

Sum

July, 1968

No. 6

Progress Report
of the
Veterans Administration - Armed Forces
Coccidioidomycosis Study Group

EDITORS

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The 12th Meeting
of the
VA-AF Coccy Study Group

Last year's meeting was considered so successful that we patterned this year's meeting after it. It was held one day prior to the meeting of the California Thoracic Society (CTS) and ran from 9 to 5 with a continual high interest to the very last minute. All arrangements (room, projectors, coffee) were supplied to us by the Tuberculosis and (NOW) Respiratory Disease Association of California (TRDAC). The room was comfortable and the "school-room" arrangement and informal dialogue were conducive to a relaxed but alert atmosphere.

Although it is too early to be absolutely certain, it appears that next year's meeting will be in San Diego at the El Cortez Hotel (?) on the following days:

Tuesday, April 15	Our VA-AF Study Group
Wednesday, April 16	The Smith Conference
Thursday, April 17	CTS and TRDAC
Friday, April 18	" "
Saturday, April 19	" "

During the CTS business meeting held Friday, May 3, 1968, it was decided by the CTS members that there should be no more concurrent medical meetings and that the Smith Conference (to include all mycoses) should be a full day's duration.

We have heard much praise about our agenda, so we will reprint it. Unless other suggestions are forthcoming we may have a similar one next year. Dr. Finegold chaired the CDC session and Dr. Leroy Hyde chaired the USC session. The few business items were sandwiched between the learned dialogues, and time was left for only two study unit reports.

VA-AF Coccidioidomycosis Study Group
Program for May 1, 1968

DEL WEBB'S TOWNHOUSE
Fresno, Calif.

8:30 a.m. Registration

9:00 a.m. Welcome by Dr. J. W. Murdoch, Jr.,
Director, VAH, Fresno.

a.m. Scientific Session

12:00 to 1:30 p.m. Intermission

p.m. Scientific Session

Reports from Study Units

Business

SCIENTIFIC SESSION

1. What are the best laboratory procedures for the diagnosis of systemic mycotic diseases? This will be a review of the work being done at the National Communicable Disease Center (NCDC), Atlanta, Georgia. The discussion will include Coccidioidomycosis, Histoplasmosis, Cryptococcosis, Blastomycosis, Actinomycosis and Nocardiosis. Coordinator: Ajello
2. Research spectrum at the University of Southern California. Coordinator: Biddle
3. Panel (Open discussion).
With the newly developed serological tests in the past several years, there is a need to know their general usage, how they compare with the older traditional tests and their clinical significance. Is there a need for a cooperative study to relieve our anxieties?
Coordinator: Salkin
4. Panel - Studies with Coccidioidins M, S, E, (Mycelia, Spherules, Endospores). Discussion leaders: Huppert, Levine, Pappagianis

STRUCTURE OF THE STUDY GROUP (1968)

<u>Study Units</u>		<u>Representatives</u>
1. VA	Fresno	Cheu, Sorensen
2. "	Long Beach	Hyde
3. "	Los Angeles	Davis, Finegold, Wright
4. "	San Fernando	Huppert, Salkin
5. "	Tucson	Wallraff
6. USN	San Diego Hospital	Bloom, Reid
7. "	Oakland Biol. Lab.	Levine, Sippel
8. USA	Fitzsimons	Cowley
9. "	Fort Detrick	*
10. USAF	Davis-Monthan	DeSando, McKay, Thimmesch
11. USPHS, NCDC,	Atlanta	Ajello
12. " , NIH,	Bethesda	*
13. Univ. Calif.	Berkeley	**
14. " " Davis		(Pappagianis)
15. Univ. Southern California		Balchum, Biddle, Brown
16. San Diego State College		Walch
17. Kern General Hospital		Einstein, Hampson, Larwood

Officers

Chairman	-	David Salkin, M.D.
Secretary	-	Milton Huppert, Ph.D.
Statistician	-	(Henry L. Jorgensen, Western Research Support Center)
Consultant	-	(Roger O. Egeberg, M.D.)
Central Office	-	(James H. Matthews, M.D.)

Guests - Omieczynski, Sun, Tu, Ruth Walch

* Status in doubt.

** UC Berkeley Coccy Laboratory transferred to UC Davis.

(---) Absent at this meeting.

Business Transactions

1. Lapsed Memberships.

- (1) Lackland AFB - No representation for several years.
- (2) U.C. Berkeley - Laboratory transferred to U.C. Davis.

2. Memberships in Doubt.

- (1) NIH, Bethesda - Have not heard of their continuing interest. Will wait another year.
- (2) Fort Detrick - Have heard indirectly that the Coccy program has been cancelled.

3. New Membership

The Kern County General Hospital Group was voted in as full-fledged members. This is a powerful group that has contributed much to the literature in the past and (of course) will continue to do so. The Bakersfield group will include Doctors Anderson, Einstein, Hampson, Holeman, Huntington, and Larwood (Coordinator). They should add considerably to our clinical and pathological knowledge.

4. Dr. Ajello informed us that there are still available the now-classic Proceedings of the 1965 Phoenix Meeting on Coccidioidomycosis. Soft covered issues are available free of charge; write to Dr. L. Ajello, Mycology Section, National Communicable Disease Center, Atlanta, Georgia 30333. Hard covered books particularly suitable for libraries are \$12.00; order from the University of Arizona Press, Tucson, Arizona.

5. The Transactions of the 10th Annual Smith Conference at Palm Springs, held in April 1967 are now available. Write to us and we will see that you get your copy from the Tuberculosis Association.

6. The Bibliography

Stephen Cheu gave us handouts of the 1965 and 1966 and a partial 1967 bibliography of publications to add to our master list. If you need any more copies, or even a copy of the master list, write to him directly: Dr. Stephen Cheu, Veterans Administration Hospital, Fresno, California. By the way to make the bibliographical list, send him a reprint of each of your coccy publications.

7. The Scientific Session

The word is OUTSTANDING! We kept no special notes and must rely on the abstracts handed us by the discussants.

8. It is with great regret that we announce the retirement of our secretary, Mrs. Cleo McCubbin, in September 1968. She is the one responsible for the sense of order in our office, the power behind all of our activities and the one who sends you all "my" nice letters and communications. Thank you, Cleo! Enjoy your "retirement" but don't work too hard.

Scientific Session

1. What are the best laboratory procedures for the diagnosis of systemic mycotic diseases? A review of the work being done at the National Communicable Disease Center (NCDC), Atlanta, Georgia. The discussion will include Actinomycosis, Blastomycosis, Coccidioidomycosis, Cryptococcosis and Histoplasmosis.

Discussant: Libero Ajello Chairman: Sydney Finegold

Unfortunately, Doctors William Kaplan, Leo Kaufman and Lucille K. Georg could not attend and their work and his own was reviewed by Dr. Ajello. Information on the characteristics of these microorganisms and on detailed procedures for isolating and identifying them can be obtained by writing to Dr. Ajello. In the following high-lights we have concentrated on the more recent information.

Actinomycosis

The 3 common pathogens are A. israelii, the one usually found in man; A. propionicus, described in 1962 and probably more common than the 5 human cases described to date would indicate, and because it can be distinguished from A. israelii only by immunological and biochemical means; and A. bovis, the common cause in animals but not found in humans. For the primary isolation enriched media and anaerobic culture methods must be employed. Recent information places the actinomycetes closer to bacteria than to fungi. In differentiating the 3 pathogens and the saprophytic A. naeslundii, the methods of value relate to morphology, biochemistry animal inoculation, and immunological procedures. Antigenic differences are present and both agar gel precipitin and FA tests are used but reagent antigens and antisera are not yet available for general distribution. Lucille Georg expects much more accurate serological tests in a year. There is no routine test for the detection of antibodies in patients' sera.

Blastomycosis

The term "North American" is no longer applicable for describing the disease caused by Blastomyces dermatitidis. Recently autochthonous cases have been diagnosed in several African countries, and NCDC prefers the term "Blastomycosis". The source of infection is not known, although a few reports of recovery from soil suggest this as the natural habitat for B. dermatitidis. Both man and animals are susceptible to natural infection, but there is no evidence for transmission from animals to man or man to man. Intradermal and serological tests are not reliable. Complement fixation tests are positive in less than 50% of proven cases and the interpretation of a positive result is complicated since B. dermatitidis antigens frequently react with sera from histo and coccy patients who do not have blasto. Some of these problems may be resolved in the near future by current studies with a specific fluorescent antibody reagent and with immunodiffusion technics.

Cryptococcosis

This disease causes an average of 66 deaths per year in the U. S. A. The number of cases of cryptococcal meningitis is estimated at over 200 annually. The number of cases infected is unknown and the possible existence of a benign form of the disease is unknown due to the lack of a specific skin test antigen. C. neoformans exists in nature as a saprophyte and has been repeatedly isolated from the soil, especially from pigeon and other bird habitats. The yeast cells are disseminated by winds and are inhaled by humans and other animals. There is no known transmission from one person to another or from animals to man. The organism is not a part of the normal flora of the body. The differentiation of C. neoformans from saprophytic cryptococci requires cultural, biochemical and animal methods. The most important recent developments include 3 tests of diagnostic and prognostic importance: The indirect fluorescent antibody test of Vogel (IFA), an agglutination test for antibodies by Gordon and Vedder, and a latex slide agglutination test for antigen in body fluids by Bloomfield, et al. The IFA test is not entirely specific but is particularly valuable where the other tests are negative. The other tests are specific. Kaufman and Blumer studied 60 proven cases: 50% were detected by the IFA test, 43% by the tube agglutination test, and 45% by the latex test. The 3 tests combined show a 92% diagnostic accuracy. These studies establish that contrary to earlier impressions, C. neoformans does stimulate antibody production in man, but that in some cases the patient is saturated with antigen.

Histoplasmosis

The definitive diagnosis rests on the isolation and identification of H. capsulatum from pathological specimens. A series of 6 daily, early morning specimens is generally sufficient. Recommended media are: brain heart infusion agar with chloramphenicol (0.05 mg/ml) and cycloheximide (0.5 mg/ml); brain heart infusion agar with 6% blood; Sabouraud dextrose agar. The blood agar should be incubated at 37°C and the other media at room temperature. The serological tests of value are: complement fixation (CF); agar gel immunodiffusion (ID); latex particle agglutination (LPA); fluorescent antibody inhibition (FAI). The CF test has been most widely used, but it is a complex and expensive procedure requiring highly trained personnel. Accurately standardized yeast phase and mycelial phase antigens must be used. Crossreactions may occur with sera from cases of other mycoses and from apparently healthy persons. Titers of 1:8 or 1:16 with either antigen are presumptive only, while titers of 1:32 or higher suggest infection. A series of specimens during the course of the disease can be most valuable since a 4-fold change in titer in either direction is a significant indicator of disease progression (increasing titer) or regression (decreasing titer). In disseminated cases, a state of anergy frequently occurs. The ID test is very useful as a screening procedure. A positive result is presumptive evidence. The presence of both "m" and "h" bands has diagnostic value. The LPA test is an excellent aid for the diagnosis of acute histoplasmosis but has lesser value for chronic disease. A titer of 1:32 or greater is strong evidence for active or recent infection. The histoplasmin used for skin testing can act as a booster injection in hypersensitive individuals. When blood for serology is obtained 2 weeks or more following a positive skin test, the serum may show an increase in titer (CF or LPA) or a conversion to positive in the ID test in the absence of change in clinical disease. The FAI test, and also the ID test, have shown excellent correlation (97%) with results of the CF test (using the yeast phase as antigen). A fluorescent antibody reagent specific for H. capsulatum has been used for detecting fungus cells in pathological specimens. Kaufman and Blumer of NCDC have reported 5 serotypes among cultures of H. capsulatum. This has important implications for future serological work in histoplasmosis.

One important feature! There is a rumor accredited to Charlotte Campbell that, if the histoplasmin for skin testing is heated to 60°C for 10 minutes, it will not affect the skin reaction adversely and will eliminate serological sensitivity. Huppert wrote to Campbell for verification, and she replied that it is not so. One should draw blood for serology prior to skin testing.

Fluorescent Antibody Techniques (FA)

This very useful technique is being adapted rapidly to successful application in the mycoses. We have summarized the current status with the following very brief notes.

Blastomyces dermatitidis: Specific fluorescent antibodies have been developed by Kaplan and Kaufman, enabling rapid and accurate detection in cultures and clinical materials.

Candidiasis: Although non-specific conjugates are used in productive investigations, there are as yet no specific tests for identification of *Candida* species.

Coccidioidomycosis: Specific conjugates are available for the tissue form of *C. immitis* and for the detection of antibodies in serum with a 90% agreement of the FA and CF tests. Studies are in progress to develop immunofluorescence techniques to detect *C. immitis* in soil.

Cryptococcus neoformans: A recently developed conjugate by Pidcoe and Kaufman is highly specific in lesion exudates and tissue sections and with many isolates of *C. neoformans*.

Histoplasma capsulatum: A specific test is now present and (again) developed by Kaufman and Kaplan.

Paracoccidioides brasiliensis: Specific test available.

Sporotrichum schenkii: Very reliable FA test now available which has a 90% agreement with cultural methods in lesion exudates.

NOTES

(1) The NCDC needs serum from proved cases of actinomycosis. Send to:

Libero Ajello, Ph. D.
Chief, Mycology Section, Laboratory Program
National Communicable Disease Center
Atlanta, GA 30333

Telephone: 404-633-3311

(2) If you need serum for cryptococcal studies, communicate with Dr. Andrew Foder of the NCDC who will send you some from the serum bank.

(3) For any details regarding the work at NCDC or for procedures and media recipes, communicate with Dr. Ajello.

(4) The fluorescent antibody reagent for staining C. immitis in tissue is available to state health departments and federal health agencies. The conjugate can be obtained from the Biological Reagents Section of the NCDC, catalogue number F2-1005-30. Each vial contains 1 ml of lyophilized reagent.

2. Research spectrum at the University of Southern California

Chairman: Leroy Hyde, M.D.

Only Doctors Balchum, Biddle and Brown were present. We missed Doctors Egeberg, Elconin, Levan, Korn and Butt. The research spectrum includes:

- (1) Egeberg and Elconin - ecologic studies.
- (2) Biddle - serological studies.
- (3) Levan and Korn - the lymphocyte transformation test.
- (4) Butt - Pathogenetic studies in monkeys.
- (5) Balchum - skin test surveys of youth camps in S. California.
- (6) Brown - Treatment of coccy meningitis.

Dr. Balchum reported on the skin test conversion rate to coccidioidin among probation groups, in the L. A. County Work Camps. These included 715 males, 14-18 years of age. The 54 boys assigned to Camp Scudder in the Saugus area had the highest conversion rate (26%). There were other camps in the vicinity with much lower rates. It was concluded that the field area in which the boys worked was the important factor, rather than the camp site in which the boys lived. The discussion focused on posting sites known to be infectious, the possible use of vaccination for these groups, permitting only skin test positive individuals in these areas, and certain legal aspects.

Dr. Brown reviewed the cases of Coccy meningitis encountered at U.S.C. and the special therapeutic problems they presented, particularly the evolution of new lesions during the course of chemotherapy.

3. Panel: With the newly developed serological tests in the past several years, there is a need to know their general usage, how they compare with the older traditional tests and their clinical significance. Is there a need for a cooperative study to relieve our anxieties?

Open discussion. Moderator: Salkin

The commoner tests in question include:

CF	-	Complement fixation
TP	-	Tube precipitin
ID	-	Agar gel immunodiffusion
LPA	-	Latex particle agglutination
FA	-	Fluorescent antibody
IEP	-	Immuno-electrophoresis
AGPI	-	Agar gel precipitin inhibition test of Ray

There was much discussion and all agreed that a cooperative study was indicated. The chairman dictatorially appointed Dr. Huppert as acting chairman to investigate the organization of and participation in a cooperative study. It was agreed that participation would be limited to those stations which would be able to fulfill the requirements of an established protocol.

4. Panel: Studies with Coccidioidins M.S.E. (Mycelia, Spherules, Endospores)

Discussants: Levine, Huppert

In the first place, Levine suggested that, since the letters are cumbersome and forgettable, we should call them simply mycelial, spherule or endospore coccidioidins. Accepted!

The original coccidioidin still in use and made by Smith is of mycelial origin. The investigation of the others is going on at present and some of the findings are most interesting. Current studies include the potential use of these spherule and/or endospore coccidioidins for serology and skin testing.

REPORTS FROM THE STUDY UNITS

Time permitted only two reports, from San Diego State College and from Fitzsimons. However, we will include those that could not be given. Those given at the Smith Conference will be abstracted under that heading.

San Diego State College

Strains of C. immitis have been produced with double nutritional markers and with variable virulence for guinea pigs and mice. Some single back mutations have been found, but there have not been any back mutations for both nutritional markers. These strains are being used to investigate the question of parasexuality in C. immitis and several stable heterokaryons have been obtained. Strains with low or with no demonstrable virulence for experimental animals are being studied for potential use as a viable vaccine. Protection against heterologous strain challenge has been demonstrated.

H. Walch

U.S. Army, Fitzsimons

Soluble Antigen Fluorescent-Antibody Technique Applied to Coccidioidomycosis
Presented by Ray Cowley

Sera from patients with coccidioidomycosis can be tested for specific antibody against coccidioidin using a modified Soluble Antigen Fluorescent-Antibody (SAFA) Technique¹. This is an indirect fluorescent antibody test localized on cellulose polyacetate membrane filter paper discs (1/4" diameter) and objectively quantitated by means of a fluorometer. Relative amounts of IgG, IgA, and IgM can be determined simultaneously which in turn may indicate the stage of infection. The SAFA results are directly related to results obtained by complement-fixation, agar diffusion, tube precipitin and immunoelectrophoresis techniques. Cross reactivity with other fungus infections has been found to be lower with the SAFA technique than with complement-fixation tests.

James W. Gautsch

¹ Toussaint, Andre J. and Anderson, Robert I., 1965. "Soluble Antigen Fluorescent-Antibody Technique". Applied Microbiology 13:552-558.

Fresno, VAH

Cheu - Continuing studies on the Bibliography of Coccy. Please send your reprints to him, large or small, important journal or otherwise (who knows which is important), paper or abstract.

Cheu - Beginning a study on the compensability of Coccy. He states that "our hospital has had about a dozen patients who have received compensation by either the Workmen's Compensation Board of Appeal or by litigation. As a result, the VA was also able to receive a claim for treatment."

(Ed. A similar study is being started by Einstein and Levan of Bakersfield.)

Sorensen and Cheu - Electrophoresis of spinal fluids.

Using acrylamide gel electrophoresis, a study of CSF and serum proteins has been started to determine if specific protein bands in these cases can be found. Preliminary efforts suggest that such bands may occur.

Sorensen - Some observations on the Ecology of C. immitis.

When growing C. immitis on Sabouraud's dextrose agar on plates in such a way that an agar portion of the plate occupies one-half of the plate and a wet cake of a soil sample the other half, the coccy growth was affected differently by different soil samples. Soil from non-coccy sources developed bacterial growth extending over the surface of the agar, preventing the growth of C. immitis. On plates with coccy-yielding soils no such aggressive occupation occurred. Instead C. immitis grew freely over the agar surface and eventually moved on to the soil surface itself. Spherules may develop in the margin of C. immitis colonies when the fungus is grown adjacent to several bacteria isolated from the inhibitory soils.

Sorensen - An unusual Variant of Coccidioides:

A strain of Coccidioides was recovered from a subcutaneous abscess in which it appeared in the mycelial phase. This strain did not stimulate production of complement-fixing antibodies as expected in disseminated disease, and it failed to grow in the presence of sodium borate. These very important differences raise questions regarding the classification of the organism.

Los Angeles VAC

Wright - Comparison of Intradermal Coccidioidin Cutter Antigen (1:100) with Lederle Coccidioidin Tine Test (1:150).

A total of 137 subjects were tested. The results follow:

<u>Cutter</u> <u>Intradermal</u>	<u>Lederle</u> <u>Tine</u>	<u>Number</u>
Neg.	Neg.	103
Neg.	Pos.	2
Pos.	Pos.	20
Pos.	Neg.	12
		<u>137</u>

Using the intradermal test as a true standard, the Tine Test showed 1.9% false positives (2/105), and 37% false negatives (12/32). The Tine Test is therefore not recommended for clinical use.

San Fernando VAH

Huppert and Peterson in collaboration with Levine and Pappagianis - Sera from vaccinated and non-vaccinated mice were prepared in Levine's laboratory. These were coded and sent to Huppert's laboratory where ID tests with mycelial phase antigens were performed. After completion of the tests, the specimens were decoded and it was found that sera from all non-vaccinated mice were negative while all those from vaccinated animals were positive. The experimental design was repeated with sera obtained by Pappagianis from vaccinated and non-vaccinated humans. The same test failed to differentiate between the two groups.

Huppert and Peterson in collaboration with Levine and Pappagianis - Levine made several coccidioidin preparations from the spherule phase of C. immitis and one from the endospore phase of C. immitis. These were tested by ID in the labs of Pappagianis and Huppert against sera from the human volunteers who had been vaccinated. Two of the spherule coccidioidins reacted with all sera, including those from non-vaccinated subjects. The other antigen preparations also failed to differentiate between sera from vaccinated and non-vaccinated groups. ID tests designed to match the reactions of the several antigen preparations for identity or non-identity revealed (1) at least two antibody responses which were different from those found with mycelial coccidioidins; (2) the spherule coccidioidins, within the limitations of the ID test, did not contain the F antigen (that which correlates with results of the CF test); (3) the endospore coccidioidin did contain the F antigen.

Huppert, Sun and Tu in collaboration with Dr. Klaus Schwarz (VAH, Long Beach) - Schwarz has been studying selenium organic compounds for metabolic effects. These have been made available for testing for antifungal activity. Preliminary studies indicate that some of these selenium compounds are fungistatic not only against C. immitis but also against many of the other known fungus pathogens. Homologous series of these compounds are being synthesized to be used in studies directed toward determining the mechanism of action and the molecular structure associated with the antifungal property. From this information it may be possible to predict the molecular composition which will produce the maximum antifungal activity.

Sun and Huppert - Mating studies have been attempted with the typical and atypical strains of C. immitis. These have been unsuccessful up to the present time. Recently, Brosbe reported the induction of spherule formation in C. immitis when cultures were grown on agar media. We have confirmed this observation and, with slight modifications in technic, have induced spherule formation in a number of strains. The procedure is being adapted further for use in studies on morphogenesis in C. immitis.

Salkin - Continuing studies on the control group of over 700 patients and their long-term follow-up. We have recently had the preliminary data completed and put on IBM cards. Imagine our indignation when we checked the cards and found a number of errors. Imagine our embarrassment when on further checking we found most of them were our own. We hope to have the data processed in the near future.

Tucson VAH

Wallraff - Recent Developments in Serological Methods for Diagnosis of Coccidioidomycosis. A slide latex agglutination test and an immunodiffusion test were employed as screening tests for split specimens of serum from suspected cases of coccidioidomycosis. If positive by either screening test, an Agar Gel Precipitin Inhibition Test (AGPI) was performed. Tests on approximately 120 consecutive serum specimens over a period of two years indicate that the results of the three more recent serological tests correlate well with those of the established tube precipitin test and the complement-fixation test reported from a reference laboratory.

THE CHARLES E. SMITH CONFERENCE

Unfortunately, due to a misunderstanding, only an afternoon was allotted to this conference and it ran concurrently in competition with the general thoracic session. It was later voted by the CTS that a full day will be devoted to coccy and other fungus diseases. We did very well at the conference and counted 60 to 75 in attendance at all times. The program follows:

1. Coccidioidomycosis in Children in Tucson - McKay
2. Evaluation of a Coccidioidin Tine Test - Wallraff, O'Bar, Wachs, Snow
3. Lymphocyte Transformation Test in Coccidioidomycosis - Kelley,
Stanfield, Dukes, Parsons
4. Effect of Cyclophosphamide on Experimental Coccidioidomycosis -
Landau, Shechter, Dabrowa, Newcomer
5. Disseminated Coccidioidomycosis in Renal Transplant patients
Receiving Immunosuppressive Therapy - Davis, Klasky, Koppel
6. Histoplasmosis: Recent Developments in Epidemiology and Control -
Tosh, Chin
7. Pulmonary Cryptococcosis: Should Amphotericin B Therapy be
Used - Perkins
8. A Blastomycosis Survey in California - Casad, Sorensen
9. Panel: Ask the Laboratory Experts - Huppert (Moderator)
Ajello, Biddle, Hampson, Huntington, Levine
10. Panel: Ask the Clinical Experts - Salkin (Moderator)
Cheu, Einstein, Evans, McKay

IN ADDITION: Two papers which could not be accepted because of lack of time.

1. Coccidioidomycosis in the VA Hospitals in New York City -
Netzer, Littman
2. Case report of a Coccidioidal Meningitis treated with an
Ommaya Valve - Escovitz

A summary of the papers we have on hand follows.

Summaries of Papers From The Smith Conference

1. Coccidioidomycosis in Children in Tucson. B. McKay

(Summary not received.)

2. Evaluation of a Coccidioidin Tine Test as a Skin Testing Technique.
Evelyn B. Wallraff, Ph.D., Paul R. O'Bar, M.D.,
Erhardt E. Wachs, M.S., and Isabelle B. Snow

A coccidioidin Tine test has been evaluated by comparison of skin reactions induced by intradermal injection of 0.1 ml of Lot 64D4 coccidioidin 1:100 with those induced by Tine test material supplied by Lederle Laboratories. Volunteers among the hospital staff (approximately 250) have been given the intradermal test on one arm and the Tine unit on the other arm. Readings of erythema and induration for each test site were recorded in mm at 24, 48, and 72 hrs. after skin testing. Results indicate that although it has certain practical advantages, the Tine test is less sensitive in detecting positive reactors.

3. Lymphocytic Transformation Test in Coccidioidomycosis.
Janet B. Kelley, B. S., A. B. Stanfield, M.D.,
C. Dean Dukes, Ph.D., and James L. Parsons, M.D.

Antigenically specific transformation of peripheral lymphocytes in vitro is a useful indicator of immunologic reactivity. Lymphocytes from coccidioidin sensitive patients exhibited blastogenesis and increased DNA synthesis following exposure to Huppert's coccidioidin. In contrast, no significant response occurred with lymphocytes obtained from skin-test negative individuals prior to skin testing. An increase in blastogenic and DNA synthetic activities of peripheral lymphocytes were observed following skin tests.

4. Effect of Cyclophosphamide on Experimental Coccidioidomycosis.
Joseph W. Landau, M.D., Yaakov Shechter, Ph.D.,
Nina Dabrowa, M.S., and Victor D. Newcomer, M.D.

The exact factors determining resistance to coccidioidomycosis are not established. Drugs with immunosuppressive activity are now used extensively in clinical medicine. Limited information is available

concerning their influence on coccidioidomycosis. This report presents an evaluation of the effect of cyclophosphamide on the course of experimental systemic coccidioidomycosis. Cyclophosphamide has a significant adverse effect which is influenced by the relationship between the time of cyclophosphamide administration and the time of establishment of infection. Various studies were performed in an effort to elucidate the mechanism of action of cyclophosphamide in coccidioidomycosis.

5. Disseminated Coccidioidomycosis in Renal Transplant Patients Receiving Immunosuppressive Therapy. Alvin Davis, M.D.,
I. Klasky, M.D., M. Koppel, M.D.

Two post-renal transplant patients have developed disseminated coccidioidomycosis at Wadsworth VA Hospital. The first patient, a 48-year old Caucasian male, was thought to have had coccidioidomycosis ten years ago. He received a cadaver transplant on April 2, 1967, and made a satisfactory postoperative recovery, receiving azathioprine and prednisone in moderately high doses. The patient was discharged from the hospital on immunosuppressive medication on May 27, but he began to develop proteinuria and azotemia during the month subsequent to discharge. He was readmitted to the hospital on July 31, in congestive cardiac failure, with evidence of graft rejection. During the subsequent two-week period he received radiation therapy and the prednisone dosage was increased to 200 mg per day for a short period, and then tapered. On August 13 the patient became febrile, and a chest x-ray two days later showed a pulmonary infiltrate. By August 18, chest x-ray showed a diffuse miliary process and the patient died that evening. Autopsy revealed generalized coccidioidomycosis.

The second patient, a 29-year old Caucasian male, had no prior history of coccidioidomycosis but he had lived in an endemic area for many years as a child. He received a cadaver transplant on October 29, 1965, and received azathioprine and prednisone subsequent to transplant with occasional rejection reactions which responded to temporary increase in prednisone dosage plus irradiation. Despite a persistent serum creatinine level between 4 and 5 mg%, the patient remained active, continued working, and in June, 1967, started on a vacation trip through areas of the southwest and Mexico, known to be endemic for coccidioidomycosis. The patient developed severe, pleuritic right-sided chest pain on June 28, and became febrile by July 12. Chest x-ray on that date showed an infiltrate on the right and questionable consolidation on the left. By July 24, x-ray findings had progressed to a bilateral, multiple nodular process, and because of

rapid progression of his disease, an open-lung biopsy was done on July 26. This revealed coccidioidomycosis and treatment with amphotericin B was started promptly. The patient then developed a meningitis but with continuing treatment he did well and has been discharged to his home. He is now being treated and followed as an ambulatory patient.

(Ed.: Dr. William Haas of Los Angeles VAC told us that up to January 1968, the literature in USA recorded 615 renal transplants with 302 successful takes, or 49%. So we may expect more such cases of coccy, and TB and others.)

6. Histoplasmosis: Recent Developments in Epidemiology and Control
Fred E. Tosh, M.D. and Tom D. Y. Chin, M.D.

Several outbreaks of histoplasmosis have resulted from work on the sites of old blackbird roosts. Investigation of these outbreaks revealed that workmen on the site were at greatest risk of infection, although many individuals living or working near the site were infected, probably due to air-borne dissemination of spores. These observations led to an investigation of blackbird roosts to determine (1) the frequency of contamination of soil under such roosts with Histoplasma capsulatum, (2) if positive roosts were responsible for infections as evidenced by increased histoplasmin prevalence, (3) if infectiousness of a roost could be related to the number of viable particles in the soil, and (4) if decontamination of positive roosts would eliminate the danger of infection.

A survey of 32 roosts in Arkansas and Missouri revealed 9 (28 per cent) positive for H. capsulatum. Five of the positive roosts were located in urban areas where large populations could possibly be exposed to wind-blown spores of the fungus. Histoplasmin skin test surveys among school children in two communities having positive roosts and in two communities not having roosts revealed significantly higher histoplasmin prevalence in the communities having positive roosts. Also, histoplasmin prevalence was highest among children residing near the positive roosts.

The number of viable particles of H. capsulatum per gram of soil in samples from different areas of the same roost were only slightly different; however, there was a wide variation in the number per gram of soil in different sites.

Two sites responsible for outbreaks of histoplasmosis have been decontaminated with 3 per cent formalin solution with apparent elimination of H. capsulatum from these sites. Yearly histoplasmin skin testing of

school children is being conducted around one of these sites. In 1965, 33 per cent of the children were positive to histoplasmin, but in 1966 and 1967 this had decreased to 31 and 26 per cent, respectively. These data suggest that decontamination of urban sites contaminated with H. capsulatum is an effective control measure.

7. Pulmonary Cryptococcosis: Should Amphotericin Therapy be Used?
Woodbury Perkins, M.D.

Nine surgical cases were divided into 4 groups:

(1) Unilateral disease - No amphotericin B used and all did well. There were 4 such cases and the oldest case is 14 years postoperative.

(2) Unilateral disease not suitable for resection but a pleurectomy was done because of pleural fluid with a good result.

(3) Diffuse bilateral disease - One case had over 3 gms amphotericin B, a second case also had 3 gms amphotericin B but died 14 months later from progressive disease.

(4) Bilateral, localized, resectable disease - An illustrative case was one where a left lung lesion cleared considerably with potassium iodide but, when it was stopped, the lesion recurred 7 months later. The patient could not tolerate amphotericin B, surgery was done but the patient died postoperatively.

Discussion was held regarding the indications and contra-indications of using amphotericin B, the use of iodides, and the tendency for recurrence after many years.

8. A Blastomycosis Survey in California. Donald E. Casad, M.D.
and Royal H. Sorensen, B.S.

A survey was made by questionnaire of 1142 physicians and hospitals with an 87% response. In all, 37 cases of Blastomycosis were treated in California. Three had inadequate data for analysis, one was an accidental self-inoculation, and the other 33 cases were traceable to the known endemic areas.

9. Coccidioidomycosis Acquired in Southwestern U. S. and Encountered in VA Hospitals in the New York City Area - Sol Netzer, M. D. and M. L. Littman, M. D.

Analysis of cases of coccidioidomycosis seen in all parts of the United States usually reveals that the patient had previously resided in or travelled through an endemic area of the southwest. Of the sporadic cases of coccidioidomycosis encountered in VA hospitals in the New York City area, it was possible to elicit a history of past residence in or travel through the southwest in every instance. Of the 9 cases of coccidioidomycosis recorded, all were in males, 7 were pulmonary and 4 of these patients had cavitory disease. Two patients had coccidioidal osteomyelitis and one patient developed coccidioidal meningitis. One patient with coccidioidal osteomyelitis had been undiagnosed and untreated for 9 years after his initial exposure. However, the usual period of time between the initial exposure and admission to a VA hospital varied from 6 months to 5 years. Coccidioidin skin tests were positive in 7 of the 9 cases, complement fixation tests were positive in 4 and Coccidioides immitis was isolated from 5 of the cases. Four patients received amphotericin B therapy and an equal number received surgical treatment. The patient with coccidioidal meningitis died.

10. Treatment of a Patient with Coccidioidal Meningitis through a Functioning Ommaya Valve W. E. Escovitz, M. D.

This 42 year old Caucasian developed signs, symptoms and laboratory tests diagnostic of coccidioidal meningitis about June 1965. On August 17, 1965 a Richam valve (an Ommaya-type valve) was inserted into the right cerebral ventricle. Treatment with amphotericin B was given intravenously, intraspinaly, intracisternally and through the Richam valve. No cerebrospinal fluid could be obtained from the Richam valve at any time. The complement fixation studies of blood and cerebrospinal fluid continued to be significantly positive despite treatment and the patient's physical condition worsened. An Ommaya valve was inserted into the left cerebral ventricle on April 4, 1967. The patient was subsequently treated with amphotericin B solely by the intraventricular route. Thereafter, remarkable clinical and serological improvement occurred.