Evans: Most of his were in the lower lobe.

Dr. Salkin: Most in his series have subsequent segmental and subsegmental surgery. Are there any comments on this?

Dr. Cotton: Doesn't think it matters. He palpates for satellite lesions to determine the extent of the disease. He doesn't worry too much about this. Can do a decortication, sometimes going right through an empyema, and still get a good re-expansion of the lung.

Mrs. Paulsen and Salkin: Have seen cavities rupture and then be successfully treated by intubation drainage. Should you drain a coccys empyema before surgery?

Dr. Hyde: What about a patient with empyema and a cavity known to exist in the lobe? Should there be a preliminary drainage here?

Dr. Evans: No. Operate as soon as diagnosed. You have to remove the cavity and also decorticate.

Other surgeons on the Panel: Concurred on the need for immediate surgery without preliminary drainage.

Dr. Winn: Has seen two ruptured cavities with simple effusion, rather than empyema. Has anybody else seen this?

No response.

Moderator: What is the role of amphotericin B in ruptured cavities?

Dr. Paulsen: The drug is too toxic, and therefore haven't used it in this type of case, and have had no trouble.

Dr. Steele: Would anyone use amphotericin B?

Dr. Salkin: Recommends local use of the drug. Doesn't believe you can get 100% recovery with surgery alone. In one difficult case, amphotericin B packs helped clear the empyema.

Dr. Steele: Won't it run out of the drainage tube?

Dr. Salkin: Some of it remains.

Dr. Cotton: What is the toxicity of local application of amphotericin B?

Dr. Salkin: Since there is little absorption of the drug there is no toxicity observed. This is the safest use of amphotericin B.

Dr. Paulsen: How long contact with the drug is needed?

Dr. Huppert: This drug does not kill, it merely suppresses the growth.

Dr. Larwood: In attempting to use intrapleural amphotericin B can you "slosh" the drug and then drain it?

Dr. Cohen: Can you use high tube irrigation with a low drainage tube?

Dr. Steele: The object is rapid re-expansion with the lung's adherence to the chest cage.

Consensus of surgeons: The technical difficulty of desired drainage of the cavity vs. need to retain the drug in the cavity precludes the routine use of amphotericin B locally in the pleural cavity.

Dr. Cohen: Has anyone instilled pulmonary cavities with amphotericin B by intrabronchial catheter?

No response.

Dr. Einstein: Distinguishes between an old and new cavity. A patient who ruptures during primary disease should have amphotericin B, but a patient with older disease with cavity rupture has less need of the drug.

Dr. Winn: Wondered how much ballooning in some of these cavities is due to secondary bacterial infection. In surgical coverage he rarely exceeds 3/4 gm. of amphotericin B and sees no significant toxicity, so intravenous drug in these cases may be worthwhile.

Dr. Evans: Has used pneumoperitoneum post-operatively to help the remaining lobe refill the hemithorax. May be selected instances where amphotericin B is called for, but has no evidence for the routine use of the drug. Good surgical management is the best bet.

Question from the floor: Cites a case of drainage without resection where the cavity disappeared and the pleural space cleared up. The sputum cultures were positive, and later the cavity reappeared. Should they reoperate and should amphotericin B be used?

Dr. Paulsen: Would treat as a standard residual cavity for resection without concern for the prior rupture. Presumes it was a primary type which is now chronic.

Dr. Salkin: Would recommend both intravenous and local amphotericin B in such a case.

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Third Scientific Session
Friday Afternoon, Nov. 30, 1962

D. Ecology and Epidemiology Papers

A. Ringle, Chairman

14. SOME CONDITIONS AFFECTING SURVIVAL OF C. IMMITIS IN SOIL

Roger Egeberg
Los Angeles, California

C. immitis has been shown to live under extremely varied environmental circumstances of nutrition, temperature, and pH. We demonstrated it could survive well in mud and in desiccated soil. It was therefore postulated that the reasons for the very spotty distribution of C. immitis in an endemic area might be due to the relative prevalence of its antagonists and competitors.

Chemical analyses were made of the soil in the area in which we intermittently find C. immitis. Soils taken in this same area at times of year when we did not find C. immitis were pooled and their chemical constituents evaluated, and compared with pooled specimens taken at a time of year when we did find C. immitis. There was a great difference in salinity. The sodium and chloride content of the soil containing C. immitis was ten or more times as high as the average of the soils not containing C. immitis.

Approximately thirty different organisms were isolated from the soil and at least eight of these showed inhibitory effects on the growth of C. immitis on Sabouraud's medium. These organisms are being investigated as to their tolerance of increasing salinity in the soil compared with C. immitis.

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Discussion from the floor:

Dr. Eckmann: The salinity has been increasing in Coachella and Imperial Valleys irrigated with Colorado River water. They are starting to over-irrigate with a special drainage situation in an attempt to leach the salt out of the soil. This problem also exists in Mexico. This suggests that there may be a potential source of increase of coccidioidomycosis in these areas.

Dr. Reed: What was the texture of the coccy bearing soil? Answer: In over 100 isolations of the organisms it was always found in fine powdery soil, never in coarse soil.

Dr. Reed: In Arizona there is an area of black, high alkali soil with poor drainage, yet these areas are not high coccy regions. Answer: The sodium concentration in those soils is probably much higher than that in the San Joaquin Valley soils tested and reported in this paper.

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15. OBSERVATIONS ON A LABORATORY EPIDEMIC OF HISTOPLASMOSIS

John F. Murray and Dexter Howard
Los Angeles, Calif.

In 1961 a medical student became ill with pericarditis and pulmonary infiltrations; later, these were shown to be due to histoplasmosis by the finding of positive precipitin and complement fixation (1:256) reactions to histoplasmin. Subsequently, several members of his class were found to have positive histoplasmin skin tests. These observations led to a survey of the chest x-rays, skin test sensitivities and serological titers of the entire class. The results on 62 of 66 members of the Class of 1963, who were exposed to living Histoplasma capsulatum in a laboratory exercise, show that 26 (42%) had positive histoplasmin complement fixation studies of 1:8 or greater and 9 (35%) of the individuals had positive chest x-rays. X-ray abnormalities consisted of solitary nodular lesions and enlarged hilar lymph nodes which progressively calcified.

The results of the skin tests were compared with similar studies on the next two classes that used heat killed cultures of Histoplasma capsulatum. The incidence of positive reactions to three dermal antigens is as follows:

	<u>Tuberculin</u>	<u>Coccidioidin</u>	<u>Histoplasmin</u>
Class '63	7.4%	7.4%	41.9%*
Class '64	10.1%	10.1%	10.1%
Class '65	8.8%	14.8%	11.1%

*Difference statistically significant (p 0.01)

It seems reasonable to conclude that the significant increase in histoplasmin skin test sensitivity in the class of '63 was the result of accidental laboratory infection. Although all of the illnesses but one were mild, severe disease

was encountered in one student in whom the possibility of late complications still exists. This experience demonstrates that all personnel should handle living Histoplasma capsulatum with care and respect. It is recommended that inactivated cultures be used for routine teaching purposes.

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Discussion from the floor:

Dr. Egeberg: Were complement fixation or precipitin tests performed on students who had negative skin tests after this outbreak? Answer: Yes, and all were negative.

Question: Did you consider amphotericin B therapy in the pericarditis and lymphadenopathy case. Answer: Yes. Considered it, but withheld it and decided to watch the case.

Dr. Biddle: Were differences noted between complement fixation titers with the histo yeast phase and the histo mycelial phase? Answer: The students varied with respect to their response to one or the other of these two antigens, but each student was fairly consistent on repeated testing with most of these antigens, in respect to the ratio of reaction to the two.

Dr. Marcus: Were Petri dish cultures of H. capsulatum used in this class? Answer: In general, people are much more cautious in handling of Coccidioides cultures than they are with Histoplasma cultures.

Dr. Huppert: What were the results of skin tests on the prior class? Answer: Not done.

Mr. Sorensen: Since some infected people do not give a positive complement fixation titer it is possible that some of the cases were missed. Answer: Yes, but the positive complement fixation figure for this group was high, that is, over 70% among those who gave a positive skin test.

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16. LONGITUDINAL STUDIES OF A LARGE URBAN SOIL
RESERVOIR OF H. CAPSULATUM

Coy D. Smith, Fred E. Tosh, Harold J. Lynch, Jr.,
Jack C. Evans, C. F. Orr, Michael L. Furcolow

In 1959 an epidemic of histoplasmosis occurred among Boy Scouts in a small city in Missouri. The source of the epidemic was determined to be an

eleven acre plot of ground near the center of the city. This plot was being converted into a city park. Repeated soil sampling for approximately three years during different seasons of the year revealed no change in the percentage of positive isolations of Histoplasma capsulatum from this plot of ground. Sixty-four per cent of 237 samples collected from this source over the three year period were positive for Histoplasma capsulatum. Clearing the area of trees and undergrowth to expose it to sunlight failed to effect the percentage of isolations.

Depth studies as determined by core sampling showed that Histoplasma capsulatum could be isolated at least a foot below the surface.

The application of two different fungicidal agents and two soil fumigants failed to have any effect upon the elimination of Histoplasma capsulatum from the area.

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Read only by title. No discussion.

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17. A LOCALIZED EPIDEMIC OF COCCIDIOIDAL INFECTION

W. A. Winn, H. B. Levine, J. E. Broderick,
and R. W. Crane
Springville, California

Within the endemic area for coccidioid disease, contamination of the soil by the fungus Coccidioides immitis is not uniform, but occurs, rather, in isolated locales. A similar situation exists in the epidemiology of Histoplasma capsulatum.

The paper reports the occurrence of primary coccidioidomycosis among a group of ten young children who played together in a single small backyard near Woodville, California. The clinical disease varied from severe in four of the children, to a subclinical infection in one. Nine of the ten children had changes in the pulmonary roentgenograms and residual pulmonary findings were present at the end of a year in six. All developed skin sensitivity to coccidioidin and nine of the ten also developed serological changes, with titers of complement fixation varying from a high of 3+ in 1:256 serum dilution down to 4+ in 1:4 dilution. All of the children recovered and amphotericin B therapy was not used.

Thirty-five soil samples were obtained from the yard and from four of these, *Coccidioides immitis* was isolated by H. B. Levine of the Naval Biological Laboratory, Oakland, California. A relatively new technic was used for identification of *C. immitis* in the soil by the inoculation of mice via the intranasal route. The strain of *C. immitis* that was isolated from this particular backyard was highly virulent for mice and a rapid grower on culture media. The backyard soil environment was favorable in that there was no lawn and no method of dust control. It is presumably impossible to eradicate the fungus from such soil environments. Control of infection might result from the use of a protective vaccine, when one becomes available. Planting of lawns and oiling of dusty surfaces would also result in partial control of human infection.

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Discussion from the floor:

Dr. Egeberg: Was surprised to hear of outbreak of coccidioidomycosis at the east side of the valley. Would like to sample some of these neighborhood yards.

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18. COCCIDIOIDIN AND HISTOPLASMIN SENSITIVITY IN SOME SELECTED GROUPS IN JAPAN

Yasue Sawaki
Bakersfield, California

Japan has not yet reported definite histoplasmosis and coccidioidomycosis. However, in view of now wide-spread world travels and transportation of freight, it has become of considerable interest in Japan to know whether this disease may have entered Japan.

Skin tests with coccidioidin and histoplasmin were carried out in 780 persons. The methods and results were as follows:

Subjects Tested	Places	Groups	Skin Test		Positive Reactors	
			TB & Cocc.	Histo Cocc.	TBC(%)	Cocc.(%) Histo.(%)
390 TB patients	Chest Hosp.	Never abroad	186		181(97.4)	
	Chest Hosp.	Had gone abroad		160		2(1.25) 2(1.25)
	TB Sana-torium	Not divided		44	0	0
390 healthy adults	Chest Hosp.	Empl. at hosp.		210	0	1(0.5)
	In Tokyo	Repatriated persons		60	0	0
Total				594	3	9

1. Incidence of induration and erythema in histoplasmin and coccidioidin skin tests were essentially identical, although there is a slightly higher percentage with histoplasmin.
2. The 12 reactors were all male and had been abroad. Their occupation and past history is related to their reason for being overseas. Chest x-ray and sputum culture study in the reactors did not demonstrate any pathological signs, except in the TB patients. Cross reaction between histoplasmin and coccidioidin were seen in 4 of 12 of the cases.
3. These studies indicated that more aggressive further studies are needed toward groups of individuals who have lived outside of Japan and returned, as well as those who are in contact with foreign transports and personnel.

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Discussion from the floor:

Dr. Wyborney: A skin test survey on individuals giving positive x-ray findings in the mobile testing units in San Diego yielded 18% positive tuberculin reactors, 12% positive coccidioidin reactors and 26% positive histoplasmin reactors. Very few histo cases are seen in San Diego, and these have been contracted in the east.

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PANEL III

CLINICAL RESULTS WITH VARIOUS MODES OF AMPHOTERICIN B TREATMENT

Moderator: Donald C. Kent, San Diego, Calif.

Panelists: Stephen H. Cheu, Fresno, Calif.
Hans E. Einstein, Bakersfield, Calif.

Moderator: There has been considerable progress in chemotherapy of coccidioidomycosis. Fiese, in the 1958 edition of his book, could record no proven effective chemotherapeutic agent for this disease. What are some of the criteria to be used in evaluating clinical results with amphotericin B?

Dr. Cheu: These include changes in clinical status by objective and subjective signs, such as, weight, temperature, etc; lab findings, including drop in leukocyte counts, sedimentation rate and complement fixation titers; x-ray improvement; biopsies, such as those showing healing of cutaneous lesions. The various complications and side effects of the drug, such as renal toxicity, must be balanced against the clinical signs of improvement of the disease itself.

Moderator: What is your experience with parenteral administration of amphotericin B?

Dr. Einstein: Uses intravenous administration most frequently and adds only heparin to the solution. Has tried mixing other ingredients with poor results. Routinely now uses oral steroids to reduce toxicity and this appears to be effective. Believes there is no apparent advantage to using the drug every 48 hours instead of daily. Believes the toxicity is a reflection of the total dose, not the rate of administration. Anticipated results with intravenous administration are:

1. Progressive primary disease: early chemotherapy with small dosage leads to spectacular results.
2. Chronic osteomyelitis: intravenous drug, plus local administration yields excellent results.
3. Draining skin sinuses: Do well under chemotherapy.
4. Meningitis: May be overtreating this form at present. Chronic meningitis is benign in terms of extension of the disease, but bad in terms of the locus. Complement fixation titers in meningitis

cases are generally lower than with other disseminated forms of the disease. The damage appears to be anatomical rather than fungal. Currently he is starting an initial intravenous course, but the patient is then maintained on intrathecal or, even better, intracisternal administration of the drug only, and this modality is holding up well. When chemotherapy is stopped the patient will usually relapse. Therefore, chemotherapy of meningitis patients should not be discontinued unless one is forced to. He may stop the intravenous use due to the renal toxicity, but not the intracisternal use. The maximum interim between intracisternal injections is 2 weeks and if one delays longer than that one is likely to get in trouble.

Dr. Cheu: In meningitis, the earliest chemotherapy is best. Once blockage starts to occur, patient is in serious trouble. With lower dose chemotherapy one needs to stop less often due to toxicity, so actually, over a period of time, the same total dosage may be achieved.

Dr. Winn: Believes one can "back down" in dosage. Uses suppressive intracisternal therapy once a week in meningitis. Even 2 years of such treatment does not result in a cure. Does not yet know how long it must be continued to result in a cure.

Moderator: In non-meningeal disseminated coccidioidomycosis, when can you stop amphotericin B treatment?

Dr. Einstein: Stop when there is clinical and serological evidence of healing. The old lesions dry up and no new ones appear and the titer falls. So far has not had to return more than a couple of patients to therapy, if he had considered them definitely cleared up with chemotherapy at the termination of the drug.

Dr. Cheu: In acute disseminated disease, start your chemotherapy and continue for 4 months, then follow the patient, and only resume chemotherapy if specific reasons are presented. In meningitis, give 3 to 6 months of intravenous therapy, then intrathecal therapy for 3 months, and then stop and follow the patient for further decision as to whether to re-treat or to maintain a permanent regimen of therapy.

Dr. Groel: An adequate intravenous course should be 3 to 6 months with respect to toxicity, etc. Has no special ideas about intrathecal administration.

Dr. Stonehill: It is hard to decide when to stop chemotherapy in non-meningeal dissemination. Short intermittent courses of chemotherapy sometimes lead to some recurrences of disease. How likely are these recurrences to develop into meningitis?

Dr. Einstein: Would rather take a chance with an intensive short course with less than 2 gms. of amphotericin B, or even better, less than 1 gram, recognizing that there is some risk of meningitis. He is betting to be able to recognize the group with the particular high hazard of meningitis. These include pregnant women, certain races, and certain types of fulminating disease in white women with very rapid meningitic death. When this latter type of disease starts to appear, treat fast and long.

Dr. Matthews: How can we measure whether it is the amount or the duration of the chemotherapy that is effective?

Dr. Einstein: We don't know this yet. In CNS syphilis it appears to be total dosage of treatment. In coccy, intermittent, that is, every 48 hours treatment, until a given total dose is achieved, is difficult, due to the prolonging of the hospitalization of a patient. A short term, high dose regimen seems equally effective.

Dr. Matthews: Is there any evidence on excretion rates of the drug? Is this repository chemotherapy or is anything known of peak vs. sustained levels of the drug?

Dr. Groel: Generally there is a plateau of 12 to 24 hours after infusion of the drug.

Dr. Winn: In severe threatening or active dissemination you have to watch out for meningitis, even though the patient appears to be doing well. Recommends routine lumbar punctures to detect early meningitis as this is much easier to treat than older meningitis.

Dr. Cheu: Routinely does lumbar puncture on acute patients.

Dr. Einstein: Agrees. Early coccidioidal meningitis often does not give classical clinical signs and symptoms that other meningitides do and the lumbar puncture is necessary for early detection of this disease. Usually recommends a half to one mgm. intrathecal or intracisternal treatment with amphotericin B.

Dr. Kent: Intracisternal administration is much easier to do than intrathecal. It sounds frightening but it actually is easier. Shares Dr. Winn's experience of archnoiditis with intrathecal administration of the drug.

Dr. Einstein: Some practitioners are still using lumbar puncture for administration of amphotericin B, but he never does.

Moderator: What is your experience with the use of amphotericin B for surgical coverage?

Dr. Cheu: Does not routinely cover surgical cases with this drug, but in selected cases this may be proper.

Dr. Einstein: Generally considers this unnecessary and may be harmful.

Moderator: What is your experience with oral administration of amphotericin B? In older studies with the insoluble oral preparation no chemotherapeutic effect was noted. Campbell has used the solublized intravenous preparation orally in mice and noted protection. Later Dr. Utz of NIH published results of such chemotherapy in humans, with 5 to 7 gms. daily administration of this form of the drug given orally, and observed detectable serum levels comparable to that with 1 to 1 1/2 mgs. per kilo intravenous administration. All of these patients got sick with diarrhea, nausea and anorexia. The longest period of chemotherapy by this route was 10 days and no effect was noted on the infection itself. Nobody else apparently has had enough of this drug to test in this manner.

Dr. Kravetz: Worked with Dr. Utz on this project and repeated this with bile salts alone, which gave the GI upset. They were not able to repeat the experiment because the patients preferred the intravenous use of the drug!

Moderator: Local treatment with amphotericin B often gives good results. Has used on a sinus from draining empyema with good results. Local use avoids renal damage. What is your experience with local usage of amphotericin B?

Dr. Cheu: Packs or infiltration or dusting of powder in local abscesses has resulted in healing of these abscesses in a number of cases.

Dr. Egeberg: When he was working with prodigiosin, washing of sinuses with the drug sometimes yielded clearing. Probably in many cases the washing with gentian violet or merthiolate, etc., would also have been good.

Dr. Kent: Has not done this in closed chest in empyema. In a case of a patient with a fistula, minimal results were achieved with intravenous therapy. Then he used several kinds of flushes in a tube with no effect. Finally he added amphotericin B by tube with drainage to the fistula and closure resulted in three weeks.

Dr. Einstein: Why hesitate in putting the drug into the closed chest? This is a good method.

Dr. Birsner: Would it be worthwhile to study the disposition of amphotericin B by using tagged drug?

Drs. Cheu, Kent and Einstein: Yes. This would help elucidate the transport of the drug and possibly the mode of its action.

Dr. Birsner: Can this body recommend that such a study should be undertaken?

Dr. Matthews: If an investigator has the facilities and interest, he should go ahead with such a study.

Moderator: Isolated limb perfusion has been used as a form of treatment in carcinoma. It is performed by cannulation of an artery with partial occlusion in the distal portion of the artery, yielding high arterial pressures to cause maximum perfusion of the drug into the tissues. High oxygenation improves this so perfusion is done with blood rather than saline. What is your experience with isolated limb perfusion?

Dr. Murray: Two cases have been so treated at UCLA. Both yielded transient improvement, and at least one of the cases relapsed.

Dr. Salkin: One patient at San Fernando was so treated for an ankle lesion with no success.

Dr. Kent: Could this be applied in some way to the lung? In dogs, no pathological changes are seen in lung due to such procedures. Has used this in carcinoma in man in a *couple of cases, isolating the lobe.*

Moderator: What are your experiences with amphotericin B plus steroid therapy?

Dr. Einstein: Has had considerable experience with cortisone. In pre-amphotericin B days, has used cortisone for amelioration of severe primary manifestations with no bad effects. This should be the place where cortisone would harm if it was going to harm at all. It is possible that it causes late dissemination, but it also can make chemotherapy more effective. He uses steroids freely with no causes for regret.

Dr. Cheu: Doesn't use steroids at all. Wonders if steroids would contribute to the hypokalemia.

Dr. Einstein: Uses cortisone, plus amphotericin B, plus potassium treatment.

Dr. Winn: Uses cortisone, with intravenous amphotericin B to cut the side effects. Gives the cortisone intramuscularly a couple of hours prior to the amphotericin B with very good results. Believes it may reduce renal damage. Does not use intrathecally.

Dr. Einstein: Uses the steroid orally.

Dr. Wyborney: Has used steroids very little so far. In one experience he mixed amphotericin B and the steroid in the infusion bottle and the patient went into shock. Later he gave steroid orally, two hours before the amphotericin B treatment with no bad reactions.

Dr. Stonehill: As in acute staphylococcal pneumonia, this may reduce inflammation and thus save lung. If it cuts the nephrotoxicity that would be good, but this is not yet confirmed. In very acute primary disease, steroid therapy is called for.

Moderator: Does anybody have any experience on aerosol treatment?

No comment.

(Ed.: Aerosol amphotericin has been used at San Fernando VAH on 4 patients with doubtful results. The blood showed no ampho. values.)

Dr. Einstein: For several years now, only amphotericin B has been discussed. A secondary drug is a major need to be used either in tandem or alternating with amphotericin B. Had started studies with nitrofurans when amphotericin B came along and the nitrofurans were dropped. Should these be resumed? What about isoniazid?

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REGISTERED ATTENDANCE

L. Larry Allen, M.D., VA Hospital, San Fernando, Calif.
Joseph E. Anderson, M.D., Bakersfield, Calif.
L. A. Baker, M.D., VA Hospital, Tucson, Arizona
Lois Beishir, UCLA Medical Center, Los Angeles, Calif.
Margaret Biddle, Ph.D., County General Hospital, Los Angeles, Calif.
John W. Birsner, M.D., Bakersfield, Calif.
H. W. Bosworth, M.D., Los Angeles, Calif.
Joseph F. Boyle, M.D., Los Angeles, Calif.
Edwin A. Brosbe, Ph.D., VA Hospital, Long Beach, Calif.
John T. Burroughs, M.D., Los Angeles, Calif.
Marilyn Capener, State Health Department, Berkeley, Calif.
Dr. M. W. Castleberry, Fort Detrick, Maryland
Stephen H. Cheu, M.D., VA Hospital, Fresno, Calif.
R. Clark, M.D., County General Hospital, Arlington, Calif.
Dr. James M. Cobb, Naval Supply Center, Oakland, Calif.
A. A. Cohen, M.D., VA Center, Los Angeles, Calif.
John L. Converse, Fort Detrick, Maryland
Bert H. Cotton, M.D., Los Angeles, Calif.
Ray Cowley, M.D., Fitzsimons Army General Hospital, Denver, Colo.
Dr. T. A. Cromartic, Porterville, Calif.
Duane Crummett, Ph.D., Los Angeles, Calif.
R. T. Cunningham, M.D., Bakersfield, Calif.
A. Davis, M.D., VA Center, Los Angeles, Calif.
H. A. Davis, M.D., U.S.S. Haven, San Diego, Calif.
Dr. R. H. Diven, Tucson, Arizona
Wilfrid J. Dixon, Ph.D., Los Angeles, Calif.
Nine Djar, Los Angeles, Calif.
A. A. Dubrow, M.D., VA Hospital, San Fernando, Calif.
Bertram H. Eckmann, M.D., Department of Public Health, Riverside, Calif.
Dr. S. Ede, U.S.S. Haven, San Diego, Calif.
Roger O. Egeberg, M.D., Los Angeles, Calif.
Hans E. Einstein, M.D., Bakersfield, Calif.
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Byron H. Evans, M.D., Fresno, Calif.
Albert Fink, M.D., VA Center, Los Angeles, Calif.
Dr. Seymour Froman, Olive View Hospital, Olive View, Calif.
Auko Fujiwara, UCLA Medical School, Los Angeles, Calif.
I. T. Gadzikowski, M.D., VA Hospital, San Fernando, Calif.
Martha Garstka, M.D., Bakersfield, Calif.
William Grindon, M.D., VA Center, Los Angeles, Calif.
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William L. Gruber, M.D., VA Hospital, San Fernando, Calif.

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 C. Ross Hampson, Ph.D., Bakersfield, Calif.
 J. L. Hansen, M.D., County General Hospital, Riverside, Calif.
 John W. Heizer, M.D., VA Hospital, San Fernando, Calif.
 Elmer F. Herring, M.D., San Fernando, Calif.
 F. E. Hesse, M.D., Berkeley, Calif.
 William L. Hewitt, M.D., UCLA Medical Center, Los Angeles, Calif.
 Gilbert Hill, M.D., Salt Lake City, Utah
 Charles W. Holeman, M.D., Bakersfield, Calif.
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 Francis T. Johnson, M.D., Monrovia, Calif.
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 Mrs. Jewel Kietzmen, VA Hospital, Long Beach, Calif.
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 Thomas R. Larwood, M.D., Bakersfield, Calif.
 Howard E. Liston, M.D., VA Hospital, Phoenix, Ariz.
 Matthew Locks, M.D., VA Hospital, Long Beach, Calif.
 Dr. E. P. Lowe, Fort Detrick, Md.
 Robert Lundigan, Eaton Laboratories, Los Angeles, Calif.
 Paul Maier, M.D., VAOP Service, Los Angeles, Calif.
 Stanley Marcus, M.D., Salt Lake City, Utah
 James H. Matthews, M.D., VACO, Washington, D.C.
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