

1953
TRANSACTIONS OF THE THIRD ANNUAL MEETING
of the
VETERANS ADMINISTRATION - ARMED FORCES
COCCIDIOIDOMYCOSIS COOPERATIVE STUDY
Los Angeles, California

The third annual meeting of the VA-Armed Forces Coccidioidomycosis Cooperative Study was held on December 4 and 5, 1953, at the VA Regional Office in Los Angeles. This year the meeting was divided into two phases. On December 4, attendance was limited to representatives of Study Units and to special Consultants and other special representatives. At this closed session, the problems of mechanics and administration of the Cooperative Study were discussed. The December 5 meeting was an open one, devoted to a scientific program on Coccidioides immitis, and on coccidioidomycosis.

RESUME OF DECEMBER 4 ADMINISTRATIVE MEETING:

1. This year, for the first time, all study units were represented. The attendance was as follows:

VAH, Minneapolis, Minn.	Dr. Wendell H. Hall
VAH, Phoenix, Ariz.	Dr. Matthew J. Noon
VAH, Albuquerque, N. Mex.	Dr. William Hentel
VAH, Houston, Texas	Dr. J. Newton
VAH, Fresno, California	Dr. Stephen Cheu & Dr. R.H. Sorensen
VAH, Kerrville, Texas	Dr. John A. Carswell
VAH, Long Beach, Calif.	Dr. Leroy Hyde & Dr. Edwin A. Brosbe
VAH, San Fernando, Calif.	Dr. David Salkin, Dr. John D. Steele, Dr. M. Huppert & Dr. L.G. Wayne
VAH, Tucson, Arizona	Dr. Solomon Netzer
VAC, Whipple, Arizona	Dr. Robert M. James
US AFBH, Lackland, Texas	Col. Robert B. Stonehill
US AFBH, Davis-Monthan	Dr. Peter R. Meis
USNH, San Diego, Calif.	Capt. Ralph G. Streeter
WBAH, El Paso, Texas	Col. R. C. Hunter
Fitzsimons AGH, Denver, Colo.	Major Joel H. Richert
VACO, Washington, D.C.	J. Williams, R & S
VACO, Washington, D.C.	Dr. Edward Dunner, Research Svce
VACO, Washington, D.C.	Dr. J.W. Raleigh, Chemo. Committee
Walter Reed Army Inst. Research	Charlotte C. Campbell
Area Office, San Francisco, Calif.	Dr. Arthur L. Ringle
L.A. Dept. Charities	Dr. Roger O. Egeberg
U.C. School Public Health, Berkeley, Calif.	Dr. Charles E. Smith

2. Retrospective Study and Case Registry Report Forms:

As of the second meeting, 414 cases of coccidioidomycosis (including 61 disseminated) had been reported in this study. The total of forms submitted has now reached 439, with essentially the same observations as in the smaller group.

At the 1953 Memphis meetings of the TB Chemotherapy Study, it was decided that the 1955-56 coccidioidomycosis retrospective study should be extended into a continuous study until further notice. This long-range program should provide some of the following information on natural history of the disease:

- a. Fate of chronic, particularly cavitary, disease. Are cavities benign, or is the danger of serious hemorrhage or other complication greater than surgical hazards of removing these lesions?
- b. Evaluation of occurrence of late dissemination. The VA-Armed Forces Study is exceptionally well suited to long term follow-up of this problem. Dr. Raleigh suggested that if we are most interested in long term follow-up, possibly our present registry report form is too detailed. A simpler initial form might be best with special forms to be filled out for cavitary disease, progressive primary disease, disseminated disease, and surgical studies. No decision was made to change the report form again, so the new form will be used until further notice.

Several changes have been made in the report form, as recommended at the second annual meeting. The new report form is available now for distribution to study units. In order to pinpoint racial differences in susceptibility, the item on racial extraction was clarified. Blocked and open cavities were defined more explicitly. It is important that it be noted on the report form whether a cavity was ever present. Since relatively few cases come to surgery, it will generally be necessary to rely on x-ray findings to determine occurrence of cavities.

In surgical cases, the report form should have a place for surgical pathology so that in these cases it will be possible to correlate actual presence of nodules, blocked cavities, and open cavities with x-ray interpretations. The new report form only has a place for additional non-resected pathology, so it will be necessary to add a special note to the report on pathology of resected tissue. Also on this form, there is no place to indicate whether the diagnosis of coccidioidomycosis was suspected, confirmed, or not suspected prior to surgery. In order to evaluate surgical complications, it will be important to know in what proportion of cases the surgeon knew he was dealing with coccidioidomycosis, so a note will also have to be appended to cover this information. A special letter will be sent out to add these items.

Since the Armed Forces do not use the VA "C" number to identify patients, it is requested that serial numbers be used for all Armed Forces patient report forms.

All report forms should be prepared in duplicate; furthermore, they should be filled out in pencil. It may become necessary to prepare more copies at a later date, and the Thermofax copying machine does not copy some types of ink; this is why pencil is preferable.

The question arose as to when the forms should be filled out in the continuing registry. It was decided that the form should be started for a given patient as soon as the diagnosis of coccidioidomycosis is made. It would be best to keep the form in the patient's folder and to submit it as soon as it is completed. Occasional reminders will be sent out to send in forms, and a supply of blank forms will be maintained by each study unit. Study unit hospitals will fill out their own forms. Material for non-study unit hospitals in the VA and Armed Forces will continue to be processed by Dr. Leroy Hyde at VAH, Long Beach, California.

3. Reference Laboratory and Culture Bank:

THE COCCIDIOIDES IMMITIS CULTURE BANK now has over 100 strains of this organism. All cultures are verified by production of disease, with characteristic spherule formation, in dba mice. Cultures are available of a wide range of virulence for mice, and anyone desiring to study C. immitis may send for representative, virulence-assayed strains. It was pointed out that there is no known correlation between virulence of C. immitis for mice and virulence for man. There is much variation in morphology, cultural characteristics, and mouse virulence from strain to strain. Miss Campbell raised the question of whether repeated passage of low virulence strains through mice would enhance their virulence. In the experience of Dr. Smith and of Dr. Huppert, repeated direct passage did not notably enhance virulence.

Cultures may be sent from study units to the Reference Laboratory for confirmation of identity, virulence assay, and/or amphotericin B sensitivity determinations. Cultures should be accompanied by the name of the study unit, name and C-number or serial number of the patient, and date of the specimen. Miss Campbell suggested that these services would be valuable to VA hospitals in the non-endemic areas, since laboratories in these areas have little experience with coccidioidomycosis. Unfortunately, an invitation to all such laboratories to send fungus cultures directly to the Coccidioidomycosis Reference Laboratory would probably result in that laboratory's being swamped with cultures "suspected" of being C. immitis. Dr. Hall suggested that this service be made available to Area Reference Laboratories, which could screen cultures from individual stations and refer doubtful cultures to the Coccidioidomycosis Culture Bank. Dr. Dunner suggested that a letter to this effect be prepared and that Dr. Raleigh could then coordinate with Dr. Callender's office for dissemination of this information.

Postal regulations concerning mailing of live C. immitis cultures have been determined and will appear in the new edition of the Laboratory Manual.

Dr. Huppert expressed his intention to add cultures of all systemic fungi to the culture bank collection. In addition to the C. immitis cultures, he now has about 200 Nocardia cultures. He would like to receive cultures of Histoplasma capsulatum, Blastomyces dermatitidis, and Cryptococcus neoformans. All such cultures submitted should be accompanied by information as to the source of the cultures.

THE COCCIDIOIDOMYCOSIS COOPERATIVE STUDY LABORATORY MANUAL is to be revised and issued in loose leaf form, to simplify later revision. The manual covers (1) Collection of specimens; (2) Direct examination and special stains; (3) Culture techniques; (4) Requirements for identification of C. immitis; (5) Serological tests and skin tests; (6) Histopathology, and (7) Safety measures for handling C. immitis.

The new Manual will recommend that all isolation cultures be made in duplicate, employing one bottle of Sabouraud agar and another of antibiotic agar (BBL's Mycosel Agar has proved very satisfactory). The Millipore Filter (MF) technique for examination of spinal fluid was re-emphasized. Filtrates can be used for sugar and chloride determinations and for the complement fixation test. The use of prescription bottles rather than tubes or Petri dishes for C. immitis cultures was restressed for safety reasons.

SEROLOGY and SKIN TESTING antigens are available from the Reference Laboratory. All VA study units are to perform skin tests with the coccidioidin from the single, large batch (No. 64D4) supplied to us by Dr. C. E. Smith, and being distributed by Dr. Huppert. The standard coccidioidin distributed by the Walter Reed Institute is from this same batch, so that Armed Forces are using the same reference skin test antigen.

The Reference Laboratory is presently producing satisfactory antigen for complement fixation and precipitin tests. There is now enough antigen available for 10,000 complement fixation tests, and this antigen is available to Study Units. The antigen represents a pool from 24 strains of C. immitis, representative of a variety of sources in a broad geographical distribution.

The Reference Laboratory is able to perform complement fixation and precipitin tests on serum from all patients on the amphotericin B protocol, for hospitals desiring to have this service. Some tests can also be run for non-protocol cases in study unit hospitals. For hospitals whose laboratories will be performing their own serology studies, the Reference Laboratory should receive occasional split specimens to check comparability of study unit laboratory and Reference Laboratory results. Limited amounts of known positive complement fixing control sera are available to enable study unit laboratories to standardize their techniques.

These sera will be distributed, on request, at a 1:16 dilution and should be run as received and at 1:32 and 1:64 final dilutions. No control serum for the precipitin test is available for distribution.

Dr. Huppert plans to start work soon on purification of C. immitis antigens. The clinical value of use of purified antigens remains to be determined.

THE ARMED FORCES INSTITUTE OF PATHOLOGY has agreed to set up a library of coccidioidomycosis gross specimens for teaching purposes. This Study has not been relying on the AFIP for diagnostic services.

A SUMMARY OF SERVICES available from the Reference Laboratory and Culture bank at VAH, San Fernando, follows:

1. Serology (Complement fixation and precipitin) on all protocol cases.
2. Serology on non-protocol cases when desired, until such time as the work load becomes too large.
3. Distribution of the special lot of skin test antigen.
4. Distribution of complement fixing antigen and precipitin test antigen to study units desiring to perform their own serologic studies.
5. Serologic tests on split specimens as a spot check on Study Units performing their own serologies.
6. Confirmation of identity of C. immitis cultures.
7. Amphotericin B sensitivity studies on C. immitis isolated from patients on amphotericin B or definitely scheduled for this drug in the near future.
8. Distribution of cultures of C. immitis to laboratories desiring to study this organism.
9. Distribution of limited amounts of positive control serum for complement fixation tests.

Specimens and requests for information or materials should be sent to:

Dr. Milton Huppert
Chairman, Coccidioidomycosis
Laboratory Committee
Veterans Administration Hospital
San Fernando, California

4. Disseminated Coccidioidomycosis - Amphotericin B Protocol Study:

We have completed the first year of amphotericin B protocol. An attempt was made to gather information on results by means of a narrative report from participating hospitals, rather than a formal report form. This was not eminently successful. A formal report form will be developed in the near future.

The definition of disseminated coccidioidomycosis came in for some discussion. Several types of involvement were mentioned as questionably definable as disseminated, for purposes of chemotherapy protocol assignment. These included empyema, paratracheal lymph nodes, mediastinal lymph nodes, new cavity formation subsequent to resection of a cavity, cervical lymph nodes, primary cutaneous lesions. The consensus was that all extrathoracic coccidioidomycosis be dealt with as disseminated disease. There will be some borderline cases (e.g. simultaneous cervical and paratracheal lymph node involvement) wherein the line is hard to draw.

Only newly disseminated cases are suitable for protocol, since patients surviving several years of disseminated coccidioidomycosis have exhibited resistance that would make drug evaluation difficult, if not impossible.

Drs. Dunner and Raleigh raised the point that amphotericin B supplied for this study should be used only on disseminated cases, due to the limited amount available from Squibb. If anyone wishes to treat non-disseminated (i.e. non-protocol) cases with this drug, he is free to do so, but should make his own arrangements for obtaining a supply of amphotericin B.

Questions were raised concerning dosage and duration of therapy. The dose is to be as recommended by Squibb and conditioned by the patient's tolerance to the drug.

Duration of therapy was not precisely defined in the original protocol, but was indicated to continue at least four to eight weeks. Dr. Cheu mentioned treatment of two meningitis cases for over a year. The patients were clinically well, but spinal fluid chemistry, cells and titers haven't reflected improvement. Dr. Meis discussed two patients who received i.v. amphotericin B for 36 days and showed clinical and serological improvement. After treatment was stopped, relapse occurred. Retreatment caused improvement, and cessation of treatment again resulted in relapse. Finally, an 3 month treatment resulted in excellent improvement without relapse. The concensus was that long term therapy should be adopted

5. Reorganization and Decentralization of the Study:

The Coccidioidomycosis Cooperative Study originated with the Executive Committee of the Tuberculosis Chemotherapy Cooperative Study. Dr. Dunner pointed out that there are presently about twenty VA cooperative studies and that it will be very difficult for VACO to administer all of them directly. Therefore it was proposed that the Coccidioidomycosis Study be made an independent cooperative study, no longer a sub-group of the TB cooperative program.

A decision was made that the records and correspondence for the Coccidioidomycosis Cooperative Study be maintained at the San Fernando VA Hospital. Mrs. Ticehurst is presently working at that hospital for the Solitary Pulmonary Nodule Cooperative Study, and for the present at least, will also be able to perform the

secretarial tasks for the Coccidioidomycosis group as well. Drs. Raleigh and Dunner constantly stressed, however, that moving the study records to an endemic area hospital will not prove to be a panacea for the communications problem which has limited effectiveness of the program to date. The burden of assembling, tabulating and analyzing data may prove too much for a field station.

In the past, the report forms were designed by the committee in the field. The codes were designed, and the cards punched and tabulated in Central Office. Some times all the desired information wasn't provided by the form, or tabulations were not made on all desired items. It is, of course, impossible for the VACO statisticians to anticipate which items of information are desired in tabulations. On the other hand, people actually involved in the study, if present during the tabulation process, can guide the selection of information to be tabulated. Thus decentralization to the field can help resolve the communication problem, but the very large statistical job may create bigger problems in the field station, which does not have the statistically trained people available. For the time being then, San Fernando will try their hand at using the forms and cards, but duplicate report forms and IBM cards will be maintained in VACO. The duplicate forms will be prepared at the study units, and transmitted to the Chairman, Coccidioidomycosis Cooperative Study at VAH, San Fernando; the extra copy will then be forwarded to VACO. Final analysis of data, after preliminary workup at San Fernando, will probably best be done by VACO statisticians. Dr. Stonehill suggested that a statistician be assigned to work with the executive committee. Mr. Williams pointed out that ultimate interpretation of results will require review of the forms themselves, not simply statistical analysis of the punched cards.

Dr. Salkin proposed that the Committee structure of the study be changed, since the present Plans Committee (consisting of representatives of all study units, as well as special representatives) is too large to assemble frequently and perform all the functions of a real working committee. What is presently referred to as the plans committee meeting is actually the annual meeting of the Study.

Considerable debate followed on the effectiveness of small and large committees and on proposed organizational structure of this program. The consensus was that a small executive committee should be formed, which can meet as frequently as needed, and the larger "Cooperative Study Group" meet once a year, as in the past. The executive committee will poll the study units on matters which require their opinions and decisions, and will notify the units of action taken during the year.

In discussing committee structure, Dr. Raleigh reiterated the fact that there will have to be some centralization in one hospital, but that San Fernando need not dominate the study since a five man executive committee will have four non-San Fernando members who can rotate on alternate years. This will provide good representation from all study units.

The following executive committee was established:

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|---|------------------------|
| 1. San Fernando VAH | David Salkin, Chairman |
| 2. Fresno VAH | Stephen Cheu |
| 3. Long Beach VAH | Leroy Hyde |
| 4. Tucson VAH | Solomon Netzer |
| 5. U.S. Air Force | Robert B. Stonehill |
| 6. Recording Secretary
(non-voting member) | Lawrence G. Wayne |

This committee will remain in office for two years, after which two members will be rotated every two years, and individual representatives will serve for two years. The Chairmanship will also be rotated every two years.

The present consultants will not be members of the executive committee, but their services will be available to the Committee.

Sub-committees will be appointed, as needed, by the executive committee. Sub-committee members should be selected from the Study Units. Among the committees considered were:

- (1) Laboratory (presently existing)
- (2) Exhibits (presently existing)
- (3) Surgical
- (4) Publications and Public Relations
- (5) Chemotherapy
- (6) Forms
- (7) Classification and Standards

Sub-committee appointments will be announced in the near future.

6. X-Ray Film Library and Panel:

The second annual meeting of the Coccidioidomycosis Cooperative Study had recommended establishment of an X-ray film library of coccidioidomycosis cases, as well as a panel to review films.

The question of an x-ray review panel was discussed at the third annual meeting. It was recognized that there is considerable lack of uniformity of interpretation of films, and also that a single individual, reading and rereading the same films at a later date will show variability in interpretation. Several alternate recommendations were made by members attending the meeting as to a panel's function and mechanics: (1) Review only cavitary cases; (2) Blind review by individual members of a panel, rather than review by the group sitting together; (3) Refereeing action on questionable cases only. Dr. Steele stressed the fact that comparability of cases was needed for decisions on fate of resected and non-resected lesions.

Arguments against a film panel centered about the tremendous amount of work involved in assembling material and panelists, and the question of whether the results would really warrant the effort and expense. The consensus was that they would not, and therefore no x-ray review panel was established.

The proposal for establishment of an x-ray film library was received more favorably. Dr. Steele recommended use of translite copies of selected films. The Solitary Pulmonary Nodule Cooperative Study has made arrangements to equip the Sepulveda VAH with equipment for making translite copies of films, and it should be possible for the Coccidioidomycosis Study to share these facilities.

It was the general feeling that films of all reported cases need not be collected. Various members expressed interest in films of certain types of cases. Dr. Richert wanted a library of complications, Dr. Noon wanted interesting cases in general and Dr. Carswell wished to see films of cases with other coexisting disease. Miss Campbell firmly pointed out that such collections, to be useful, must include some usual cases for comparative purposes.

Most agreed that the film collection would be more useful for teaching purposes than as an actual source of information on the study itself.

The library will be established for interesting cases. Cases for inclusion in the collection are to be decided on by individual Study Units; the selected films on these patients will be sent to San Fernando VAH, where arrangements will be made for translite copies to be prepared, and the original films returned to the Study Units. Packages containing the films should be clearly marked:

"Attention: Chairman, Coccidioidomycosis Cooperative
Study. Not to be sent to X-Ray Department"

7. Follow-up Mechanics:

Opinions were expressed on the follow-up needs of various forms of the disease. Dr. Cheu follows up on primary cases about once a month until the chest clears and the titer falls below 1:16. Dr. Netzer follows the primaries every two weeks for three months, and the cavitary cases every three months. No limit was set on follow-up periods; it will probably be a number of years before we can decide on cut-off dates.

The follow-up mechanics of VA non-service connected cases represents a serious problem. The CBOC program is difficult at the local level. The use of a P-10 admission is simpler and more satisfactory if the patient will cooperate. VACO had no suggestion on the best method, and it was felt that each study unit should work out its own follow-up mechanics.

Follow-up forms for the protocol cases will be prepared and distributed soon. Other follow-up forms will be designed and distributed as various phases of the program develop.

3. Exhibit Committee:

Dr. Netzer was appointed, after the second annual meeting, as Chairman of the Exhibits Committee, and Drs. Brosbe and Cheu served on this committee with Dr. Netzer. The committee met twice in 1953 and, with the cooperation of other study units, have developed an excellent exhibit. A mock-up of the exhibit was shown to the group; Mr. Flory, in VACO, is preparing the actual exhibit. Present plans call for presentation at the NTA meeting in Chicago, and the AMA in Atlantic City.

Dr. Netzer proposed that his committee be discontinued after the presentation of the exhibit in 1959.

RESUME OF DECEMBER 5 SCIENTIFIC SESSIONS

Scientific papers on the medical and epidemiologic aspects of coccidioidomycosis, as well as laboratory studies on the disease and its causative organism, C. immitis, were presented by invited participants from Study Unit Hospitals and from unaffiliated hospitals and universities. Abstracts of their papers follow:

9:30 A.M. Session: Dr. A. Ringle, Moderator.

1. "Clinical Reports" by R. Cohen, M.D., Bakersfield, Calif. (Dr. Cohen was unable to attend to present his paper personally, but in fine dramatic style, a telegram arrived at the 11th hour, containing a summary of the material he wished to present. The text of the telegram follows:)

"I deeply regret that I cannot be with such a group having a common desire to conquer coccidioidomycosis. I wanted to state that I was using another approach to the problem such as using wood-rot fungi such as *Fomes officinalis*, *Fomes pinicola*, and *Lensites trabea* and *Sclerotinia fructicola* which love cellulose of which the coccy's capsule is composed. Anyone interested in this approach may obtain the fungi from plant pathology department, University of California, or "Schimmelbureau" Holland. I have been trying in vitro griseofulvin, a penicillin product produced in Glasgow. Both reports are too embryonic yet. Clinically I have been using Furadantin, 100 mgm q.i.d. on primary cases. I reported that it inhibits coccy's at less than 300 microgram per millilitre. It shows some merit. Fungizone in meningitic cases fails to lower the cell count under 150 and I am not too happy about it when we have shown cases living two years without therapy. Someone has the right answer to our common problem, and such a gathering will bring it out. Thank you. Robert Cohen, M.D., Bakersfield, Calif."

2. "Observations on the Clinical and Serological Effects of the Treatment of Disseminated Coccidioidomycosis with Nitrogen Mustard". by N.B. Kurnick, M.D., VA Hospital, Long Beach, California.

Since disseminated coccidioidomycosis is a granulomatous disease associated with hyperplastic reaction of the reticuloendothelial tissues, whose complement fixing antibody response reflects the severity of the disease rather than appearing to serve an effective immunological protective mechanism, it appeared of interest to test the effect of a depressant of the reticuloendothelial system. Nitrogen mustard was selected for this purpose. Three patients with disseminated coccidioidomycosis but without cerebrospinal involvement, each showed healing of lesions and concomitant fall in the complement fixing titer. Each of these individuals had had persistent and recurrent disease. One of them, a negro male, has now been asymptomatic and has had a negative complement fixing titer for over 2 years. The second, a Mexican male, has had a remission lasting not quite 1 year thus far. The third, a white male, died of a coronary approximately 4 months after therapy. Three other patients with coccidioidal meningeal lesions were also treated. One of these died 2 months after the institution of nitrogen mustard therapy, of a collapsed cervical vertebra due to coccidioidal involvement, with resultant quadriplegia and respiratory failure. The second died of internal hydrocephalus due to his disease. The third patient was quadriplegic at the time of institution of therapy, was thought to be going rapidly down hill. After several courses of nitrogen mustard his state is considered to be stable. In these patients fall in the complement fixing titer of blood and spinal fluid were noted, but not as consistent as with the first 3.

It is recognized that the depression of the complement fixing antibody titer is probably due to the direct action of the nitrogen mustard on the reticuloendothelial system. The concomitant clinical improvement confirms the fact that this antibody exerts no effective protective action. Although our results suggest that nitrogen mustard is not a definitive fungicidal agent, the improvement noted, at least transitorily, in all 6 patients, and marked and sustained improvement in 2, indicates that the therapy with nitrogen mustard deserves more extensive trial. Evaluation of nitrogen mustard therapy in so variable a disease as disseminated coccidioidomycosis will be possible only after much wider experience has been obtained than it is possible to achieve within our own institution.

2. "Some Clinical Notes on Disseminated Coccidioidomycosis" by David Salkin, M.D., VAH, San Fernando, California.

A report was made on 61 cases of disseminated coccidioidomycosis collected during the 1955-56 retrospective study. The highlights were as follows:

1. Time of Occurrence: 90% disseminated within 6 months from time of primary infection, 95% within 12 months, and 5% more became clinically evident within one to six years.

2. Prognosis: Of the total group, 16% could be considered inactive, 44% were still active, and 40% were dead. Of the 42 non-meningeal cases, 20% were inactive, 20% dead, and 60% still active in some degree. Of the 19 meningeal cases, 16 were dead, and 3 still alive at 27 months, 39 months, and 120 months.

3. Results of treatment: The 61 cases had every variety of treatment, including no treatment, with a total figure of 27% improved. Knowing the ineffectiveness of all the therapies, this figure may be taken as a good control.

4. Amphotericin B Treatment: To date we have 32 cases so treated, with definite improvement ranging from 20 to 35%. This includes 5 cases of meningitis, 13 cases of other disseminations, 2 cases of primary disease, 10 residual cavities, 1 granuloma, and 1 post-operative empyema. Although these figures are not better than the control, there is no doubt in the minds of most clinicians that amphotericin B is of definite value in some cases. How much value and how durable remains to be determined. It is possible that later figures may give us even a mathematically statistical difference. To date it appears to be the general impression that, used intravenously in its present form, it is certainly the best palliative drug we have. However, one must not use it too casually because of its toxic effects.

11:00 A.M. Session, Col. R. Hunter, Moderator.

4. "Ecology of Coccidioides Immitis in San Diego County". Henry A. Walch, Ph.D., James F. Pribnow, Lt. (USN) and Ruth K. Walch, B.A., San Diego State College, U.S. Naval Hospital, San Diego, and University of San Diego, College for Women.

The probable existence of Coccidioides immitis in the soil of San Diego County was first indicated by the San Diego County Public Health Department investigations of four apparently locally acquired infections.

The present study was planned for the isolation of the fungus from the soil of this county. Case investigations were carried out to provide information that might pinpoint particular geographic areas in the county. This was done by follow-up of cases from which a culture isolate had been referred to us for identification. Other sources were serologically and symptomologically positive cases that had been brought to our attention by interested physicians.

Soil sampling has been done in various areas of this county. C. immitis has been isolated from the soil of an old Indian campsite east of the city of Lakeside (since this paper was given the fungus has been re-isolated from this area and confirmed by laboratory animal infection.)

In this report only three of the many cases investigated are reported. These three cases are of interest since they occurred in children ranging in age from 1-1/2 to 5 years. Two of these children were culturally positive and the third

was serologically positive and displayed typical symptoms which were confirmed by a physician experienced with the disease. Two of the children had no history of travel from their homes. The third had traveled to the Los Angeles area via the coast route about one month prior to onset. These children live in different areas of the county several miles apart. It was noted that all of the children were active "diggers" in the soil of their yards. All of them had sandboxes and two of the yards had been supplemented with topsoil.

(This study is supported by a grant from the San Diego County Tuberculosis and Health Association. Laboratory space for this project has been provided at the Zoo Hospital by the San Diego Zoological Society. We are grateful to Leon Gardiner, M.D., of the San Diego County Health Department, who provided us with information of past occurrences of this disease in this area.)

5. "An Epidemic of Coccidioidomycosis at Edwards Air Force Base." Bernard Jaffe, Capt., USAF (MC), Edwards AFB, California.

Edwards Air Force Base is situated 120 miles northeast of Los Angeles on the edge of the Mojave desert. During the summer the temperature ranges to 120 degrees during the day with an attendant low humidity. During the winter the temperature reaches 60 to 70 during the day and the nights are cool and dry.

The average yearly incidence of coccidioidomycosis at Edwards Air Force Base has been one to two cases. Rainfall during the winter of 1957-58 was extremely heavy and desert flowers bloomed more profusely than they had in eight to ten years on the desert. Starting September 1958, construction of 500 new homes, using bulldozers which turned up the earth to one to two feet below topsoil was started at Edwards Air Force Base.

Shortly thereafter, the first of 35 cases of adult and 15 cases of pediatric coccidioidomycosis was seen. The cases have been characterized by unusually high incidence of erythema nodosum; 50% of the males and 65% of the females presenting with this complaint. History of exposure in endemic areas is difficult to obtain, as at an Air Force Base most of the patients have moved in and out of the endemic region several times over a period of years.

Public health statistics from Kern County show 192 cases of coccidioidomycosis in the country this year as opposed to 176 cases last year. This in no way equals the increased incidence at Edwards. It is concluded that a combination of two factors, increased rainfall and dust production, are probably responsible for the increased incidence of coccidioidomycosis at this base. All cases are being followed in the routine way with serial x-rays and complement fixation titers. So far, no case of extension beyond the lungs has been documented. However, the morbidity in several flying personnel has been extreme in that they have been off flying status for two or more months.

6. "Summary of Research Studies on *Coccidioides immitis* by the Arizona State Department of Health" H. G. Crecelius, Ph.D., Arizona State Dept. of Health, and K. T. Maddy, D.V.M., M.P.H., U.S. Public Health Service, Phoenix, Arizona.

The research studies on *C. immitis* being conducted in the Arizona State Department of Health have been in progress for 18 months. The basic epidemiological studies on cattle utilizing skin sensitivity and observations at post mortem have been completed and reported elsewhere by Dr. K. T. Maddy.

The laboratory studies have progressed in two directions: (1) on the development of coccidioidin suitable for complement fixation studies, and (2) the elucidation of the meteorological factors which contribute to the propagation of the organism in nature. We have accomplished the first objective and have sufficient antigen to do diagnostic studies for Arizona.

No new knowledge has been gained concerning the culturing of the organism from soil, however our results are somewhat better when the sediment is used for inoculation rather than the supernatant fluid from soil suspensions. The media devised by Ajello and his group, using actidione, has yielded the greatest number of isolations. We have found essentially the same results reported by Plunket et al in soil samples of California, namely that there are "hot spots" which repeatedly show the organism. The ease with which they can be isolated depends on seasonal variations.

7. "Serologic Positive Coccidioidomycosis Cases from Non-Endemic Areas". Charlotte C. Campbell and Grace Binkley Hill, Walter Reed Army Institute of Research.

During the eight year period from 1 January 1950 to 31 December 1953, ser (1 to 20 specimens per patient) from a total of 337 cases with a presumptive or culturally verified diagnosis of coccidioidomycosis were received in the Medical Mycology Laboratory, Walter Reed Army Institute of Research, for complement fixation tests with coccidioidin*. Precipitin tests were not carried out.

The CF titer was negative at the lowest serum dilutions employed (1:4 or 1:8) in 71 cases in which *Coccidioides immitis* (according to accompanying data) was isolated from the patient (Table I). In 9 additional culturally verified cases, the single serum specimens received were anticomplementary. Sera from the remaining 257 cases (76.2%) were positive with coccidioidin at CF titers ranging from 1:4 to 1:2043. In 35 of these cases, *C. immitis* was also isolated, whereas in 172, it is not known whether the organism was recovered or not, and in many instances, whether attempts were made to do so.

* Lots 47-54 and 47-63 generously supplied by Dr. C.E. Smith, Univ. Calif.

TABLE I

Summary of complement fixation reactions in 337 cases of coccidioidomycosis during eight years of serologic study of mycotic diseases (1 January 1950 to 31 December 1953). Walter Reed Army Institute of Research

<u>Basis of Diagnosis</u>	<u>No. Cases</u>
A. <u>Coccidioides immitis isolated:</u>	
1. Complement fixation test - Neg.*	71
2. " " " - AC **	9
3. " " " - Pos.	85
B. <u>Coccidioides immitis isolation - unknown ***</u>	
1. Complement fixation test - Pos.	<u>172</u>
Total	337

* CF titer less than 1:4 or 1:8. (It should be emphasized that in many instances date of serologic testing did not coincide with active stage of disease.)

** Single serum received - Anticomplementary.

*** The organism doubtless was isolated from many of the cases. However, the laboratory is not always apprized of this fact.

Of greater interest, however, is the fact that 203 (60.2%) of these cases were in persons hospitalized in geographic areas that are not endemic for coccidioidomycosis (Table II). Sixty-six were veterans.

TABLE II

Summary of Serologic and Cultural Data in 337 Cases of Coccidioidomycosis, Walter Reed Army Institute of Research, Washington, D.C., 1950-1958, Inclusive

Year	<u>No. Cases in Non-endemic Areas*</u>		<u>No. Cases in Endemic Areas**</u>		Total
	Service	Veterans Administration	Service	Veterans Adm.	
1950	2	1	2	1	6
1951	14	2	1	2	19
1952	20	1	6	0	27
1953	17	0	9	1	27
1954	19	2	13	3	37
1955	19	12	3	6	45
1956	16	19	16	10	61
1957	11	14	17	7	49
1958	19	15	17	15	66
	<u>137</u>	<u>66</u>	<u>89</u>	<u>45</u>	<u>337</u>
	203		134		

*Endemic Areas: California, New Mexico, Arizona, Texas, Southern Utah.

**Non-endemic Areas - Medical installations in all other states.

In view of this laboratory's own location in a Middle Atlantic area, it is perhaps not surprising that the majority of cases coming to its attention are also from nonendemic areas. Nevertheless, these data do emphasize the fact that the infection is present in a number of persons not currently residing in endemic areas, and that clinicians in nonendemic areas, especially those dealing with chest diseases, should be continuously cognizant of this situation.

1:30 P.M. Session. Dr. R. Egeberg, Moderator.

8. "The 'In Vitro' Spherules of Coccidioides Immitis." A. M. Breslau, Research Service, V.A. Center, Los Angeles, California.

Coccidioides immitis is a diphasic imperfect fungus. In nature it exists as a saprophytic mycelium; in the host it takes the form of a spherule or sporangium producing endospores. The filament is composed of multinucleated cells containing mitochondria whose cristae may run longitudinally, lipid inclusions and reticular cytoplasm. The wall is doubly contoured with an electron transparent content which contains polysaccharide and is stained by P.A.S.

The tissue spherule has a thin wall which stains with P.A.S. In later stages of development an external phospholipid layer is present. The protoplast is rich in ribonucleo-protein and mucopolysaccharide. The latter passes into the cytoplasm only when the phospholipid layer is absent or broken as in exsporulation.

Two strains, of seven tested, transform into spherules "in vitro". There is no correlation between virulence and transformation. Nor is reduced oxygen tension necessary. There are two methods of transformation. In the first and more common one, the cells of the filament enlarge, round up and develop into sporangia. Endospores are produced by cleavage. The phospholipid layer is lacking. It is probable that in the host, it is produced by the invaded tissue. Instead of a single structure, the spherule wall is lamellated. The nuclei are double contoured and contain a large electron opaque nucleolus. The cytoplasm is filled with mitochondria containing cristae. A vacuole may be present which is filled with a substance less dense than cytoplasm (mucopolysaccharide?). During spore formation walls grow in from the peripheral spherule wall.

In the second form of metamorphosis, the filament thickens. Numerous transverse divisions produce very short but very wide cells. Longitudinal and oblique divisions then give rise to a cluster of endospore-like cells, which grow into sporangia and produce endospores.

9.. "Characteristics of Polysaccharides Derived from Coccidioides Immitis."
Earl G. McNall, Ph.D., Dept. of Medicine, Univ. of Calif. School of Medicine,
Los Angeles, California.

It is well known that most coccidioidin preparations are not satisfactory for the complement fixation test and that those which are, usually have been obtained from mycelial cultures which have undergone extensive autolysis. It is our finding that the soluble polysaccharides derived from cultures of C. immitis, as they are initially elaborated, have higher molecular weight than the usual complement fixation antigens and are anti-complementary. Satisfactory reagents for complement fixation tests contain lower molecular weight polysaccharides than those elaborated during the log phase of growth. Chemically modified chromatography paper was developed in our laboratory which was utilized for the characterization of complement fixing antigenic polysaccharides as to molecular weight class and homogeneity.

There are isolated reports in the literature which indicate that the mycelial wall of C. immitis consists largely of chitin and/or cellulose. It is our finding, however, that the cell wall polysaccharide is made up of an insoluble polysaccharide (PlX) and a lipopolysaccharide (PlL), neither of which contained cellulose or chitin. Galacturonic acid, mannose, glucose and glucosamine in the acid hydrolysate of PlX were demonstrated by chromatography in several solvent systems. The soluble polysaccharide present in the culture media of C. immitis contains the same sugar moieties as PlX. Complement fixing antigen appear to range from 16-40 residues/molecule, whereas the size of the polymer in the organism cell wall is 10-20 times larger. The insoluble cell wall polysaccharide (PlX) from C. immitis contains approximately 400 residues/molecule. The infra-red spectra of the PlX polysaccharide indicates that beta bonding predominates and only about 25% alpha bonding between sugar residues. The predominate inter-linkages between residues are 1, 3.

A preliminary study of antigenic character of PlX clearly demonstrated that detectable antibody titers appear within one week following a single I. P. or I. M. injection of antigen at a level of 5 mgm/kg. A series of 3 injections at this level spaced 3 days apart followed by a single injection at 2 weeks produced a higher titer of antibody as demonstrated by exhaustive precipitation in the zone of slight antigen excess.

Relatively low levels of polysaccharide (1-10 mg/kg) conferred partial protection to C. immitis infection in mice, whereas the highest level (100 mg/kg) enhanced the death rate. It is well known that the administration of high levels of pneumococcal polysaccharides induce immune paralysis and perhaps this is the explanation for the enhanced death rate when high levels of C. immitis PlX are administered.

We are currently evaluating the protection induced by this polysaccharide in several species of animals after a series of immunizing injections in both chronically and acutely infected animals.

10. "Preliminary Growth Studies on Histoplasma Capsulatum." Charlotte C. Campbell and John P. Reilly, Walter Reed Army Institute of Research.

Histoplasmin preparations currently used in both skin test and serologic studies in histoplasmosis require an incubation of two to four months. However, in preliminary studies to eventually prepare more specific materials for both of these procedures, it has been found that by the simple expedient of shaking or aerating the cultures, this incubation period can be reduced to 14-21 days. Asparagine broth filtrates of cultures harvested at this time are comparable in serologic activity to those incubated for the more extended periods.

Glycerol, one of the ingredients of the asparagine broth medium which has long been employed for the production of both histoplasmin and coccidioidin, was also found to inhibit the growth of some strains of H. capsulatum. Eleven of twenty-two strains grew more luxuriantly, with or without shaking, in asparagine broth medium from which only the glycerol was deleted.

The depressed growth rate in the strains inhibited by glycerol did not appear to be associated with the time interval between isolation of the organisms and their use in the experiments. Some of the strains inhibited were recent isolates, still laden with "tuberculate chlamydospores" while others were "stock" cultures, isolated as long as ten years ago, in which the chlamydospores were few or absent.

Nor did the isolation source appear to be of importance. Some of the strains inhibited by glycerol were isolated from humans, others from soil and others from dogs.

Further investigation of these preliminary findings are in progress.

2:15 Session. Dr. David Salkin, Moderator.

11. "The Sensitivity and Resistance of Different Types of Coccidioides Immitis Cells to Amphotericin B." Milton Huppert, Ph.D., and Leila J. Walker, M.S., Mycology Research Laboratory, VA Hospital, San Fernando, California.

Since the initial reports in 1955 of the antifungal activity of amphotericin B, there have been many studies concerned with the use of this antibiotic against fungi causing systemic disease. The in vitro studies, however, have been done only with a limited number of strains of each species. The present report is concerned with testing the antifungal effect of amphotericin B against over one-hundred strains of Coccidioides immitis, and with the variability of sensitivity to this antibiotic by single strains depending upon the type of fungous cell used as inoculum.

The fungus, C. immitis, can be divided into at least three distinct types of cells. These are the vegetative or hyphal cells, the arthrospores, and the spherules which, in the mature form, contain endospores. Techniques were developed for preparing three different inocula. As far as could be determined, one type of inoculum contained only the hyphal cells, a second was made up exclusively of arthrospores, and the third was composed of spherules and endospores only.

When over one-hundred strains of C. immitis in the hyphal cell type of inoculum were tested against amphotericin B, there was a wide range of sensitivity, some strains being resistant to 100 ug/ml of the antibiotic. When cultures representing resistant and sensitive strains were tested in the arthrospore and in the spherule-endospore stages, it was found that these cell types differed in their susceptibility to the antibiotic as compared to their corresponding hyphal cell type. Since it is believed the spherule-endospore stage-inocula provided the most significant information, it is recommended that these be used for amphotericin B sensitivity testing.

12. "The Development of Strains of Candida Albicans and Coccidioides Immitis Which are Resistant to Amphotericin B." Lloyd J. Sorensen, Ph.D., E. G. McNall, Ph.D., V. D. Newcomer, M.D., and T. H. Sternberg, M.D., Dept. of Medicine, Division of Dermatology, Univ. of Calif. Medical Center, Los Angeles,

Three strains of Candida albicans and two strains of Coccidioides immitis became resistant to amphotericin B by continual passage in media containing gradually increasing increments of this antibiotic. Two of the Candida albicans were not completely inhibited by 1000 mcg/ml of amphotericin B although partial inhibition was observed at lower levels.

When these amphotericin B resistant strains were tested against their susceptibility to nystatin and pimaricin, cross resistance was evident when nystatin was used, but not when pimaricin was used. This cross resistance assumes clinical significance since both amphotericin B and nystatin are used for the therapy of candidiasis.

13. "Incidence of Coccidioidomycosis in Arizona in 1953." Keith T. Maddy, D.V.M., M.P.H., U.S. Public Health Service and Arizona State Department of Health.

With only about 47 percent of Arizona physicians sending in their weekly morbidity report cards to the Arizona State Department of Health, and with only a proportion of these reporting coccidioidomycosis, by December 5, 590 cases had been reported. This compared with 553 cases the same time one year before.

By several methods of estimation we believe that at least 5,000 persons in Arizona became ill enough with this infection this year to seek a physician's attention. In addition to the above figures, over 50 cases have been diagnosed

at Davis Monthan Air Force Base, about 25 cases have been seen at Williams Air Force Base, and about the same number at Luke Air Force Base.

By the end of the year we may have reports on about 10 disseminated cases. Arizona has a little over 1,000,000 persons in the state and most live in the endemic area.

The dog population of the state is about 150,000. In our animal disease reports up to December 5, we have had 102 cases of disseminated infections in the dog reported and we know of many more cases. Obviously the disseminated infection is far more common in the dog than in man, and as such, the dog is a much more available subject for some studies on this infection.

Almost 2,000 cases of this infection were reported or seen at post mortem inspection of cattle slaughtered in the state so far this year.

It appears that 1953 was a normal year for this infection in Arizona.

13. b. "A Study of a Site where a Coccidioides Immitis Infection was Acquired." Keith T. Maddy, D.V.M., M.P.H., U.S. Public Health Service and Arizona State Dept. of Health.

A family and their dog went for a picnic in the desert. The family spent the day resting, but the dog was very active digging in the rodent burrows and inhaling deeply for possible animal scents. Eleven days later the dog developed coccidioidomycosis which became disseminated two months later. The people did not become ill.

From the rodent burrows and adjacent areas soil samples collected at various times over a period of three years were checked for C. immitis by mouse inoculation techniques. Soil temperatures were taken at various depths, and rainfall data were recorded. C. immitis was isolated a number of times. These isolations were more common following the late summer rains, and at this time were more common near the surface in rodent burrows. After long periods of dry weather, isolations could usually be made only deep in the rodent burrows. The rodent burrows provide an environment with much more constant temperatures and moisture content than the desert topsoil, and are therefore a better environment for the fungus C. immitis.

13. c. "Artificial Infection of Cattle with Coccidioides Immitis." Keith T. Maddy, D.V.M., M.P.H., and Raymond E. Reed, D.V.M., U.S. Public Health Service and Arizona Dept. of Health.

Two experiments were planned to determine if there is a clinically-evident phase of illness in coccidioidomycosis in cattle, and to check the correlation of coccidioidin reactions with infections.

The first experiment was conducted on 95 head of coccidioidin-negative Hereford steers and heifers with an average age of 1-1/2 years which were brought from their home range at 3,000 ft. altitude in central Arizona to Tucson, Arizona. 43 of the cattle were inoculated intratracheally with saline containing 436,000 viable fragments of hyphae and arthrospores of C. immitis. The animals were retested with coccidioidin after a 90 day observation period and then were given a post mortem inspection when sent to slaughter.

No symptoms of illness were observed. 42 of the 37 head available at the end of 90 days were coccidioidin positive. 10 of the positives were in the uninoculated controls; this was about the expected natural rate of conversions for the area. 32 of the 42 of the available inoculates were coccidioidin-positive. At post-mortem 7 of the naturally infected cattle showed respiratory tract lesions. Of the artificial inoculates, one showed a subcutaneous lesion at the site of inoculation. Previous studies have indicated that coccidioidal granulomas in cattle lungs heal almost entirely in 30 to 60 days after the infection is acquired. Previous studies had also indicated that small reactions to coccidioidin were difficult to detect in Herefords due to their thick skin. The first study was performed on the Herefords in the endemic area because these cattle were available and owned by a state agency, and we would have had less reason for alarm had clinical illness and/or deaths occurred.

The second experiment was conducted at Woodruff, Arizona, at 5200 ft. altitude on 65 coccidioidin-negative young cattle. 25 were inoculated intratracheally with 1,400,000 viable fragments each in saline and 17 received 700,000 each. 23 were left for controls. None of the inoculated animals developed any clinically evident illness.

90 days after inoculation, these animals were coccidioidin-tested again. 63 were available for study, two having died of a post-castration septicemia during the study. None of the control animals became coccidioidin-positive. All of the inoculated animals were positive to three coccidioidins. All of the animals were negative to haplomyacin at both the beginning and the end of the study. All of the animals were negative to histoplasmin at the beginning of the study. Previous studies had shown that cattle infected with coccidioidomycosis consistently give small or negative histoplasmin reactions.

This study was useful in establishing the specificity of coccidioidins that had already been used in skin-testing home-raised cattle in all counties in Arizona. This last mentioned study had established the specific natural infectivity of the various parts of the state assuming the coccidioidins used gave a specific reaction. The second experiment, outlined above, established that specificity to our satisfaction.

14. "Treatment of Canine Coccidioidomycosis with Amphotericin B". R. E. Reed, D.V.M., College of Agriculture, Univ. of Arizona, Tucson, Arizona.

The objectives of a trial using amphotericin B in dogs infected with coccidioidomycosis were to determine the value of the dog in such investigations and to determine if the antibiotic is effective against the disease when administered intramuscularly at the level of 1 mg/kg on alternate days. The same level of amphotericin B administered intravenously in greater concentration and at a more rapid rate than recommended for man (because of the difficulty in restraining dogs during treatment) had regularly produced fatal azotemia in healthy and infected animals.

Thirty-six young, healthy dogs with negative complement-fixation and precipitin serology and negative coccidioidin sensitivity tests were infected by intratracheal insufflation with a saline suspension containing 100,000 viable particles of *C. immitis* from dry Sabourad's agar culture. Five of this group were maintained as infected, untreated controls, and an additional 5 dogs were kept as healthy, untreated controls. Of the 31 infected dogs intended for treatment, 6 died of the disease before treatment could be started. Treatment was started 7 days (7 dogs) and 14 days (13 dogs) after infection. In the group started at 7 days, 4 survived to receive 10 injections given over 20 days, and in the group started at 14 days, 11 were alive 20 days after treatment began.

Serums taken from all dogs before treatment and from those still alive after the seventh injection of amphotericin had normal urea nitrogen levels. Serums collected from dogs after the seventh injection contained no amphotericin as indicated by a complete lack of inhibition of *C. albicans**. At the time these data were presented, 3 of the 5 infected, untreated control dogs were still alive and were being held with 3 surviving treated dogs. Necropsy of all dogs that died before, during and after treatment, and all killed for necropsy after treatment, revealed extensive coccidioidal disease in thoracic tissues as well as dissemination to tissues outside the thorax in many, and the fungus was recovered in culture from various tissues in each. The uninfected control dogs remained healthy.

* After the method of Taylor, et al., Am. Rev. Tuberc. and Pulm. Dis., 77, (1953).