

Transactions

of the

EIGHTH ANNUAL MEETING

of the

VA-ARMED FORCES COCCIDIOIDOMYCOSIS

STUDY GROUP

December 5 - 6, 1963

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## Foreword

The eighth annual meeting and these Transactions are dedicated to the memory of Doctor Arthur Levi Ringle who died November 11, 1963. He was a founder of this group and his hard work and encouragement were prime factors in developing this Conference. We have lost a friend.

This meeting was attended by 133 students of the disease. Excellent papers were read on almost all phases of the subject. A number of reports dealt with ecology and epidemiology. Interesting developments dealt with unusual forms of *C. immitis*, the fluorescent antibody test, and Huppert's immunodiffusion screening test. Intriguing experiments were presented in the development toward a human vaccine.

The clinical sessions dealt with laboratory methods, a review of coccidioidomycosis of the skin, bones, and meninges, a panel on Amphotericin B and a panel on pulmonary surgery.

We are indebted to many people for the success of this meeting--to our Central Office colleagues Drs. James H. Matthews, Edward Dunner and William B. Tucker; to the Los Angeles Medical Association for the use of its facilities; and especially to our co-workers Cleo McCubbin, Meryle Acosta and Johnsie Bailey.

I am personally indebted to my associate, Dr. Milton Huppert, whose tireless efforts made this meeting possible.

DAVID SALKIN, M.D.

Chairman

## Introductory Remarks

D. Salkin

I welcome all of you, official representatives and others, to the 8th annual meeting of the Study Group. Although our meetings are not publicized, fully 133 are attending, including 52 from the VA-AF and 81 from civilian life. Many important developments have occurred in the past 8 years and I am gratified that some of them resulted directly from discussions at these conferences. To name a few, they are: (1) the Lower Sonoran Life Zone concept of Maddy, (2) ecological studies, such as those of Egeberg and associates, (3) the introduction of Amphotericin B, (4) a better understanding of the relationships of laboratory and clinical findings, (5) excellent experimental pathology, (6) the progressive developments toward a vaccine, (7) a crystallization of the place of pulmonary surgery, (8) the monograph on coccidioidomycosis in 1958 by Fiese, (9) the introduction of new concepts dealing with pathogenesis, clinical classifications, and the clinical assessment of the activity of the disease.

Probably our most valuable accomplishment is the very existence of this Group and these meetings with the resultant communication of opinions, cross fertilization of ideas, and a development of a more comprehensive portrayal of the whole subject.

In the past year, over 50 articles on the subject appeared in various journals. New editions of texts by Hinshaw and Garland and by Greer have revised chapters on coccidioidomycosis; a new text by Emmons, et al, has a chapter on the subject. Dr. Cheu is continuing his Bibliography. In the next several months a new book edited by Dr. Steele will appear with the title "The treatment of mycotic and parasitic diseases of the chest", published by C. C. Thomas; chapters on coccidioidomycosis have been written by Huppert and by Salkin and Evans.

The 1962 Transactions appeared late, unfortunately. It was printed by the Tuberculosis and Health Association of California - a total of 700 copies. One copy was sent to each member of the California Thoracic Society and the remaining 300 copies were sent to our registrants and others who requested one. The demand was quite heavy and we were left with only several copies for our file. You may be pleased to know that Dr. Myrnie A. Gifford wrote to tell us how much she enjoyed reading the Transactions.

## ECOLOGY AND EPIDEMIOLOGY

Chairman: R. O. Egeberg

### 1. Coccidioidomycosis in a university archeological group.

W. C. Riley and Gertrude T. Huberty

During the month of September 1962, a group of 16 persons, including two children, sponsored by the U. C. L. A. Anthropology Department, travelled to the Los Banos area at the northern end of the San Joaquin Valley to engage in anthropological investigation of the Yokut tribe of Indians. Most of the group were active at some time with digging, sifting and screening fine silt.

Subsequently, every one in the group developed primary coccidioidomycosis, varying from a benign upper respiratory illness in 3 to pneumonitis and cavity production in 13. Most of them developed symptoms within 14 days from exposure. One interesting feature was the appearance of a positive skin test in 2 patients 2 and 3 months after exposure although their precipitins became positive in the third week.

Students participating in field trips in the endemic areas should have skin tests prior to and following exposure and should be made aware of the possibility of coccidioidal infection.

(Authors' summary)

Discussion from the floor:

Dr. Melick: How many developed cavitory lesions out of the group of 16?

Dr. Riley: There were 6 or 7 cavitory lesions.

Dr. Meis: Any follow up studies?

Dr. Riley: Yes, all have been followed. Two or 3 have nodular lesions.

Dr. Meis: Was surgery required?

Dr. Huberty: No surgery was required.

Dr. Newcomer: Shouldn't skin tests be given beforehand to insure that only skin test positive people do this type of study? We should use a team of positive reactors in field studies.

Dr. Egeberg: It would probably take a decade before they would accept that.

Dr. Huberty: We urge everyone to get a skin test. It would be hard to find a positive team.

Dr. Einstein: The Feather River Dam project will cause quite a public health problem. Contractors will be hiring non-positive workers, and the illness will be compensable.

Dr. Huberty: This would be a good opportunity to try the vaccine.

Dr. Levine: The vaccine has not been approved yet for trial in humans.

Dr. Proctor: The hazard of occupational exposure is already a fact. Dusty conditions from soil moving activities have earned workman's compensation for people from back east. A water development project should automatically qualify for compensation those negative reactors who develop coccidioidomycosis.

2. Household epidemic of coccidioidomycosis with recovery of *Coccidioides immitis* from the soil.

Yasue Sawaki and C. R. Hampson

In April, 1963, eight people in Bakersfield, California, became ill with pulmonary coccidioidomycosis. A history of these cases pointed to the yard of the home and to the adjacent vacant lot as the source of the infection. Soil samples were taken from 13 sites in these locations.

Saline suspensions were made of the soil samples, allowed to settle, and the supernatants were handled by 2 different methods. In one, the supernatants were planted directly on a nutrient agar medium containing cycloheximide and chloramphenicol. In the second method, mice were inoculated by intranasal instillation using 4 animals per sample. Spherules were observed in the lung of one mouse. This was a minimal lesion, however, and the entire lesion was used for microscopic studies. Therefore, no culture was recovered from this mouse.

The culture method for isolation of *C. immitis* from soil was entirely negative. This had involved 78 cultures and 92 subcultures.

The results suggested that intranasal inoculation of mice may be more sensitive than the cultural method for recovery of *C. immitis* from soil.

(Authors' summary)

Discussion from the floor:

Dr. Levine: Were two mice used for each locus?

Dr. Sawaki: No. Four mice per locus.

Dr. Levine: Was there only one positive mouse from location #4?

Dr. Sawaki: Yes.

Dr. Egeberg: In defense of the plate technique, we find both methods used together to be a better approach. The plates give an idea of other fungi which are prevalent in the sample. If, of course, you are interested only in finding *C. immitis*, then the injection into mice is better.

Dr. Levine: The point to be made is that the number of positives by the mouse method is higher with intranasal instillation than with intraperitoneal injection. The study with Winn proved this.

Dr. Egeberg: Point well taken.

Dr. Newcomer: Are there any methods to prove that the people were infected from *C. immitis* in the soil at location #4, or whether they may have been infected from a different source?

Dr. Salkin: Dr. Huppert may be able to answer that.

Dr. Huppert: The question raises the point, "Is there enough difference between strains of *C. immitis* so that they can be separated into identifiable varieties?" Such information is not yet available. In the study of a small hospital epidemic reported by Dr. Eckmann, we believed that the four cultures obtained were sufficiently different from the usual cultures of *C. immitis* to warrant a cautious statement that they all originated from the same source. These four cultures all developed a tan pigment and a powdery type of surface growth. Later today, Mrs. Walker will report on another unusual type of *C. immitis*. These are only instances indicating that in the future we may have enough knowledge of different types of *C. immitis* to begin a study of epidemics of coccidioidomycosis.

3. Observations on *Coccidioides immitis* found growing naturally in soil.

K. T. Maddy

At one previously identified positive soil sampling area, one of a periodic series of soil samples resulted in 9 isolations of *C. immitis* from 36 soil samples. These identified 9 sites and 3 of the negative sites were partially excavated for a depth of 6 inches. They were moistened with the equivalent of one inch of rainfall, and then they were covered with glass and canvas. The pits were examined daily for fungal growths. Fifty-seven suspect fungi were collected during the next 12 days. Three of these were eventually identified by the mouse intraperitoneal injection technique as *C. immitis*. All three had been found growing on pieces of decaying vegetation in the soil.

(Author's summary)

(Ed. note: Dr. Maddy's paper was read by Dr. Huppert.)

Discussion from the floor:

Dr. Greer: What type of soil was this? There are two things that affect growth in the soil, the living and the non-living, and there is a big difference between culturing in the test tube and in soil. What was the pH of this soil?

Dr. Egeberg: Since Dr. Maddy is not here, I suggest you contact him by letter. In our experience, we have not seen the red soil such as that mentioned by Dr. Maddy.

4. Coccidioidin skin test survey by the Los Angeles County Tuberculosis and Health Association.

J. B. Masters, D. Crummett, R. Gaines and C. Robinson

In 1962 the Tuberculosis and Health Association of Los Angeles County initiated a five-year study to determine the level of coccidioidin skin sensitivity among high school students. The project has two general purposes: (1) to learn the percentage of reactive students in selected geographical areas of the County at a given time; (2) to ascertain the changing rate of infection in certain areas over a period of time. During the first year, eight high schools

in widely scattered regions were chosen to participate. All students with parental consent were given an intradermal test using a 1:100 dilution of Smith antigen 64D4 administered by physicians using disposable syringes. The tests were read 48 hours later by public health nurses. Reactions of 5 millimeters or more were interpreted as positive. Positive reactors received a minifilm chest x-ray at the school. Results of the testing and x-rays were sent to the parents, with detailed findings made available to the family physician upon parental request. Record cards included a brief residence and travel history.

In the first year 11,292 students were tested and read in eight high schools--Banning, Canoga Park, Cleveland, Los Angeles, Antelope Valley, Covina, Palmdale and South Pasadena. The first four are a part of the Los Angeles Unified School District and the others are in separate districts. The average participation was 64.1% with a range from 37.3% to 85.9%. There was a higher degree of cooperation for girls (69.2%) than for boys (59.2%).

The average percentage of positive reaction was 7.5% but the range varied from 5.0% in Banning to 11.3% at South Pasadena. Percentages were higher in boys (8.4%) than girls (6.7%). Sensitivity was similar for the various ages. The majority of indurations were between 5 and 15 millimeters diameter but low-grade reactions under 5 millimeters were present in over one-third.

Forty percent of the students gave no previous residence outside of Los Angeles County, and 30% had lived only in other states in which *C. immitis* is not considered endemic. Most of the students (91.4%) had traveled outside of Los Angeles County at some time and nearly three-fourths (73.7%) mentioned endemic regions or states. The percentage of positive reaction was consistently higher in all schools among students who previously had been in the San Joaquin Valley, Arizona, Nevada, New Mexico, or Texas than for those who had lived or traveled elsewhere. However, reaction percentages at South Pasadena High School were considerably higher for both groups.

(Authors' summary)

Discussion from the floor:

Dr. Egeberg: How qualified were the people giving and reading the tests?

Dr. Masters: Experienced Health Department teams did the testing and reading.



Dr. Newcomer: Beautiful study representing four years of planning. I am puzzled as to why the fourteen year old group has the same rate as the seventeen year old group.

Dr. Masters: Travel might explain age group similarities.

Dr. Hyde: Great mobility means little. We know from our experience at Long Beach that 13-14% of the adult male population here lived in known endemic areas.

Dr. Egeberg: Any idea why the incidence was higher in South Pasadena?

Dr. Masters: The South Pasadena school was selected because it was new and because of a great deal of ground breaking at the school and at a nearby housing tract.

Dr. Riley: Regarding the similarity between the fourteen and seventeen year age groups, one might find a higher percentage of positive reactors in the grade schools. These little people, literally and figuratively, are closer to the soil.

Dr. Locks: One of the problems in coccidioidin skin testing is that you may lose some of the positives when the readings are at 48 hours. Some patients begin to show a reaction at 18 hours, peak at 24 hours, and fade out at 48 hours.

##### 5. Attempts to eliminate *Histoplasma capsulatum* from soil.

C. D. Smith, M. L. Furcolow, and F. E. Tosh

*H. capsulatum* probably does not grow wide-spread in nature but in localized foci. Bird manure seems to enhance the growth of the fungus; the sources of infection for several urban epidemics of histoplasmosis are known to have been heavily contaminated with bird manure. The potential danger of human infection from such foci has been pointed out by recent reports of urban epidemics.

Several attempts to eliminate the fungus from the soil at the source of an epidemic have been unsuccessful. Physical changes such as removal of trees, brush and leaves had no effect in eliminating the fungus. Covering one area of the source with fill dirt reduced the number of isolations for a short time only. The application of four different chemical fungicides failed to eliminate the fungus from the soil.

There may be several reasons for the failure to rid this site of *H. capsulatum*. Since the organism has been found more than a foot below the surface, physical changes on the surface may have little effect on it. The chemicals used may not be effective against the fungus or else were inactivated by constituents in the soil. Perhaps insufficient concentrations of the chemicals were used or insufficient quantities were applied to insure contact with all of the spores. The chemicals may have failed to penetrate to a sufficient depth to reach all the spores.

(Authors' abstract)

Discussion from the floor:

Dr. Salkin: An article in the New York Times stated that there were 30,000,000 histoplasmin positive skin test reactors in this country and 160,000 clinical cases per year. Did you mean these figures?

Dr. Tosh: No. There are possibly one-half million cases.

Dr. Hyde: What happened to the birds in the park?

Dr. Tosh: They left the park when we started clearing the underbrush, and moved to other areas where *H. capsulatum* developed.

Dr. Huppert: Have you ever recovered *H. capsulatum* directly from the birds?

Dr. Tosh: No.

Dr. Kong: Have you considered adding the amphotericin B producing organism to the soil?

Dr. Tosh: No. We have not considered it.

Dr. Brosbe: What were the practical problems involved in the application of chemicals to the soil?

Dr. Tosh: The chemicals applied were inexpensive, but their application required 2 to 3 individuals. It would not be economical.

Dr. Newcomer: Have you tried gas sterilization methods such as those used on golf courses and football fields?

Dr. Tosh: It would have to penetrate to at least a one foot depth.

Dr. Newcomer: I believe it does penetrate that deep.

Dr. Tosh: It is worth trying then.

#### PANEL I - CURRENT STATUS OF LABORATORY METHODS

Moderator: M. Huppert

Panelists: Margaret Biddle  
C. R. Hampson  
D. H. Howard  
R. Huntington

Dr. Huppert: Several weeks ago I notified each of our panelists that we would focus our attention on laboratory problems and on current methods for processing pathological specimens. I will present a question to each panelist in turn and then leave the panel at your disposal for questions and open discussion.

(Question directed to Dr. Biddle)

What is the current status of serology for systemic fungus infection and what are the most frequent problems you encounter?

Dr. Biddle: The studies of C. E. Smith established the correlations between diagnosis and prognosis of coccidioidomycosis, and complement fixation and precipitin tests. These correlations remain valid. With other laboratories performing these tests, problems arise concerning the production of the antigen, critical standardization of the antigen, and interpretation of the titer reported. With histoplasmosis, there is less correlation between complement fixation titer and clinical disease. There are no serological tests in general use for cryptococcosis, candidiasis, or nocardiosis. The acute problems facing us today are: 1) antigen standardization; 2) obtaining geographical histories; 3) interpretation of serological results; 4) lack of specificity of fluorescent antibodies in fungus infections.

Dr. Huppert: (Question directed to Dr. Hampson).

What methods do you prefer to use for the isolation of fungi causing systemic infection?

Dr. Hampson: Much depends on the type of specimen. In general, we streak the material received on Sabouraud's media and Mycosel agar and incubate

at room temperature and at 37°C. The following methods can be used for processing a spinal fluid specimen:

1. Filter through a membrane filter and transfer the filter to a culture medium.
2. Centrifuge and culture from the sediment.
3. Leave the spinal fluid stand at room temperature.

From biopsy or surgical specimens, one should first make wet mounts from several selected areas. Pieces of the tissue should be ground in a mortar with sand and cultures made from the homogenate. The most serious problem is obtaining a satisfactory specimen. We have received specimens to which formalin had been added, and in one case, Clorox was used. If possible, obtain the specimen yourself and use aseptic technique. When material is to be sent through the mails, it is advisable to add anti-bacterial antibiotics. In general, the major problems may be listed as:

1. Obtaining a proper specimen.
2. Receiving a sufficient amount of specimen.
3. Control of contamination.
4. Finding spherules in the specimen but obtaining no growth in culture, as in old nodules.

Dr. Huppert: (Question directed to Dr. Howard.)

What is your approach to identifying a fungus culture with consideration of the possibility of finding pathogenic fungi other than *C. immitis*?

Dr. Howard: Dr. Orda Plunkett taught me to identify the pathogenic fungi by looking at them. There is usually very little difficulty in identifying *C. immitis* when it is isolated from clinical material. Characteristic arthrospore formation, the cardinal feature of this fungus, can be delayed in some cultures and animal inoculation may be of value. We have noted, however, that simply waiting for the arthrospores to appear is as efficient. One must be aware that there is some variability in arthrospore shapes.

Two culture varieties of *Nocardia asteroides* are recognized; an orange to yellow, waxy, cerebriform colony and a chalky-white powdery form.

The latter is similar in appearance to *N. brasiliensis*. The two may be distinguished in that *N. brasiliensis* will hydrolyze casein and liquefy gelatin, while *N. asteroides* does not.

Isolation of members of the genus *Cryptococcus* from sputum presents a perplexing problem. Such cultures which hydrolyze urea and grow at 37°C can be identified as *C. neoformans* only on the basis of animal virulence. One wonders about the surety of such a criterion inasmuch as there is as yet no compelling evidence that strains which lack virulence for experimental animals are also incapable of producing human disease. If we find encapsulated yeasts in spinal fluid we make a direct report of cryptococcosis.

Dr. Huppert: (Question directed to Dr. Huntington)

What are the problems you encounter in the histopathological study of tissues for fungus infection, and what are some of the unusual findings you see?

Dr. Huntington: Contrary to the beliefs of many, morphology is certainly not a dead subject. One may mention four interesting problems now being studied; first, the pathology of amphotericin toxicity; second, pathologic confirmation of clinical diagnosis or suspicion of coccidioidal meningitis; third, the recognition of the mycelial phase of *C. immitis* in infected tissue; and fourth, distinguishing small *C. immitis* from large *H. capsulatum* in arrested primary lesions.

It has recently been shown that irreversible chronic glomerulonephritis may result from prolonged treatment with amphotericin, and we have had the opportunity to corroborate these findings. From the theoretical standpoint, the demonstration of chronic glomerulonephritis referable to a drug is novel and of extraordinary interest. The initial renal lesion of amphotericin is tubular and reversible. However, with long-continued administration, an irreversible nephropathy develops which is characterized by very striking glomerular lesions. Although we originally shared the fear that prolonged intrathecal administration might result in the accumulation of large amounts of the drug along the spinal cord and cauda, with resulting foreign body arachnoiditis, such a process has not been evident in our material.

The clinical diagnosis of coccidioidal meningitis may offer some difficulty when, in the presence of active or disseminated coccidioidal infection, one gets an increase of cells and protein in the spinal fluid without positive spinal fluid cultures or elevated spinal fluid complement fixation. In such cases, one may wonder whether the meningitis is coccidioidal or coincidental and due to something else, particularly if the patient recovers. To get good illustrative blocks of coccidioidal meningitis may be quite difficult,

even when the process was amply identified during life. Leptomeninges may not stick to the brain during preparation unless the meningitis is pretty well advanced. New techniques, such as stripping, rolling, and embedding a lot of pia arachnoid, should be tried for the study of possible coccidioidal meningitis at autopsy.

For careful study of coccidioides and other fungi in tissue, special stains should be used, particularly in caseous, calcified, or cavitary lesions. The Grocott-Gomori methenamine silver stain, though lacking the handsome variegated colors of the PAS stain, is more useful in our experience. What must be assumed to be mycelial phase *C. immitis* turns up rather often in infected tissue, particularly in pulmonary cavities. A characteristic mycelium with arthrospores has to be called coccidioidal if associated with good coccidioidal spherules, or if the culture shows *C. immitis* and nothing else.

The Grocott-Gomori stain shows that small spherular forms of *C. immitis* are even more common in heavily infected tissue than I had realized. Many workers have emphasized the occurrence of large forms of *H. capsulatum*. Thus a good deal of study seems necessary in the attempt to determine whether, in subjects who have resided in both the coccidioidal and the histoplasmal areas, we can decide what peripheral caseo-calcific lesions are due to one or the other or both fungi. Old lesions, particularly if calcified, whether histoplasmal or coccidioidal, frequently give negative cultures, and with many such lesions, if the differentiation can be made, it will have to be from microscopic sections.

#### Discussion from the floor:

Dr. Anderson: Dr. Huntington, can *C. immitis* cells occur in sufficient number in a cavity so that they might be transferred from one person to another?

Dr. Huntington: People are not able to transfer *C. immitis* directly from one to another. The Riverside case reported here last year was a shocker, illustrating what can happen by fomite contamination and transmission. People shouldn't hack on the floor but into cups.

In reference to Dr. Hampson's report, we have found in postmortem studies that where spherules were present in nodules, they failed to grow in culture 90% of the time. How long a time is required before reproduction ceases in these old spherules?

Dr. Salkin: In our VA-Armed Forces Study, there were 112 proven cases of coccidioidal nodules. We have not been able to establish how old they have to be, but many one year old nodules have given positive cultures. Thirty per cent of the nodules contained endospores and hyphal forms.

Dr. Huntington: This shows that one sees only what one is interested in or prepared to accept.

Dr. Newcomer: In a study with rats, we noted that filamentous forms were related to those cells which could, in effect, get away from the host. In cavities, the organisms can get away and they do produce mycelium, but there are so many other fungi that one must question whether the hyphal form he is seeing is actually that of *C. immitis*.

Dr. Reid: We are very concerned about animal disease and the lack of success in culturing lesions. One must be careful not to disregard tissues which may be involved even though they may look clear grossly. Dr. Swartz, in his work with histoplasmosis and coccidioidomycosis in over 100 dogs, cultured hilar lymph nodes even if they did appear normal. Quite a few yielded positive cultures.

Dr. Marcus: Dr. Howard, I have heard about organisms which were morphologically indistinguishable from *C. immitis*, but which were not virulent when injected into mice. Are there any organisms besides *C. immitis* which produce spherules in mouse tissue?

Dr. Howard: Dr. Huppert can answer the first part of the question better than I. In animals *Haplosporangium parvum* can produce spherules. This fungus was found in wild rodents by Emmons. There are some isolates of *C. immitis* that are apparently unable to produce any spherules. Non-budding *Blastomyces brasiliensis* may look like *C. immitis* in tissue under H and E stain.

Dr. Huppert: We have 3 cultures that could be considered as *C. immitis* on the basis of colonial and microscopic morphology. They have never produced disease in experimental animals, but they can be recovered from the animal tissues even 60 days post-infection. What shall we consider to be virulence? Must an organism produce overt disease, or is survival within the animal sufficient? We still don't know whether these 3 strains should be classified as *C. immitis*.

Dr. Pappagianis: Dr. Smith's strain 46 was obtained from a fatal disseminated case and is relatively avirulent for the laboratory animal except when given by intranasal instillation.



- Dr. Wyborney: In San Diego we have had 3 documented cases of histoplasmosis where the fungus was recovered only by animal inoculation.
- Dr. Levine: Strain 46 will produce spherules after intranasal passage through mice.
- Dr. Biddle: Egeberg and Conant have recovered from soil samples strains of fungi which resemble *C. immitis*. These were not active in the complement fixation test and they were antigenically different from known cultures of *C. immitis*.
- Dr. Hampson: Miss Capener, how are your histoplasmosis serologies? What antigen are you using? Are you finding a high incidence in California?
- Miss Capener: C.D.C. antigen and technique. The only incident of histoplasmosis was a case which originated in Mexico and was discovered in California.
- Dr. Hyde: Evaluation of histoplasmosis serology depends on whether a physician does a skin test before drawing a blood sample. If there is a delay of several weeks before obtaining the blood, there may be a rise in titer which is related to the stimulus from the skin test.
- Dr. Spence: Will skin test affect both phases?
- Dr. Huppert: There may be a variation in titer with either antigen, yeast or mycelial phase.
- Dr. Meis: It may remain for 2 or 3 months.
- Miss Capener: Many physicians want to know how often specimens should be sent in.
- Dr. Biddle: In primary pulmonary cases, once every three to four weeks until you get a negative that is continuous. In disseminated cases once every three months is adequate.



## IMMUNOLOGY AND MYCOLOGY

Chairman: Margaret Biddle

6. Two drugs for coccidioidomycosis. Methylene Blue and the sodium sulfate of cinnamic acid.

R. Cohen

Methylene Blue has been used for bacteriological staining, as an antidote for cyanide poisoning, for idiopathic methemoglobinemia, for outlining sinus tracts, for urinary infections, and even for malaria. It is safe to use orally at dosages up to 300 mg. and intravenously up to 1.5 mg. per kg of body weight. It is fungicidal for *C. immitis* at 25 ug per ml. Though it is too early to evaluate clinically, it has been given to rabbits, intravenously and intramuscularly, with ease. Experience with the drug is 88 years old and it may be tried safely in humans if one stays with the therapeutic doses that have been used for years in other conditions.

The sodium salt of cinnamic acid has been used as a test substance in the evaluation of liver function. It can be given at oral dosage of 40 mg. per kg or intravenously at 5 mg. per kg. A 15 per cent solution, both intravenously and intramuscularly has been used without any sloughing. The drug is fungicidal at 6000 ug per ml.

(Author's summary)

7. Studies of *Coccidioides immitis*.

D. Gale, Elizabeth A. Lockhart, Ethel Kimbell, and  
Nancy R. Evans

The dissemination and survival of *C. immitis* in the organs of 4 strains of outbred mice injected intraperitoneally with hog gastric mucin or broth, respectively, has been ascertained with 3 strains of *C. immitis* of known virulence. The strains of mice used are 2 albino strains, one of which was used by Dr. C. E. Smith's laboratory in assaying the virulence of the *C. immitis* cultures, and 2 pigmented strains, 1 pink and 1 brown.

Inoculation of 680 viable particles of C47, a strain of intermediate virulence, with mucin, produced approximately 50% deaths within 48 hours in both pigmented mouse strains, with 20% deaths in 1 albino strain and 9% deaths in the

other albino mice. Dissemination and survival was best in the peritoneal organs, spleen, liver, omentum, and mesentery, and relatively poorly to lung, kidney, heart, and heart's blood. The pigmented mice gave best results. In contrast inoculation of 6 viable particles of C46 and 65 viable particles of C42, both strains of high virulence, produced essentially the same death pattern in 48 hours, but more extensive and rapid dissemination to all organs cultured.

The data support the hypothesis that a toxic component is present in *C. immitis* as shown by the high death rate in the pigmented animals in 48 hours. Experiments are in progress to demonstrate such a component and to ascertain its nature.

(Authors' summary)

Discussion from the floor:

Dr. Walch: Have you ever tried mucin in culture medium? When we used mucin in class, it caused rapid growth.

Dr. Gale: Mucin generally inhibits the growth of an organism.

Dr. Reid: Did the survivors have an increased protection?

Dr. Gale: Yes, they showed some protection which was eventually lost.

Dr. Wallraff: Did you inject mucin in mice without filtrate and was there any protection effect of the mucin alone?

Dr. Gale: Yes, but only for the first few days.

Dr. Levine: Albino mice have a spectrum of susceptibility from about 100 to 500 arthrospores, but some die from 2 or 3 arthrospores. How can you attribute this to pigment? I hope you are not trying to relate this to man.

#### 8. An unusual form of *Coccidioides immitis*.

Leila J. Walker, O. A. Plunkett and M. Huppert

An atypical form of *C. immitis* has been isolated from the Los Banos area of California where several anthropology students had been infected. Three

cultures were recovered from soil and all produced arthrospores in a cluster of hyphal branches which formed acute angles with the main branch. This was compared with the more characteristic strains in which arthrospore-bearing hyphae developed at right angles to the main branch and were distributed evenly throughout the colony.

Variations in color and texture of colonies make these properties inadequate for identification of *C. immitis*. Infection of experimental animals with subsequent demonstration of endosporulating spherules is the most reliable basis for identification of *C. immitis*.

Discussion from the floor:

Dr. Warren: Did you do any serology on the mice?

Mrs. Walker: No.

Question: Did you recover the same culture from the people who were infected from this area?

Dr. Hubberty: We took lots of cultures but did not recover the organism.

Dr. Larwood: Did these cultures kill mice?

Mrs. Walker: No.

Dr. Kong: Were you able to develop spherule forms in vitro with these cultures?

Mrs. Walker: Not yet.

9. Development of fluorescent antibody reagents for the specific detection of *Coccidioides immitis* in clinical materials.

W. Kaplan and Mary K. Clifford

The most accurate method for diagnosis of coccidioidomycosis is the isolation and identification of the causal agent. However, the procedures employed for this purpose are time consuming and entail the use of animal pathogenicity tests. Hence, the direct microscopic examination of clinical specimens is generally carried out to expedite a presumptive diagnosis of this

disease. This examination entails a search for the diagnostic endosporulating spherules of *C. immitis*. Frequently, these or other tissue forms of this pathogen are not found, and elements that are observed may resemble other pathogenic fungi. Such difficulties led us to investigate the possibility of applying the fluorescent antibody (FA) technique to the diagnosis of coccidioidomycosis. For full realization of the diagnostic potentialities of this technique, it is necessary to utilize FA reagents specific for the tissue form of *C. immitis*. The preparation of such products and the results of their preliminary evaluation for diagnostic use are the subject of the present report.

Five different lots of rabbit *C. immitis* antiglobulins were tagged with fluorescein isothiocyanate. All five reagents stained brightly the endospores and contents of spherules formed *in vivo*. The labeled antibodies, however, cross-reacted with *Histoplasma capsulatum*, *Blastomyces dermatitidis* and other heterologous fungi. Adsorption of each conjugate with formalin killed yeast cells of *H. capsulatum* eliminated all heterologous activity. Two of the adsorbed reagents still reacted strongly with the tissue form of *C. immitis*. These conjugates had been prepared from antiglobulins produced by rabbits infected with this fungus and by rabbits immunized with killed arthrospores. The staining titer of the former adsorbed reagent for the tissue form of *C. immitis* was 1:4; that of the latter was 1:2. Adsorption of the remaining 3 reagents resulted in a marked reduction in their ability to stain *C. immitis*. Hence, their residual staining powers for this organism were of too low an order to be of practical value.

A specific reagent of value for practical use could also be prepared by dilution (1:6) of the labeled antibodies produced by the infected rabbits. Specific reagents for practical use could not be prepared by dilution of the other 4 conjugates. This failure was due to the fact that their staining end points for the heterologous fungus, *H. capsulatum*, was as high or almost as high as those for the homologous fungus.

The 3 specific reagents were successfully used to demonstrate *C. immitis* in clinical materials from 8 of 9 confirmed cases of coccidioidomycosis. While these findings are most encouraging, an expanded evaluation is required before the fluorescent antibody technique can be recommended as a reliable procedure for the routine diagnosis of coccidioidomycosis.

(Authors' summary)

Discussion from the floor:

Dr. Warren: Is it possible that the antigenic sites on the walls of the *in vivo* formed spherules were taken up by naturally formed antibody, thereby inhibiting staining by the conjugates?

Dr. Kaplan: Yes, it is a distinct possibility.

Dr. Levine: The walls of the in vitro grown spherules are highly immunogenic.

Dr. Kaplan: Yes. Immunogenicity and the demonstration of antibodies are not necessarily the same.

Dr. Hampson: Do you have trouble detecting the unstained elements?

Dr. Kaplan: We have no trouble seeing them. They are pink-gray in color.

Dr. Newcomer: Are the fluorescent antibodies the same as the complement fixation antibodies?

Dr. Kaplan: I don't know. Serum that has a high complement fixation titer has good staining properties.

(The following paper was inadvertently omitted from the Transactions of the 7th meeting of the VA-Armed Forces Coccidioidomycosis Study Group, 1962.)

10a. Correlations among test results following graded parenteral challenge of guinea pigs with *Coccidioides immitis* arthrospores.

S. Marcus, G. A. Hill and W. Wu

Employing albino (Hartley strain) guinea pigs, weighing 500-750 grams at the start of the experiments, it has been possible to obtain a graded dose-response mortality curve which is dependent on the number of *C. immitis* arthrospores injected intraperitoneally. Furthermore, a dose resulting in death of about 10 per cent of animals within 60 days after i.p. challenge (ca. 100 viable arthrospores) induced a practically uniform degree of skin reactivity (hypersensitivity) in these animals in 30 days. This experimental model was employed to begin explorations of relationships among test procedures usually used to help diagnose coccidioidomycosis and the actual extent of disease in minimally to heavily infected animals. The animals have also been employed to determine the practicability of bio-assay and standardization of coccidioidin and coccidioidin polysaccharide for skin test use.

Complement fixation reactions with serum of all animals that were challenged ( $10^1$  to  $10^4$  spores) reacted with a mycelial phase cell free extract antigen. These reactions, obtained with serum from animals sacrificed

51 days after challenge, were negative with 3 of 3 normals; 1:4 in 3/3 animals challenged with 10 spores, and 1:32 in all other animals ( $10^3$  and  $10^4$  group).

Ouchterlony tests were carried out originally with undiluted coccidioidin and yielded no reactions. When the coccidioidin was concentrated 10 times by evaporation in the cold and used as central well antigen, good reactions were obtained with some but not all sera. The relative value of this procedure with various antigens is being explored.

Skin test results suggested that polysaccharide from coccidioidin, in microgram amounts, is as sensitive a reagent as coccidioidin. *C. immitis* infected animals that showed some degree of cross reaction to histoplasmin gave no response to *H. capsulatum* polysaccharide.

Skin tests on 19 guinea pigs carried out 31 days after i.p. challenge with 100 viable Silveira strain arthrospores in an experiment designed to test for differences in reagent sensitivity yielded the following results: Mean diameter of reaction in mm; Lot 64D3 at 1:100-5.4 (4.4-6.4); Lot UU-3 at 1:100-8.8 (7.8-9.8); polysaccharide 10 ug-9.4 (8.4-10.4); polysaccharide 50 ug-11.0 (10.0-12.0). Analysis revealed that within 95% confidence limits: 64D3 was less sensitive than the other 3 reagents; 64D3 was less sensitive than UU-3; UU-3 was less sensitive than 50 ug of polysaccharide but not less sensitive than 10 ug; 10 ug was less sensitive than 50 ug of polysaccharide.

(Authors' summary)

10b. Rapid production and standardization of coccidioidin  
for skin tests.

S. Marcus, Y. Aoki, and G. A. Hill

Following leads suggested by Pappagianis and by Ajello in the rapid preparation of serologically active antigens, we were lead to devise methods for more rapid production of skin test material. Although all of 4 available strains of *C. immitis* were found to grow well in solid and liquid substrates at either room temperature or  $37^{\circ}\text{C}$ , the work described involved sensitin prepared only from strain Silveira. Shaker culture on modified Sauton's medium incubated at  $37^{\circ}\text{C}$  yielded a highly active filtrate after 21 days. Cultures were then formalinized, checked for sterility after 5 days, filtered and merthiolate (0.01%) added. Guinea pigs, sensitized by a procedure described last year at these meetings, were employed for standardization. A standard U. S. P. bio-assay design, adapted to an IBM computer for calculations, was used. Lot 64D3 (Smith) was employed as standard. Results

were read independently by three observers. The potency ratios obtained were: 3.17 (2.23-6.64), 4.42 (2.61-27.33), 5.49 (2.76-426.01). These results, which are not significantly different, suggest with confidence, that the new lot may be diluted 1:3.17 to 5.49 and then employed like the standard lot.

(Authors' summary)

Discussion from the floor:

Dr. Levine: Do you have any idea of the relative dry weights of the coccidioidins?

Dr. Marcus: No.

Dr. Pappagianis: Dr. Smith has stated that there is no reason to confine the routine skin test concentration to the 1:100 level. Each batch of coccidioidin is carefully standardized and successive lots have been used in the majority of testing for some thirty years now.

Dr. Marcus: My purpose was not to knock the available coccidioidin, but to propose a controlled and reproducible method of standardization.

CLINICAL I      Chairman: W. H. Oatway, Jr.

11. Coccidioidomycosis of the skin: A review.

R. T. Wright

The review deals with actually infected skin and not with such secondary manifestations as erythema nodosum. Although the skin is commonly involved in disseminated cases, it is not commonly seen in the presence of meningitis. A simple classification of skin lesions would be: (1) skin lesions, per se (2) secondary to underlying lesions (3) primary inoculation.

Skin lesions, per se: The commonest is the verrucous granuloma, single or multiple, with especial involvement of face and scalp. Other lesions may be solid, disseminated papular, or papulo-pustular.



Skin lesions secondary to underlying infection: Skin ulcers and sinus tracts may develop from lesions of the subcutaneous tissues, bones, and viscera. The ones most commonly seen are over the joints of the extremities.

Primary inoculation: This is rare and, although a number of cases have been reported, there are 3 cases which are unequivocal. (Case report presented which had been described in the January 1959 issue of Archives of Dermatology).

The criteria for a diagnosis of a primary lesion should include: (1) no history of previous disease (2) a definite break in the skin (3) resemblance to a chancre (4) short incubation period of 1 to 3 weeks (5) positive precipitins (6) positive skin test (7) positive complement fixation test (8) development of regional lymphangitis and lymphadenopathy (9) spontaneous healing.

In all cases, the definitive diagnosis is based upon a positive culture or biopsy.

(Ed: The number of primary cases were under 20 but over 3).

#### Discussion from the floor:

Dr. Salkin: In the Control (1955-1958) group of the Coccy Study Group, there were 43 patients with skin lesions out of 103 disseminated cases. In this group, 5 had only skin lesions (only one primary described by Dr. Wright), 6 had meningitis, 6 had lung lesions, 22 had lung and other lesions, and 4 had miscellaneous non-pulmonary lesions. The racial incidence appeared to be highest in Negroes and Filipinos. The short term prognostic figures (2 to 5 years) show 16% dead, 44% active, 19% quiescent, 21% inactive (see 1962 Transactions). Deaths are due to non-skin lesions.

Dr. Larwood: Kern County General has had two cases of primary skin coccidioidomycosis. Our two residents will give a short summary of these cases.

Dr. Anderson: A Filipino male was admitted with a long history of a granuloma on the left ear. A biopsy demonstrated spherules. Patient was treated with IV amphotericin B. The lesion healed, patient became asymptomatic, and complement fixation became negative.

Dr. Lee: A Negro male was admitted with left eye pain. While working in the fields he was caught in a dust storm and got dust in his eye. Tuberculin and coccidioidin skin tests were negative. He responded to treatment with



scapolamine. At a later date, when picking peaches, his eye was traumatized by a twig, and he developed a granuloma on the iris. Skin tests were still negative, but spherules were demonstrated in fluid from the eye. After a superimposed staphylococcus infection, the eye was removed and spherules were found in the iris and cornea.

## 12. Review of coccidioidomycosis of the bones.

M. Iger and J. Larson

The report included 72 patients seen at the Kern County General Hospital and in private practice.

### Bones involved:

Skull, face, vertebrae, mandible, pelvis, clavicle, scapula, sternum, ribs.  
Upper extremity - humerus, radius, ulna, carpals, metacarpals, fingers.  
Lower extremity - femur, patella, tibia, os calcis, tarsal, metatarsal, toes.

Sex incidence - Female - 26 patients (36%)                      Male - 46 patients (64%)

### Racial incidence:

Male - 53% Negro, 20% White, 14% Filipino, 10% Mexican, 3% Chinese.  
Female - 60% Negro, 22% White, 18% Mexican.

There is a predominance of bone lesions in the Negro.

**Treatment:** It is felt that amphotericin B has decreased the extent and duration of the bony lesion. It is possible that chemotherapy alone may be enough in very early cases, but in advancing lesions one should institute adequate surgical drainage. The most effective therapy is the combined use of intravenous and local amphotericin plus surgery where indicated.

### Discussion from the floor:

**Dr. Salkin:** In the Cooperative Study, the Negro also showed the highest racial incidence of bone lesions. In a group of 44 cases, 9 showed only active bone foci and in the other 35 cases there was also active involvement in other sites (38 lungs, 7 meningitis).

### 13. Coccidioidal meningitis: A review.

J. E. Anderson, H. E. Einstein and Yasue Sawaki

The 12-month period from July, 1962 to July, 1963, afforded an excellent opportunity to study many of the manifestations of coccidioidal meningitis over a short period of time. This was possibly due to the large number of acute coccidioidal infections that occurred in Kern County, California, during this time. Immediately preceding and during this 12-month period conditions were optimal for spread of the disease. The winter months had been unusually wet and were followed by a dry, hot summer. This report is concerned with the diagnosis and treatment of eight cases of coccidioidal meningitis which were managed at the Kern County General Hospital in Bakersfield, California from July, 1962, to July, 1963.

#### Diagnostic observations:

Presenting symptoms suggesting meningitis were present in only one patient, although all patients had some type of symptoms from 3 to 20 weeks prior to the time the meningitis was discovered. Four patients had evidence of extra-pulmonary disease prior to the onset of meningeal symptoms. The coccidioidin skin test was of no value in establishing the diagnosis. Abnormalities noted on the routine chest x-ray were found in all patients. Sputum cultures were positive in all but two patients but no spinal fluid cultures were positive. The serum complement fixation test was positive in all cases with values ranging from 1:32 to 1:512. The precipitin test was negative in all cases except one. The spinal fluid complement fixation test was initially negative in three patients. There was universal elevation of spinal fluid proteins and white blood cells, mainly lymphocytes. There was a first zone colloidal gold pattern in all patients with a positive complement fixation test. The Levinson test was positive when it was performed.

#### Therapeutic observations:

Three patients died eight, four, and five weeks after the initiation of therapy. The surviving patients are all leading normal lives. There is no good indicator of effective therapy and until further knowledge is at hand therapy should be continued indefinitely. There is some question of the necessity for intrathecal amphotericin B therapy, particularly in patients who tolerate intravenous therapy satisfactorily. The toxicity resulting from intravenous administration of amphotericin B has a large range of

individual variation with no clear cut dose relationship. The role of corticosteroids is uncertain, but their use is not contraindicated.

The problems of anemia, hypokalemia and nausea associated with amphotericin B therapy are best handled with blood transfusion, oral potassium supplements and chlorpromazine. Phlebitis can be avoided by the use of small needles, distal veins and the addition of heparin in the intravenous medication.

14a. Bacterial infection in a ventriculo-atrial shunt in a patient with coccidioidomycosis.

A. Davis, J. C. Ramseyer, and J. M. Passman

A 42 year old white male developed a primary infection of coccidioidomycosis in August 1960, with a rapid development of meningitis. Amphotericin B was started about 4 months after the onset and was given intravenously and intrathecally. Since the disease progressed and hydrocephalus developed, a right ventriculo-caval shunt was done with the insertion of a Spitz-Holter valve. (Feb. 1961). The patient did well for 5 months, then developed fever and hepatitis. One year later (August 1962) blood cultures showed a staphylococcus. This was treated with penicillin G, chloromycetin, vancomycin, cloxacillin and oxacillin with no control of the infection. The patient expired October 1963.

Autopsy showed widely disseminated lesions especially in the lungs and meninges with coccidioidal spherules in tissue sections.

The authors collected data on 6 other patients in whom ventriculo-atrial shunts were done and, in only one case, was there suspicion of such a complication but no blood cultures were available. This complication has been described following ventriculo-atrial shunt for other causes of hydrocephalus, but this case appears to be the first one for coccidioidomycosis.

14b. Cortisone and coccidioidomycosis.

H. E. Einstein

The report concerns two teenagers who contracted primary pulmonary coccidioidomycosis while receiving cortisone for other reasons. The first case is a 17 year old boy who was on 60 mg of prednisone daily for acute hemolytic anemia. While on this medication, he developed a large hilar

left axillary node and shotty inguinal nodes. A chest x-ray showed a reticular infiltrate in both lower and right middle lobes. He admitted only occasional coughing. A previous film made in 1959 showed the right infiltrate. Pulmonary function studies revealed a restrictive pattern with an MBC of 53%. A lung biopsy was done with tissue sections showing yeast cells. Culture was negative. The lung tissue was injected into mice intraperitoneally and smear of the peritoneal fluid showed cryptococci which was passed serially to other mice. Fluorescent antibody blood serology for cryptococcosis was weakly positive.

The patient received 1940 mg amphotericin B intravenously and 957 mg by aerosol with no change. When last seen (November 1963) he was still asymptomatic and his x-rays were unchanged.

(Authors' summary)

#### EXPERIMENTAL PATHOGENESIS

Chairman: J. H. Matthews

Dr. Matthews: The next three papers are all related. We will hold the discussion of all three after the third presentation.

15. Immunization against experimental lethal Simian coccidioidomycosis using whole killed and fractionated arthrospores.

J. T. Sinski, E. P. Lowe, N. F. Conant,  
H. F. Hardin and M. W. Castleberry

Aerosol and subcutaneous vaccination with killed arthrospores and subcutaneous vaccination using a boivin-type fraction were compared for their efficacy in protecting Rhesus monkeys against lethal challenge with *C. immitis*. Skin test results, visible at 24 hours by mild erythema and induration, developed only in the animals subcutaneously vaccinated with killed arthrospores before challenge. Challenged animals developed definite indurated skin reactions that lasted 48 hours. Complete protection against lethal challenge was produced in the six monkeys subcutaneously vaccinated with killed arthrospores. Four of the six monkeys in each of the other immunized groups also survived. Only two of the six challenged controls survived. All animals were infected as a result of challenge, but only the animals immunized with the fractionated antigen were free of extrapulmonary lesions.

(Authors' summary)

16. Therapy in experimental coccidioidomycosis.

J. L. Converse, M. W. Castleberry,  
E. M. Snyder and E. P. Lowe

An extension of the work of Campbell and Hill (1959), in which Fungizone, the presolubilized form of amphotericin B (intended for intravenous therapy), was used as an oral therapy for experimental coccidioidomycosis in mice, was initiated to further study dose level, length of treatment, time of initiation of treatment, and comparative histopathological changes in treated and untreated animals. An extensive search also was made for evidence of renal damage or nephrotoxicity resulting from oral use of the antibiotic.

Seventy-four per cent of mice receiving a 10 day oral treatment (7, 14, or 28 mg/kg/day) with Fungizone in their drinking water survived (for 5 months) i. p. challenge doses of 150 or 1500 *Coccidioides immitis* arthrospores, strain Silveira, as compared with 7% of the untreated controls. The treated animals exhibited 50% less histopathological changes than the controls, however, 30% of them still harbored the organism 5 months after challenge. Serial sacrifice of animals from each dose group, at 30 day intervals, revealed an increase in histopathological changes with time, following cessation of treatment.

No significant difference in survival was noted (1) among animals receiving the 3 treatment doses, or (2) between treated animals receiving the low or high challenge dose. Similarly, no difference in amount of histopathological changes was noted among animals receiving the 3 treatment dose levels, however, the pathological involvement was greater in those receiving the higher challenge dose.

Further experimentation included a single dose level (12 mg/kg/day), given for 20 days instead of 10 days, and a single challenge dose (1500 arthrospores) with therapy initiated at challenge, or 5, or 7 days post-challenge. Initiation of the 20 day treatment at the time of challenge with *C. immitis* resulted in almost no evidence of disease and the absence of positive cultures 5 months after challenge. Initiation of therapy as late as 5 or 7 days postchallenge exerted a very beneficial effect on the course of the disease. Per cent mortality and positive cultures of the mice rose in direct proportion to the delay in treatment.

No histological evidence of nephrotoxicity or renal damage was noted, in any of the animals receiving therapy.

(Authors' summary)

17. Coccidioidomycosis: Canine vaccination and therapy studies.

M. W. Castleberry, J. L. Converse, J. T. Sinski,  
E. P. Lowe, S. P. Pakes and J. E. DelFavero

A three-fold study of vaccination and antibiotic therapy in experimental pulmonary coccidioidomycosis of dogs was made to determine: (1) the efficacy of various routes of inoculation of a formalin-killed arthrospore vaccine; (2) the combined effects of vaccination and oral amphotericin B therapy administered immediately following respiratory exposure to *Coccidioides immitis*; and (3) renal damage or nephrotoxicity resulting from oral amphotericin B therapy. Neither of the pulmonary routes of vaccination (aerosol or intratracheal) provided protection against a subsequent respiratory challenge of approximately 80,000 *C. immitis* arthrospores, either singly or in combination with oral amphotericin B therapy (150 mg per day for 20 days following challenge); nor did subcutaneous vaccination or therapy alone. However, 8 of 12 dogs receiving both subcutaneous vaccination and therapy completely resisted the respiratory challenge; the remaining 4 exhibiting very minimal, self-contained disease. Histopathological examination revealed no renal damage or nephrotoxicity in any of the dogs receiving amphotericin B therapy (total dose in excess of 3 gms); their blood urea nitrogen levels remaining within normal limits.

(Authors' summary)

Discussion from the floor:

Dr. Groel: Did you find any appreciable serum concentrations of amphotericin B? In North American blastomycosis cases which responded to 4-6 grams a day orally, there were no detectable serum concentrations. There seems to be a favorable response in animals, but this is not consistent in man.

Dr. Converse: We were unable to find serum levels in the dogs.

Dr. Salkin: Would it be economical to try massive oral doses in humans and is amphotericin B available in sufficient quantity?

Dr. Groel: Yes. We have sufficient quantities of the insoluble oral form as has been used in blastomycosis.

Dr. Cheu: We have given oral doses of 8 grams a day with no adverse effect. Topical treatment of skin lesions has been very effective.

- Dr. Hampson: How long were the monkeys allowed to live after challenge and how much lung involvement did they have? Why did you use arthrospores rather than the more effective spherule vaccine?
- Dr. Sinski: The monkeys were kept for 80 days postchallenge. There were no differences in lung involvement between the several groups. I had arthrospores available, and aerosol is easier with arthrospores.
- Dr. Egeberg: How do you decide the time interval between the immediate and challenge doses?
- Dr. Sinski: These were based on previous work by Cohen and Hardin on mice.
- Dr. Brosbe: What dilutions of coccidioidin did you use?
- Dr. Sinski: We used undiluted coccidioidin on the dogs and the monkeys since dogs do not respond to the diluted antigen. We used our own coccidioidin, not Smith's.
- Dr. Kong: When Freund's adjuvant is used with the vaccine, there is a strong skin reaction.
- Dr. Converse: Exactly what we found in the positive skin test.
- Dr. Kong: We have been doing skin tests in the foot-pads of mice and there is a reaction in 6 to 24 hours with or without Freund's adjuvant.
- Dr. Reid: We are all puzzled about the interpretation of equivocal skin test results. Swartz and Straub sectioned the skin test area from dogs, and were convinced that doubtful tests were true reactions.

18. Onset and extent of immunity in mice induced  
by killed coccidioidal spherules.

H. B. Levine and Yi-chi Kong

The LD<sub>50</sub> value in mice vaccinated intramuscularly with 1.8 mg of formalin-killed spherules of *Coccidioides immitis* and challenged intranasally with arthrospores exceeded 3000. In nonvaccinated mice, the LD<sub>50</sub> value was approximately 50-100 arthrospores. A multiple-dose vaccinating regimen was superior to one in which the same weight of vaccine was administered on one occasion. However, when vaccine was administered on one occasion but was apportioned equally to three different intramuscular sites, the



resulting immunity paralleled that obtained by a multiple-dose regimen. Subsequent to a single dose of vaccine, a strong immune response was observed only after 26 days; immunity to low challenging doses (more than 100 arthrospores) was demonstrated as early as 7 days after vaccination.

The magnitude of the immune response, assessed by the capacity of mice to survive challenge with increasingly severe doses of arthrospores, was also related to the morphological development of the spherule. Internal endospores (non-released) were nonimmunogenic when separated from mechanically-disrupted spherules. However, naturally-released endospores were immunogenic and their efficacy as killed vaccines increased as they, in turn, developed into sporangia which contained endospores. The relationship between the cycle of antigenic maturation and endospore to spherule morphogenesis was apparently associated with changes in the spherule wall which, earlier, was shown to be the primary residence of the sporangial immunogens.

(Authors' summary)

Discussion from the floor:

Dr. Huppert: In your 96 hour preparation of spherules which had recently ruptured and released endospores was any attempt made to separate the wall residues? Have you considered that the low immunogenic response with this preparation might possibly be due to an immune paralysis resulting from excessive amounts of the immunogen present in spherule walls?

Dr. Levine: No. The spherule walls were not separated.

Dr. Kong: We have used purified endospore preparations. It is impossible to separate the spherule walls in this preparation because they disintegrate very rapidly.

Dr. Egeberg: You have done a splendid job pin-pointing the time between inoculation and challenge. Have you used any recently isolated cultures from disseminated human cases? Have you tried different sites for immunization?

Dr. Levine: Thank you. We have tried a culture which came from the soil which was associated with Dr. Winn's disseminated case. The intramuscular and subcutaneous sites were tried and the results were comparable. The intranasal method was not as good, probably because the spherule had difficulty getting into the lung.



PANEL II - CURRENT STATUS OF AMPHOTERICIN B THERAPY.

Chairman: D. Salkin

Panelists: S. Cheu, A. Cohen, H. E. Einstein  
W. Winn and R. Stonehill

Dr. Salkin: Dr. Winn, what are your indications for use of amphotericin B as of December 1963?

Dr. Winn: I can best answer this by giving you an excerpt from my article in the Medical Clinics of North America.

Summary of indications for intravenous and intrathecal amphotericin B

"The indications for intravenous amphotericin B are summarized in Table 2. It must be emphasized in referring to this table that no single clinical or laboratory finding can itself be an indicator for such therapy without consideration of the other factors listed. Each may modify the decision and make it either a matter of urgency, as in impending or full-blown dissemination, or a rather routine procedure in the prophylactic control of acute primary coccidioidal pneumonitis. An overall concept of the problem is required in each instance before committing the patient to a course of intravenous amphotericin B, and especially before setting the total dosage. The factors itemized in Table 2 are the guideposts, any combination of which will, by proper interpretation, indicate the need for such specific antifungal therapy. For example, the persistence of serum precipitins alone, or the existence of a primary coccidioidal infection in a Negro or Filipino, would not, in themselves, be sufficient indication. But, given a Negro patient with primary coccidioidomycosis who is obviously toxic and ill and with a titer of complement fixation already at a 1:64 serum dilution, early intravenous amphotericin B would clearly seem to be needed. A maximal dosage of 5 gm should not ordinarily be exceeded unless the infection is severe or has disseminated. The administration of over 10 gm will gravely increase the likelihood of irreversible and toxic renal changes. The necessity to preserve life, however, would justify committing the patient to some loss of renal function."

"Table 2. Indications for intravenous therapy with amphotericin B in coccidioidal disease.

1. Severe primary coccidioidomycosis with persistent fever, prostration, extending or persisting pulmonary involvement, hilar or mediastinal adenopathy, elevated erythrocyte sedimentation rate, leukocytosis over 15,000 and eosinophilia over 15 per cent.

2. Unstable serology, manifested by:
  - (a) Rising titer of complement fixation (above 1:64 dilution).
  - (b) Persisting precipitins.
  - (c) Incomplete complement fixation.
3. Evidence of spread of the infectious agent from the primary pulmonary focus to other systems, such as lymphatic, cutaneous, skeletal, pleuroperitoneal, cardiac, genitourinary or meningeal.
4. A weak or negative skin reaction to coccidioidin (1:10 dilution).
5. Racial susceptibility: Negro, Filipino.
6. For surgical coverage, given two to three weeks before operation as in removal of large pulmonary cavities, destroyed areas of lung, ruptured cavities with threatened empyema, other excisional or drainage procedures, i. e., removal of infected bone, gonads, lymph nodes, sinus tracts, drainage or abscesses.
7. For prophylactic coverage when primary coccidioidomycosis occurs during pregnancy or when active coccidioidal disease occurs in the diabetic. During administration of corticosteroids."

Dr. Salkin asked the other panelists and all of them had similar general indications.

Dr. Salkin: How much renal damage do you see with amphotericin?

Dr. Einstein: I see kidney damage in every case, but with up to 2 gm one may expect reasonable normal function.

Dr. Rhoades: With patients receiving amphotericin B administration, the inulin clearance is variable. Some patients have glomerular deterioration even after therapy. We have found some evidence of renal damage even after only one gm amphotericin B. With ammonium loading there also is indication of damage. We obtained pre-treatment renal biopsy in four of six patients and they had evidence of renal pathology before therapy. Interpretations of amphotericin B kidney damage must include study of pre-treatment histology.

Dr. Cheu: We had two cases that could not tolerate even 5 mg amphotericin B. It is unpredictable and there is variability in individual susceptibility.

Dr. Winn: We are willing to sacrifice some renal tissue when amphotericin B is needed to save a life.

Dr. Salkin: In supervising the treatment of a fair number of cases, I have not seen the gross kidney damage so many people have described. We tend to use amphotericin B every other day in the average case. We work the dose up very gradually and never exceed 50 mg per dose. The total

dosages per course rarely exceed 2000 mg without a pause before the next course, and we watch the BUN very closely and lower the dose immediately if it rises.

Dr. Salkin: In treating meningitis intrathecally do you prefer the lumbar route, the cisternal route or do you vary them?

Dr. Einstein: I prefer cisternal for three reasons: (1) it may prevent block between cisternal and lumbar areas, (2) putting the drug closer to the lesion increases the concentration at that point, and (3) it is easier to perform and the patient tolerates it better.

Dr. Cheu: I am still old fashioned and, unless there is a block, I prefer the lumbar route. In one patient with an atrial ventricular shunt, putting the drug directly into this area produced a very severe reaction and, in addition, a psychotic reaction of 2 weeks duration.

Dr. Salkin: We have used both approaches, but, in general, we have used the lumbar area in the average case. We have seen no evidence of arachnoiditis and we do not feel that the results are better by the cisternal approach. As a matter of fact, it is very easy, by dissolving the drug in 10 to 20 ml of removed spinal fluid, and reinjecting with a moderate degree of force to thus bathe the cisternal area with the drug very rapidly. This is analogous to the development of a high level of analgesia with spinal anesthesia technique. In one of our cases, we injected 1.6 mg of amphotericin B in the lumbar region. Five hours later, we did simultaneous lumbar and cisternal puncture. The lumbar area showed 6ug/ml, and the cisternal 2 ug/ml. However, the drug was injected very gently without any force. It would seem that the direct cisternal puncture would give a higher concentration of the drug in the first several hours; we need more work on this subject. If it is shown that the difference is significant, then perhaps we should do only cisternal punctures.

Dr. Hyde: Did you have 25 meningitis cases with four deaths?

Dr. Winn: Yes, 21 of 25 survived, and some are still under treatment. The paper will be published soon.

Dr. Anderson: We noticed a complication in four patients receiving amphotericin B therapy. They showed elevated uric acid levels and symptoms of acute gout one month later. After treatment with colchicine and benemid the level came down while they were still receiving amphotericin B. This might be confused with bone involvement.

Dr. Locks; The problem Dr. Davis reported gives insight into the fear of the patient for this type of therapy. When using the A-V shunt you introduce problems particularly the possibility of reseeding the lung from an infected area of the brain.

## CLINICAL II

Chairman: J. Jones

### 19. The pathologic diagnosis of granulomas resected as solitary pulmonary nodules.

J. D. Steele and P. J. Melick

In the VA-Armed Forces Cooperative Study, there were 474 granulomas: 164 histoplasmosis, 122 tuberculosis, 98 coccidioidomycosis, 5 cryptococcosis, and 1 each of actinomycosis, blastomycosis, and aspergillosis. In 82 granulomas, the organism could not be identified because of insufficient tissue or advanced healing.

A pathologic diagnosis is of importance since cultures alone may be inadequate. For example, in the granulomas cultured, positive results were found in 60% of tuberculosis, 5% of histoplasmosis, and 32% of coccidioidomycosis.

The traditional hematoxylin-eosin stain is often inadequate in identifying the organisms in tissues and special stains should be used which include the acid-fast stain for tubercle bacilli and Gomori's methenamine silver (GMS) technique, Gridley stain and the Periodic Acid-Schiff (PAS) reaction for fungi. The results follow.

#### Histoplasmosis

<u>Stain</u>	<u>Total</u>	<u>Pos.</u>	<u>Neg.</u>	<u>Not done</u>	<u>% Pos.</u>
GMS	122	118	2	2	98
Gridley	126	32	58	36	35
PAS	127	24	46	57	35

The GMS stain was most useful and the 2 which were negative were positive on PAS.

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Coccidioidomycosis

<u>Stain</u>	<u>Total</u>	<u>Pos.</u>	<u>Neg.</u>	<u>Not done</u>	<u>% Pos.</u>
GMS	76	63	11	2	83
Gridley	75	60	2	13	96
PAS	78	64	3	10	94

The stains are similar in value. Only one coccidioidal granuloma was diagnosed by culture and not by staining.

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In comparing the cultures of the granulomas and the tissue sections, the following results were obtained.

Tuberculosis: In 61 granulomas cultured, 41 were positive. Of the 20 negatives, 4 were positive on smear and 16 were positive by tissue sections treated with the acid-fast stain. For adequate study, one should therefore do smears, cultures, and tissue stains.

Histoplasmosis: Of the 89 granulomas cultured, 5 were positive, and 84 negative. The value of the special stains is apparent.

Coccidioidomycosis: Of the 68 granulomas cultured, 22 were positive including 1 case where the special stains were negative. In the 46 negative cultures, 42 were positive by the special stains and 4 by the H & E stain.

Conclusion: Although all granulomas should be subjected to bacteriological smears and cultures and the traditional H & E stain, they should also be studied by the special stains mentioned for a high percentage diagnosis. Despite their value, they are not used as widely as they should be.

(Authors' summary)

Dr. Jones: We will hold the discussion of Dr. Steele's paper after the next presentation which also is concerned with surgery in coccidioidomycosis.

## 20. Review of surgery in coccidioidomycosis.

R. T. Cunningham

Surgery is reserved for the small group of patients with residuals of the primary disease and is usually done in the chronic stages of the illness. The surgical lesions include: (1) granulomas or solid lesions, many of which prove to be blocked cavities, or nodules with liquid or purulent centers; (2) cavities; (3) cavities associated with infiltrates, and (4) such complications as ruptured cavity, empyema and bronchopleural fistula.

The indications for surgery are:

1. Granulomas - for diagnostic purposes; enlarging lesions; large size of 4 cm or greater; presence of symptoms.
2. Cavities
  - (1) Hemorrhage - especially when persistent or recurrent.
  - (2) Secondary infection with recurring cough and expectoration.
  - (3) Enlarging cavity.
  - (4) Cavities over 4 cm diameter.
  - (5) Diagnostic.
  - (6) Combined TB and Coccidioidomycosis.
  - (7) Miscellaneous - Flying status in Air Force; certain occupational and recreational habits; travelers.

In reviewing 50 consecutive cavitory cases from private practice, 39 patients had pulmonary resections. Of the 50 cases, only 15 patients (30%) had a symptomatic and diagnosed primary phase; the other 35 either had no symptoms or such mild ones as to be insignificant. Skin test was positive in 84% and the serology was extremely variable. Symptoms occurred with the following frequency: Cough - 20 patients; hemorrhage - 16 patients; cavity rupture - 4 patients; pain - 5 patients; increase in size - 2 patients; difficult diabetes control - 1 patient; and malaise - 2 patients.

The type of surgery performed included: Lobectomy 15, segmentals 18 (single 10, multiple 8), subsegmentals 5 (single 4, multiple 1), and pleural in one patient.

Complications included:

Nodules - a different and smaller series but no complications.

Cavities (1) Mortality - 1 patient (2.5%) - pulmonary embolism.  
(2) Spread of the disease to the contralateral lung following a ruptured cavity. Subsequent healing under amphotericin B.

- (3) Persistent air space and skin sinus tract - cured by thoracoplasty and local amphotericin B.
- (4) Cavity formation in the right apex after resection of a left cavity in a case of bilateral disease. (Ed. note: Is this a complication of surgery or the disease per se?)
- (5) Continued disease in the lobe after a wedge resection for a bleeding cavity.
- (6) Coccy wound infection following a lobectomy. Treated locally with amphotericin B with healing.

Conclusions: With increasing experience, the surgical complications can be reduced to a low level.

(Ed. note: This group of 6 complications forms a rate of 16%; omitting case #4 the rate is 13%. The specific coccy complication rate (4 cases) is 10%. What, no B-P fistula? D.S.)

Panel Discussants: J. N. Briggs, B. H. Evans,  
G. A. Paulsen, J. D. Steele

Dr. Jones: Does the panel agree on the indications given by Dr. Cunningham?

Dr. Paulsen: Yes.

Dr. Evans: Yes.

Dr. Steele: Yes.

Dr. Briggs: Yes, but you have to get rid of the nodule for some employment purposes.

Dr. Jones: People cannot get insurance because of the lesion.

Dr. Paulsen: Reminds me of a young male turned down by the Navy. The nodule was resected and the Navy took him.

Dr. Cunningham; Should we resect a coccidioidal granuloma about the size of a quarter?

Dr. Evans: No.

Dr. Briggs: Yes.

Dr. Steele: If the etiology is unknown, you have to remove the lesion.

Dr. Jones: What do you think of the use of amphotericin B before resection? I have no quarrel with its use in disseminated cases, and if there is no acute infiltration, with good technique, amphotericin B is not required. Use of the drug is not justified because of its toxicity.

Dr. Paulsen: We do not use it routinely for surgical coverage but only when complications are known in advance.

Dr. Evans: I don't use it routinely; only in cases with large cavities and satellite lesions. I don't see that there is much difference now than prior to amphotericin B.

Dr. Briggs: No.

Dr. Steele: No.

Dr. Winn: Not routinely; only in cases that might cross the fissure or produce satellite lesions.

Dr. Paulsen: In thick wall abscess, the drug can't get to the organism.

Dr. Cunningham: I have a case at present with an enlarging cavity and a high titer. He is a Negro male and I did not use amphotericin B. I made a wide excision, and the patient did well.

Dr. Evans: I would have used amphotericin B in low concentrations.

Dr. Jones: Do you wait for amphotericin B therapy in cases with ruptured cavities and empyema?

Dr. Evans: I would not wait.

Dr. Cunningham: We don't see these cases until they have been ruptured a couple of days.

Dr. Evans: I would not wait.

Dr. Jones: During the war a cavity in aviators was an indication for surgery.

Dr. Salkin: Does the Air Force still use it as an indication for surgery?

Dr. Meis: The Air Force has a rule that they may not fly with a cavity.

Dr. Rhoades: This has nothing to do with coccidioidomycosis. This rule applies to any cavities.

Dr. Salkin: I would like to bring up an historical point. It has always been felt that the first resection was done in 1940 by Holman and Smith, but I believe, Dr. Jones, you performed the first resection in April of 1937, and the second was performed by Brunn and Goldman in July 1937.



Dr. Spence: What do you think of a 4 cm cavity that has persisted for 14 months?

Dr. Paulsen: Take it out.

Dr. Evans: Should be removed.

Dr. Briggs: Remove it.

Dr. Steele: I would observe it a little longer.

Dr. Cunningham: Take it out.

Dr. Winn: I believe that we use the term cavity loosely. We frequently include abscesses. The little 2 cm cyst, I'll go along with leaving it alone, but abscesses should come out.

Dr. Salkin: I agree with Dr. Winn. We do use the term loosely.

Dr. Greer: I am interested in whether amphotericin B should be used in preoperative cases. I believe it should be considered on the same basis as in North American Blastomycosis where it is used.

Dr. Marcus: When is drainage and irrigation with amphotericin B used postoperatively?

Dr. Paulsen: No occasion.

Dr. Evans: It might be used in chronic empyema.

Dr. Jones: If there is space left, we use it routinely, but this is poor technique. The drug is irritating. One must be careful in evaluating the effect of treatment.

Dr. Einstein: We had an 18 year old, white, male treated for hemolytic anemia with prednisone. In 8-12 days he developed a large coccidioidal abscess. He received no treatment and in one month the abscess regressed with no interruption of cortisone therapy.

Dr. Cheu: In a draining abscess, I used 50 mg of amphotericin B directly into the cavity and aspirated daily. It helped the patient.

## GENERAL STUDY GROUP SESSION

### A. Formal Reports

Chairman: D. Salkin

#### 1. Status of VA-Armed Forces Coccidioidomycosis Study Group.

D. Salkin

At the present time, my own work is devoted to a review of the "control" patients in the 1955-58 period which we plan to follow indefinitely. This involves the personal review of every chart and every individual x-ray film ever taken, and a request for a recent followup examination and x-ray. The amount of work in this phase and in obtaining the charts is considerable.

In addition, new forms were developed to include additional information with the aid of Mr. Jorgensen of the VA Western Research Support Center at Sepulveda VAH. The 3 forms are registry, disseminated and surgical.

The steps in reviewing the control group are: (1) review of all charts and x-rays as described, (2) correction and completion of old forms and adding new data, (3) copying summaries and pertinent data (surgical sheets, autopsy, pathology, etc.), (4) selection of representative x-rays and making negatives and 8 x 10 inch translites, (5) transposing the data on new forms, (6) putting the data on IBM cards.

At this date, 185 cases were reviewed and 18 were dropped because of inadequate diagnostic data. It may be added that each processed case takes 2 to 5 hours.

#### 2. Time lapse cinephotomicrography of the behavior of *Coccidioides immitis* in vitro.

E. A. Brosbe and Jewell Kietzman

Preliminary cinephotomicrographic observations of the behavior of *C. immitis* in vitro are presented. Enlarged mesenteric nodes of mice infected with strain Silveira served as the source of spherules. Cultures were maintained in a Rose Chamber at 37°C employing tissue culture medium 199 supplemented with 30 to 40 per cent inactivated guinea pig serum. The medium also contained 100 units penicillin and 100 ug streptomycin per ml. Cinephotomicrography was carried out at 8 frames per minute using the 40x phase contrast objective.

The first sequence, a continuous 36-hour recording, shows the development of an immature spherule. The protoplasmic activity is marked and vacuolization is especially noteworthy. Cleavage with endosporulation does not occur. The second sequence, a 9-hour film record, shows a rather thin-walled mature sporangium which appears to rupture. Germination of the endospores is observed.

Modifications and improvement in the culture system used at present are needed. However, results of preliminary trials suggest that time lapse cinemicrophotography may be a useful tool to employ in chemotherapeutic and immunologic studies of *C. immitis* on the cellular level.

(Authors' summary)

Discussion from the floor:

Dr. Huppert: In these preparations have you seen mature endosporulating spherules rupture and release endospores which then germinate?

Dr. Brosbe: No, but we do find endospores with mycelial forms in tissue.

Dr. Huppert: I have never seen endospores germinate through an unruptured spherule wall.

Dr. Levine: We have seen germ tubes coming through the spherule walls of our *in vitro* preparations.

Mrs. Bailey: Hadn't the spherule wall already ruptured when the second film started?

Dr. Brosbe: Yes, we thought it had, but we weren't positive.

Dr. Egeberg: Dr. Plunkett thought that *C. immitis* grew better at night. Is this speeded up 180 times? If so, in one minute, we see three hours of development. Did you make sure the stage was equilibrated as far as temperature?

Dr. Brosbe: No. The temperature in the room was stable.

3. Cooperative project on immunodiffusion as a screening test for coccidioidomycosis serology.

M. Huppert and Johnsie Bailey

Five units are represented in this study: Fitzsimons, Fresno, Los Angeles, San Fernando, and Tucson. A total of 347 patients were studied and of these 28 were eliminated for not fulfilling the criteria which had been established in the beginning of the study. The laboratory at San Fernando was used as the reference and the other four laboratories split their serum specimens, sending one portion to the reference laboratory and retaining the

second portion. Immunodiffusion tests were performed at each laboratory with antigen supplied by the reference laboratory. Complement fixation and tube precipitin tests were done in the reference laboratory.

The results were analyzed for correlation between (1) immunodiffusion and complement fixation tests, and, (2) reproducibility of the immunodiffusion results between laboratories. These are shown in tables I and II.

Table I

Comparison of immunodiffusion and complement fixation results.

Results		Total Cases	Agreement
*ID	*CF		
Neg.	Neg.	276	
Pos.	Pos.	36	312/319 (98%)
-----			
Pos.	Neg.	5	
Neg.	Pos.	2	

\*ID - immunodiffusion test.

\*CF - complement fixation test.

Table II

Comparison of immunodiffusion results between San Fernando VAH and other units

Unit	No. of cases with *ID Pos.		Total Cases	Agreement
	SFVAH	Other		
Fitzsimons AH	0	0	31	31/31(100%)
Fresno VAH	11	9	19	17/19(90%)
Los Angeles VAC	5	6	60	59/60(98%)
Tucson VAH	6	6	6	6/6 (100%)

\*ID - immunodiffusion test

Discussion from the floor:

Miss Capener: Are you using concentrated antigen?

Dr. Huppert: Yes, we concentrate it by ultrafiltration.

Miss Capener: Do you keep the tests four days before throwing them out?

Dr. Huppert: Yes.

Dr. Kong: What are the maximum number of lines, and how many lines of identity do you get? How do these lines compare with rabbit antiserum?

Dr. Huppert: We have seen up to five lines with human serum. We have seen lines of identity between human serum and both rabbit and monkey serum. There is at least one line common to practically all these sera.

Dr. Wallraff: Have you tried 37°C incubation?

Dr. Huppert: Yes. The lines appear slightly earlier than at room temperature, but the final result is the same.

Dr. Marcus: How do you standardize your antigen?

Dr. Huppert: With three sera, a high titer, a low titer, and an intermediate titer.

Dr. Levine: What strains do you use?

Dr. Huppert: We use a group of 24 cultures containing strains isolated from soil, animal and human sources.

Dr. Howard: When using the immunodiffusion test, can we eliminate either the complement fixation test or precipitin test, or both?

Dr. Huppert: The test is used as a screening test for complement fixation positive sera and does not as yet replace the precipitin test. Only positive immunodiffusion or positive precipitin sera need to be titered by the complement fixation test.

Miss Capener: Are you setting up the immunodiffusion test for histoplasmosis?

Dr. Huppert: Yes. But we see very few positives in this area.

#### 4. Coccidioidin skin reactions.

Evelyn B. Wallraff and I. B. Snow

Two coccidioidins prepared by different methods (Smith's Lot 64D4 and Huppert's Lot XVB 52F) were compared for skin test activity by repeated testing in two series of guinea pigs (total 144 test comparisons).

In the first series, litter mates were distributed in three groups. Group 1 received autoclaved mycelial fragments of the M-11 strain of *C. immitis*, Group 2, Huppert's CF antigen mixed with an equal volume of Bacto's complete Freund adjuvant and Group 3 served as controls. The guinea pigs were skin tested with Smith's and Huppert's antigens 8 and 13 days after injection. Readings were taken at 18 hours and 42 hours.

Results of the first series indicated possible immunogenicity of Huppert's coccidioidin when injected i. p. in Freund's complete adjuvant. In the second experimental series, Smith's and Huppert's coccidioidin were compared by injecting each with and without Freund's complete adjuvant.

Results of the guinea pig studies indicate one i. p. injection of coccidioidin in Freund's produces an enhanced skin test response and the skin test activity of coccidioidins prepared under controlled conditions may be directly related to their N content. In both series, 18 hour readings for skin reactions were clearly more sensitive indicators than 42 hour readings.

When Huppert's coccidioidin was diluted so that its N content equalled that of Smith's, skin test reactivity in rabbits and humans was equal to that of Smith's.

Discussion from the floor:

Mrs. Bailey: Did you use a control of uninoculated asparagine broth?

Dr. Wallraff: No.

Dr. Hyde: Have you skin tested the same animal with the same dose of the same antigen.

Dr. Wallraff: That is what we did.

Dr. Hyde: Did you repeat tests in the same site as previously tested?

Dr. Wallraff: We cannot really say. We could not be sure we were getting into the area we previously tested.

Dr. Brosbe: Did you have 18 hour and 24 hour readings and did they differ?

Dr. Wallraff: Yes, the 18 hour readings were good.

5. A comparison of the germination, growth and sporulation of *Coccidioides immitis* on three media.

D. T. Omieczynski, S. W. Becker, and F. E. Swatek

Autoclaved 0.5% yeast extract agar, in these preliminary studies, is very encouraging as a new medium for the primary isolation of *C. immitis* from sputum.

The pour plate technique, using 0.5% yeast extract agar, appears superior to the streak technique, on Sabouraud's agar. The pour plate technique increases the possibility of isolating *C. immitis* by suppressing the over growth with *Candida*. The 0.5% yeast extract agar appears to provide a stimulus for early sporulation and to suppress the growth of *Candida* to isolated and restricted colonies.

1.8% yeast extract agar is an effective medium for the direct isolation of *C. immitis* from human and animal tissues, with a significant saving in time.

1.6% yeast extract agar is extremely useful in the isolation of *C. immitis* from naturally infected soil samples.

Experiments are now under way using yeast extract agar and the pour plate technique, to isolate *C. immitis* and other systemic fungi from the soil.

(Authors' summary)

B. Business session

Chairman: D. Salkin

1. A request from the American Medical Association for a professional report based upon the Exhibit was deferred until the completion of the Control Study.
2. Exhibit: Somewhat revised recently, especially the x-rays. Shown at:
  - (1) American Medical Association, clinical meeting, Los Angeles, Nov. 25-28, 1962.
  - (2) American Industrial Health Conference, Washington, D. C., March 18-21, 1963.
3. Dr. Cheu's bibliography: Suggestion to make it loose leaf and add one sheet per year; also to cross index authors' names.
4. Dedication of this years Conference and Transactions to the memory of Dr. A. L. Ringle - adopted unanimously and resolution sent to Mrs. Ringle. (Dr. Ringle died November 11, 1963.)
5. Reports of the research activity at each unit of the VA-AF Study Group were then given.

OFFICIAL REPRESENTATIVES ATTENDING

David Salkin, M.D., Chairman

Milton Huppert, Ph.D., Secretary

James H. Matthews, M.D., Chief, Research in Pulmonary  
Disease, VA Central Office

Roger O. Egeberg, M.D., Consultant

Henry Jorgensen, Statistical Consultant



UNITS AND REPRESENTATIVES

V.A. Hospital, Fresno, Calif.	Stephen Cheu, M.D. Royal Sorensen
V.A. Hospital, Long Beach, Calif.	E. A. Brosbe, Ph.D. LeRoy Hyde, M.D.
V.A. Center, Los Angeles, Calif.	A. Davis, M.D. Sydney Finegold, M.D. B. Warren, Ph.D. Edwin Wright, M.D.
V.A. Hospital, San Fernando, Calif.	M. Huppert, Ph.D. D. Salkin, M.D. J. D. Steele, M.D.
V.A. Hospital, Tucson, Ariz.	Evelyn B. Wallraff, Ph.D.
Keesler AFB Hospital, Miss.	James Rasch, M.D.
Lackland AFB Hospital, Tex.	E. R. Rhoades, M.D.
Travis AFB, Calif.	H. R. Schumacher, M.D.
Davis Monthan AFB Hospital, Ariz.	Peter R. Meis, M.D.
Fitzsimons Army Hospital, Colo.	C. W. Arrington, M.D.
William Beaumont Army Hospital, Tex.	Alan R. Hopeman, M.D. Paul B. Solnick, M.D. Charles C. Hunter, Jr., M.D.
U.S. Navy Hospital, San Diego, Calif.	W. F. Spence, M.D.
Naval Biology Laboratory, Oakland, Calif.	H. B. Levine, Ph.D. Yi-Chi M. Kong, Ph.D.
U.S. Army Res. & Devel. Laboratory, Md.	John L. Converse E. R. Lowe, Ph.D. J. T. Sinski, Ph.D.

REGISTERED ATTENDANCE

Josef Adriany, M.D., VAH, San Fernando, Calif.  
L. Larry Allen, M.D., VAH, San Fernando, Calif.  
Joseph E. Anderson, Jr., M.D., Bakersfield, Calif.  
Yoduo Aoki, Ph.D., Salt Lake City, Utah  
Clifton W. Arrington, M.D., Fitzsimons Army Hospital, Denver, Colo.  
Dr. M. Barfatani, Los Angeles, Calif.  
Miss Mary Lou Barrett, Berkeley, Calif.  
Marjorie Biddle, Ph.D., Los Angeles, Calif.  
John W. Birsner, M.D., Bakersfield, Calif.  
H. W. Bosworth, M.D., Los Angeles, Calif.  
Miss Jacqueline E. Briggs, VAH, San Fernando, Calif.  
John N. Briggs, M.D., Encino, Calif.  
Edwin A. Brosbe, Ph.D., VAH, Long Beach, Calif.  
Miss Marilyn Capener, Berkeley, Calif.  
Stephen H. Cheu, M.D., VAH, Fresno, Calif.  
David Chernof, M.D., Los Angeles, Calif.  
Mr. Harold Cohen, Los Angeles, Calif.  
Robert Cohen, M.D., Bakersfield, Calif.  
Mr. Darrell Comstock, Oakland, Calif.  
Mr. John L. Converse, Fort Detrick, Maryland  
Duane Crummett, Ph.D., Los Angeles, Calif.  
R. T. Cunningham, M.D., Bakersfield, Calif.  
A. Davis, M.D., VAC, Los Angeles, Calif.  
G. A. Deauville, M.D., Fort Detrick, Maryland  
Nine Djare, Los Angeles, Calif.  
Mrs. Dova, Hollywood, Calif.  
Roger O. Egeberg, M.D., Los Angeles, Calif.  
Hans E. Einstein, M.D., Bakersfield, Calif.  
A. F. Elconin, M.D., Los Angeles, Calif.  
Byron H. Evans, M.D., Fresno, Calif.  
David J. Evans, M.D., Bakersfield, Calif.  
Sydney Finegold, M.D., VAC, Los Angeles, Calif.  
Albert Fink, M.D., VAC, Los Angeles, Calif.  
Mrs. Annie Fry, Los Angeles, Calif.  
Auko Fujiward, Los Angeles, Calif.  
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