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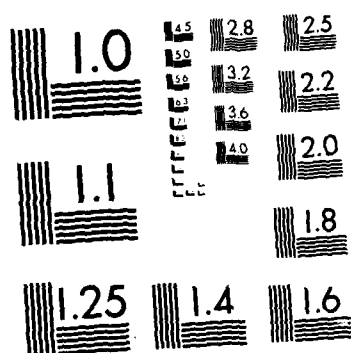
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19. ABSTRACT (Continue on reverse if necessary and identify by block number) In California, most of the old-time pioneers and many of their children and grand-children recount episodes of a disease once referred to as "soil-breaking fever". They recall that one to four weeks after a severe dust storm, or after plowing the sun baked soil, illness sometimes occurred and victims suffered a prolonged flu-like episode. Occasionally, deep seated disease or death terminated this unhappy respiratory experience with San Joaquin Valley Dust. The immigrants recognized that the illness was one that did not occur in their former abodes. They coined the terms "Valley Fever" and San Joaquin Valley Fever" to describe it; the lexicon also included "Desert Rheumatism" and "Desert Fever". Ecologic studies have not determined clearly the reason for the preference that Coccidioides immitis has for the San Joaquin Valley, the Southwest of the United States and specific locations in Mexico, Central America and South America. The fungus resides only in soils of the New World and its failure to spread beyond its well defined boundaries is a mystery.					
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THE SIXTH IAN MURRAY MEMORIAL LECTURE

Spores, Dust and Valley Fever

H. B. Levine



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BRITISH SOCIETY FOR MYCOPATHOLOGY
The Sixth Ian Murray Memorial Lecture

Spores, Dust and Valley Fever

by

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Sheffield, England
March 28, 1983

I recall discussing coccidioidomycosis with Ian Murray in 1963 and 1964, during visits to Britain. We shared pleasant and stimulating times, exchanging ideas on the role of immunity in fungal disease. Dr. Murray's death in 1971 occurred during the very year Dr. Gene Scalarone and I published on the induction of optimal protective responses in the mouse. I'm honored to dedicate this lecture to Dr. Murray's memory, at a time when we're seeking to determine the extent of such responses in human beings. I thank Dr. Evans warmly for inviting me and the British Mycopathologic Society for the opportunity to speak to you on this subject.

In California, most of the old-time pioneers and many of their children and grand-children recount episodes of a disease once referred to as "soil-breaking fever". They recall that one to four weeks after a severe dust storm, or after plowing the sun baked soil, illness sometimes occurred and victims suffered a prolonged 'flu-like episode. Occasionally, deep seated disease or death terminated this unhappy respiratory experience with San Joaquin Valley dust. The immigrants recognized that the illness was one that did not occur in their former abodes. They coined the terms "Valley Fever" and "San Joaquin Valley Fever" to describe it; the lexicon also included "Desert Rheumatism" and "Desert Fever".

Ecologic studies have not determined clearly the reasons for the preference that Coccidioides immitis has for the San Joaquin Valley, the

Southwest of the United States, and specific locations in Mexico, Central America and South America. The fungus resides only in soils of the New World and its failure to spread beyond its well-defined boundaries is a mystery. After a violent wind storm, in the San Joaquin Valley in 1978, Coccidioides did produce infections in non-endemic areas. These included San Francisco, Oakland, Monterey, and a host of locations hitherto free of the fungus, but the organism did establish itself in the soils of these regions.

Aridity, sparsity of nutrients, the absence of microbial competitors, the occurrence of divalent cations in the soils, seasonally high temperatures --- all of these have been studied to understand the organism's geographic tastes. All of them appear to be important, but the determining critical features of Coccidioides' ecologic preferences still remain unclear. The fungus has, on occasion, been recovered from rather damp locations, not characterized by alkaline soil or the absence of bacterial competitors. Yet other soils, seemingly replete with all the features we think Coccidioides appreciates, do not harbor the organism, even in locations close to those where the organism is found.

In its most gentle form, coccidioidomycosis is symptomless. Approximately 60% of all coccidioidal experiences fall into this category. Such episodes are detected when an individual acquires delayed dermal sensitivity to spherulin or coccidioidin in the absence of any illness. The remainder of coccidioidal infections usually causes moderate to

severe pulmonary disease; approximately one-quarter of such patients require medical attention. In 3% of all coccidioidal experiences, very prolonged chronic sequelae, sometimes leading to death, ensue. Extrapulmonary involvement is common in severe disease; it occurs in 0.5 to 1% of all infections. The striking public health importance of these figures becomes clear when it is appreciated that between 50% and 80% of residents in the areas of Bakersfield, California, Phoenix, Arizona, and El Paso, Texas, react to spherulin and coccidioidin. Approximately 85,000 conversions to skin reactivity occur annually in the United States with an associated 35,000 symptomatic coccidioidal experiences of which about 8000 are serious. The numbers of deaths seldom exceed 70 each year in the United States.

Transmission of coccidioidal infections by fomites is a problem of world-wide significance. We are aware of fomite-mediated infections in Ireland, England, and on the European Continent. Additionally, large numbers of visitors to the Southwest United States, to Yosemite National Park, to the lovely desert wonderlands of Arizona and New Mexico sometimes return home with problems unheard of in their native lands. Recently, a culture isolated from an American traveler in Vienna produced serious coccidioidomycosis in a Viennese hospital technician who was unfamiliar with the fungus.

At the turn of the 20th century, William Ophuls demonstrated the fungal etiology of coccidioidomycosis. Prior to Ophuls' work, the disease

was thought to be caused by a protozoan similar to one causing coccidiosis of chickens. The error was understandable, because the coccidioid spherule resembles parasites of the genus Coccidia. The first episode of human coccidioidomycosis was described by Posadas in 1891, who concluded that his patient, Domingo Escurra, died of coccidiosis. The diagnosis of coccidioidomycosis was made about 54 years later when Flavio Nino of the University of Argentina came upon the preserved remains of the patient. Histologic examination showed clearly that Escurra's lesions harboured the fungus, Coccidioides immitis.

The second patient was Jose Texeira Pereira, who died in 1895, again of what was thought to be coccidiosis. Rixford, Gilchrist and Thorne treated the third patient: Joas Furtado Silveira, who had earlier emigrated from the Portuguese Azores to a small town near Bakersfield, California. The date was 1893 and when the spherule form of Coccidioides was seen in biopsies of his skin lesions, Rixford and Gilchrist, familiar with the earlier studies, naturally assumed the etiology to be protozoan. Ironically, they thought the fungus cultured from his lesions was a contaminant.

We now pass over other important events in the history of coccidioidomycosis to recount findings that bear more directly on present-day research. In 1936, Myrnie Gifford reported that Valley Fever patients developed a skin sensitivity reaction when a Coccidioides-derived antigen (coccidioidin) was injected intracutaneously.

Her studies, as well as clinical surveys in the Bakersfield area by others, pointed to seasonal variations in the incidence of disease. A hot, dry summer preceded by a wet winter had ominous implications. The fungus grew readily in the soil during a damp winter and subsequent disarticulation of the dry mycelial chains of arthroconidia brought problems.

Dr. Gifford's skin test studies, covering a 35-year period, revealed that there were benign cases of coccidioidomycosis, even symptomless, in addition to those that were fulminant. In general, when the fungus was contained within the lungs, eventual recovery could be anticipated. If extrathoracic dissemination had occurred, the prognosis was more grave. Gifford also found that dissemination ratios among persons of Mexican, Black, and Philippino ancestry were higher than such ratios among those of European descent and the resultant mortality picture was in accord with the dissemination profile. More recently, Pappagianis has observed that Asians also show a greater propensity than whites to suffer disseminated coccidioidomycosis. In California's multi-racial society the problem of Valley Fever was therefore very important and had always attracted the attention of dedicated research-oriented physicians and scientists.

Perhaps the most prominent name in the history of coccidioidomycosis is that of Charles E. Smith. He had begun his medical career at Stanford University near Palo Alto, California, and

continued it at the University of California, Berkeley. His interest in the epidemiology and ecology of coccidioidomycosis moved him to spend almost as much time in the San Joaquin Valley as the Bay Area. He made such frequent trips to the Valley that his colleagues referred to his automobile as "The Flying Chlamydospore". Dr. Smith's skin testing surveys with an improved coccidioidin showed the close relationship between acquired delayed dermal sensitivity and a coccidioidal experience. He determined that the incubation period ranged from 7 to a maximum of 28 days, and he demonstrated the diagnostic values of the complement fixation test in the disease as well as the usefulness of the precipitin test in establishing the time that an infection was contracted. Precipitins disappeared in most patients within a matter of weeks after the onset of symptoms and so their presence indicated that an infection was of recent onset. Complement fixing antibodies tended to persist once they arose and Smith found that a declining titer was reassuring and a rising complement fixation titer was alarming.

During World War II, the Armed Forces of the United States became intimately aware of Valley Fever when illnesses arose among airmen at Mintner Field (Bakersfield, California), and at Basic Flying Training Fields in California's San Joaquin Valley. It was then that Dr. Smith, with the collaboration of the Western Flying Command (later called the West Coast Training Center), instituted procedures to spread heavy oil on training fields. Coccidioidomycosis had now become a military problem as well as a civilian public health problem. Dr. Smith was

consulted because Air Force personnel, needed urgently overseas, were being incapacitated on the training fields of California and Arizona. Smith did all that was possible at the time. He knew that the offending fungus was in the soil and that dust, raised by aircraft or sports, or even the most gentle of winds, was laden with arthroconidia. The solution, to oil the dusty fields and to ban certain sports, had unhappy ecologic and morale ramifications but it was effective.

After the War, Dr. Smith was concerned primarily with the clinical manifestations of the disease, its control by new drugs, and the concept that a vaccine could be effective. This consideration arose indirectly from his studies on skin testing antigens. He had observed that if an individual had had a coccidioidal exposure, even an asymptomatic exposure, one detected by delayed skin sensitivity, then later symptomatic disease seldom occurred. He inferred that even a modest encounter with the fungus induced strong immunity. He and Dr. Demosthenes Pappagianis, Dr. Lorraine Friedman, and Dr. Lee Gordon began a program on vaccination that has continued until this day at the Naval Biosciences Laboratory in Oakland, California.

Several years before his untimely death in 1967, Dr. Smith and I discussed the possible reasons that experimental vaccines in animals had not been as effective as might have been anticipated from the epidemiologic findings in man. I suggested that perhaps the form of the fungus that was employed to vaccinate animals was at fault. Formalin-

killed mycelia and arthroconidia had been used because both are easy to prepare. What was overlooked, in immunologic and physiologic contexts, was that the spherule and the endospore growth phases of the dimorphic agent are the structures that colonize the host. Within a matter of hours after arthroconidia produce a lesion, they undergo striking morphologic alterations. They become round, as distinct from the barrel-shaped appearance they exhibited when the mycelium disarticulated. In several days they enlarge and develop internal particles, endospores. The subsequent course of an infection, both in animals and in human-beings, is characterized by the presence of spherules and their released endospores, the agents of metastatic disease. To be sure, mycelial strands are often seen within the lesions, but the numerically dominant form is the spherule-endospore phase of the fungus.

We proposed to Dr. Smith, in 1959, that perhaps the vaccine should be comprised of spherules and endospores. Earlier, John Converse had developed a chemically defined medium that supported spherule growth. The medium consisted of ammonium acetate, glucose and mineral salts. We modified it somewhat to accommodate our strain Silveira and, in time, were able to prepare very pure spherule harvests. The first experiment was exciting: All mice vaccinated with formalin-killed spherules and endospores withstood challenging doses up to 318 arthrospores given intranasally. Most of the control animals succumbed to a dose of 79 arthrospores. Mycelial and arthroconidial

vaccines, killed by formalin and used at the same doses as the killed spherule-endospore vaccine, were relatively ineffective.

As experience and familiarity with the vaccine increased, successively more and more potent batches were produced. At the present time, the vaccine protects all mice against challenging doses of 1000 to 5000 arthrospores, given intranasally. The majority of non-vaccinated animals or placebo-treated animals succumb to doses of 50 to 70 arthrospores.

The vaccine had a marked capacity to preserve life, but it did not prevent infection. Virtually all of the vaccinated animals that survived severe challenging doses continued to harbor Coccidioides for years. What they had acquired was an increased capacity to contain the fungus within the lungs and to restrict its multiplication considerably. Dissemination was infrequent. In time, the fungal burden in the lungs tended to lessen, but complete eradication seldom occurred.

Studies covering a 15-year period showed that the immunogens resided almost exclusively in the spherule wall. The spherule structure was immunologically more efficacious than the endospore, but the latter afforded very measurable protection. Unlike many bacterial and viral vaccines the spherule vaccine induced immunity quite slowly. It became maximal at 60 days post-injection. This late response may have been associated with the chitinous structure of the spherule wall; perhaps

mammalian chitinases are so inactive that immunogen release from the wall matrix is very slow. In respect of this hypothesis, it was shown that the vaccination site in a mouse could be removed surgically after more than 30 days, ground finely, and used to immunize a recipient mouse successfully.

It was also found that when the vaccination site was surgically removed within 30 days, immunity waned. This was not the case when the vaccine depot was removed at 62 days. Studies now in progress suggest that variable early immunity may be related to T-cell suppressor and T-cell activator components of the spherule wall. The activator components appear to establish the dominant picture over a period of time, but soon after vaccination, at least with ground spherules, suppressor activity may occur.

During the studies on immunity development in mice, we noted that not all of the animals that had been vaccinated acquired delayed dermal sensitivity to coccidioidin. Only about 60% did. Nevertheless, those animals that did not become dermally reactive were as well protected as those that did. The first hypothesis was that the animals were, in fact, appropriately stimulated but that the reaction was not manifested peripherally in the skin. A second hypothesis, that coccidioidin was inadequate to elicit the reaction in all animals, was explored.

We digress momentarily from the vaccine to discuss skin test reagents because of the importance of delayed sensitivity in the immunology of coccidioidomycosis. Coccidioidin is prepared from the mycelial phase of growth. Our experience with mycelial and spherule vaccines suggested that a spherule coccidioidin might be more efficacious than mycelial coccidioidin. Earlier, Dr. Yi-Chi Kong demonstrated that such a product had skin-test eliciting properties but was somewhat toxic. We altered the methodology and produced a non-toxic spherule reagent which is now in use for human beings. Dr. Kong's designation "Spherulin" was kept. Spherulin often does detect human reactors unresponsive to coccidioidin but occurrences of the converse situation are now documented. It also elicits sensitivity reactions in vaccinated mice where coccidioidin fails to do so.

Returning to the theme of the vaccine, we believed, in 1963, that studies in mice and parallel studies in cynomologous monkeys, sustained Dr. Smith's hypothesis that immune responses to Coccidioides were profound and life-saving. We also believed that the vaccine could be considered for use in a human trial. Dr. Smith and I decided to take the first step: we injected it into the deltoid muscle of each other. Swelling developed at the site of vaccination but, after 3 and 4 doses, we felt that the vaccine was reasonably well-tolerated.

Other brave volunteers in our laboratory then came forward, six of them, and they too were vaccinated with gradually increasing doses of

killed spherules. In all instances, they tolerated the doses well but sore arms were not uncommon between the third and sixth days after injection. Lest those transient marks be their sole record, we note here with thanks, all of their names: Leonard Goldberg, Melvin Hatch, Nelson Newton, Hyman Wolochow, John Eiselein and Jack Campbell.

Dr. Pappagianis extended the study by vaccinating volunteers who were non-laboratory workers. With very few exceptions, the local tenderness was tolerable. Some of these volunteers were inmates at a Correctional Facility in Vacaville, California. Dr. Pappagianis enjoys reminding me that one of the volunteers later escaped; an unusual property of the vaccine, he comments.

We had contemplated the possibility of a vaccine trial in a human population as early as 1965. Also, we had been urged along this course by several physicians in Arizona and California whose practices included many patients with coccidioidomycosis. However, funds for such a trial were difficult to obtain. Additionally, the mills of the gods grind slowly and Investigational New Drug status for the vaccine was granted by the Food and Drug Administration only in 1977. Then, thanks to Dr. Paul Williams at Lemoore Naval Air Station, in California, a preliminary safety trial of the vaccine was conducted with U.S. Navy volunteers. Dr. Williams' study was in collaboration with numerous individuals who deserve recognition here for their contribution: Mr. Stephen P. Sorgen,

Dr. David L. Sable, Dr. Stephanie K. Brodine, Dr. Byron W. Brown, Dr. F. Carl Grumet, Dr. David Massa, and Dr. David A. Stevens.

One hundred and fifty one healthy, skin-test-negative adult volunteers received the vaccine or a placebo. The responses we were concerned with related to tolerability and safety. The vaccine was given as three intradeltoid doses over an 8-week period. There were no severe systemic reactions but individuals who had received 3.5 mg doses had severe local reactions. The dose was then reduced to 1.75 mg. Half of the vaccinees only converted their skin reaction to spherulin. Delayed sensitivity persisted for at least 6 months. However, almost all of the vaccinees developed augmented T-cell responses and showed boosting of lymphocyte transformation activity in vitro. Dr. Williams concluded that a regimen of three 1.75 mg doses was safe and immunogenic.

After Dr. Williams' report was filed, the Food and Drug Administration permitted us to extend the trial. However, once again, the trial was held back for lack of funding. At this juncture, Dr. Ralph Cunningham, Dr. Royce Johnson, Dr. Tom Larwood, Dr. Hans Einstein, and others from Bakersfield, approached their State Assemblyman, Mr. Don Rogers. Mr. Rogers convinced the California State Assembly that such support was warranted and money was made available. It was appropriated under California State Assembly Bill No. 2969, signed by Governor Jerry Brown on August 30, 1980, and a trial was organized.

Money from industry was also obtained later. The study is randomized and double-blind and is still underway. There are no results to communicate to you, but the aims of the study may be of interest.

Three thousand volunteers will receive either vaccine or placebo; there will be approximately 1500 in each group. All of them will be skin-test negative persons between the ages of 18 and 55 who reside in zones endemic for coccidioidomycosis. Females anticipating pregnancy within 3 months of vaccination will be ineligible. Over a three-year period the volunteers will be checked medically whenever any symptoms reminiscent of coccidioidomycosis occur. After the third year, an evaluation will be made on the adequacy of the vaccine. The earlier work in animals indicates that the vaccine will not prevent infections. What we do hope is that the serious sequelae of infections will be prevented. It is clear therefore that the randomized, double-blind procedure is essential because evaluations on the severity of illness will be necessary.

A trial of this nature cannot be accomplished without the help of many physicians, (all of whom have volunteered their time and effort) and medically-trained personnel. In addition to those mentioned earlier in conjunction with obtaining funds for the vaccine study, and who also help in the vaccination clinics, there are other participants who carry the burden in Bakersfield; Tucson, Arizona; Visalia, California; and Lemoore, California. Among these are Dr. Ross Hampson, Dr. Richard Whitfield, Dr. Larry Borgsdorf, Dr. Wes Holeman, Mrs. Rose

Tessandori, Mrs. Martha Falgatter, Dr. John Galgiani, Dr. Scott Tidball, Mrs. Jean Higgins, R.N., Mrs. Cathy Thiroux, R.N., Mrs. Julie Nesbitt, R.N. and Mrs. Joanne Walker Nichols, R.N. At this juncture, approximately 2,000 individuals have been injected with vaccine or placebo and the long follow-up period has begun. In 1986, we should know if the effort has produced a preparation to help human beings or if it only protects California's mouse population.

My job is to prepare the vaccine. The burden of coordinating the trial rests on the capable shoulders of Dr. Pappagianis. The development of the vaccine is hardly the work of a single person or group. We acknowledge with warm affection the contributions made during the years by the late Dr. C. E. Smith, Dr. Yi-Chi Kong, and, above all, Mr. James M. Cobb. We are encouraged that, whatever else, the studies have led to a wider awareness of coccidioidomycosis than was the case a decade ago. This has attracted people from industry who now understand the mycologic problems of our area. In particular, I close by mentioning the names of Dr. Jo Brugmans and Dr. Paul Janssen who directed much of their company's efforts into a study of new drugs for the therapy of the deep mycoses. They helped me to look at experimental therapy in addition to prophylaxis and to participate in the pre-clinical evaluation of new imidazoles in coccidioidal disease.

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