HUMAN COCCIDIOIDOMYCOSIS[1]

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Coccidioidomycosis as a respiratory infection will be considered first with respect to its "biological gradient" or "infection spectrum." Then its epidemiology will be reviewed in the pattern of factors of the etiological agent, *Coccidioides immitis*, of host effects in man, and of the environmental factors which affect these interrelationships. Finally, we shall assess plausible means which its epidemiology indicates for meeting this problem. Basic to this entire consideration is Karl Meyer's (40) fundamental observation that *C. immitis* is a "pathogenic saprophyte." Thus, although we shall refer to man as the *host*, in reality he is not a part of an infection chain. Whereas our interest centers on man and ultimately we shall have to focus on long-range protection for him, this merely will have the effect of minimizing the "pathogenic" component of "pathogenic saprophyte."

Biological Gradient: Infection Spectrum

Among animal hosts the biological gradient or infection spectrum of coccidioidomycosis varies widely. Avians wholly resist infections. Hogs, sheep, and cattle never manifest other than localized lesions either in nature or, only very exceptionally, when infected by man (5, 28, 38). Rodents in nature likewise show localized lesions (16), but "natural" dissemination would be difficult to demonstrate. Dogs, like man, show great variability, some breeds being highly resistant, whereas others show rapidly fatal dissemination (38, 47). Laboratory animals also show considerable variation, with rats and rabbits fairly resistant to widespread disease, guinea pigs intermediate, and mice and hamsters manifesting severe pathogenicity. Even strains of mice differ in this regard (22).

Primary Infection

When we focus our attention on man, we note that most infections are symptomless. Because the only adequate "sample" in which this biological gradient has been observed was that of young adults and age may well affect it, we can say only that in that age group approximately 60% are "inapparent" infections (56, 62). However, in view of apparent universal susceptibility, over half the adults moving into endemic areas eventually can be expected to become ill from coccidioidomycosis. In an 18-month period during 1938–1939 in only two counties of California, the 432 cases of erythema nodosum we saw must have represented fully 2,000 acute respiratory coccidioidal illnesses (54). The 753 soldiers hospitalized in four station hospitals during 1941–1945 in the San Joaquin Valley airfields had an average hospital stay of 1 month (62). This also approximates the average duration of hospitalization mentioned for Arizona by Hugenholtz (29) at Williams Air Force Base and Scoggins (50) at Luke Air Force Base for 1957 and by Lee (33) for the entire Western Flying Training Command during World War II. Thus from the viewpoint of *illness* coccidioidomycosis is a significant problem.

A recurrent question is whether the dosage of infecting coccidioidal spores influences the biological gradient of coccidioidomycosis. We know that in the experimental infections of animals, increasing dosage increases severity as indicated by deaths (22). Since inocula are not quantitated in human infections, only indirect evidence is available. Using as our "standard" for the infection spectrum the experience of the young adults of the Army Air Force, as indicated, over half of the infections are symptomless. In one point-source epidemic we investigated (12), a university student dug a rattlesnake from a ground squirrel hole. Subsequently the fungus was readily recovered from the soil. Of the seven students

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infected, six had symptoms and the student who wielded the shovel had the most severe illness. Another situation in which heavy dosage logically is expected is seen in laboratory-acquired infections. In our review of some 12 years of such experiences in the institution where we worked, 28 (90%) of 31 infections were clinically manifest. One person developed an extrapulmonary lesion. These two settings suggest that increased dosage is associated with increased pathogenicity in man.

Pulmonary Residuals

However, certain other aspects of the "infection spectrum" may loom up more significantly than the symptomatic clinical course. One of these is the pulmonary residual which frequently remains after the infection has been arrested. The vast majority of these lesions do not cavitate and merely constitute vexatious diagnostic enigmas, although very rarely they can produce pressure syndromes. Those which are traced from the antecedent-proved primary infection, so that there is no doubt of the etiology, seldom worry us. However, those detected de novo are diagnostic problems which differential skin tests, serological tests, and cultures of sputum or bronchial washings only infrequently can diagnose. Thus, exploratory thoracotomy to differentiate a coccidioidal pulmonary residual lesion from malignancy frequently is necessary, but the permanent damage to health is negligible. However, when such pulmonary lesions cavitate, the problem is more serious. The proportion of those with coccidioidal infections who have permanent residual cavities is unknown. Estimates of the proportion of those with clinically manifest primary infections who develop cavities range from 2 to 8% (58, 61), but most of these cavities close spontaneously. Even less possible of estimation are those who develop cavities after an inapparent infection. However, the eventual virtual certainty of coccidioidal infection results in literally thousands of cavities and their management is a problem. It should be recalled that nearly all these cavities are the legacies of the primary infection and only very rarely if ever are they comparable to the "secondary" activities of "reinfection" type tuberculosis (58, 68). Extrapulmonary dissemination is very rare and, unlike tuberculous cavities, even intrapulmonary spread is infrequent. Neither do they present the hazard of contagion (18, 58, 61). Their principal problems are those of hemorrhage, secondary infection, progressive increase in size or, with those located peripherally, bronchopleural fistulae. Although deaths due to coccidioidal cavities are rare, morbidity is a problem. With persistent cavitation the patient should continue under medical supervision even if he is symptomless. However, fatal hemorrhage due to coccidioidal cavities has occurred, fortunately, very rarely. One patient developed coccidioidal meningitis after bronchoscopy. Frequently, (and some would say *always*) thoracotomy and excision are necessary, with considerable risk of recurrence of the cavity and occasionally of complicated bronchopleural fistulae. One patient developed meningitis after lobectomy due to a bleeding coccidioidal cavity (7) as did another after a residual noncavitary lesion was excised. Although these complications are rare, they point up the difficulty of management of these occasional cavities. Unfortunately, treatment with amphotericin will not close them. Amphotericin is being tried now in "covering" surgery when excision is indicated although the toxicity and difficulty of administering this drug are limiting factors.

Disseminated or Progressive Primary Infection (Coccidioidal Granuloma)

The major concern of coccidioidal infection is the serious manifestation of extrapulmonary spread referred to as coccidioidal granuloma, or disseminated or progressive primary coccidioidomycosis. Until the advent of amphotericin, there was a 50% mortality when this complication occurred. Fortunately, in absolute numbers, deaths do not loom large. As Table 1 indicates, annual deaths in the United States range from 50 to 85, with an 8-year average of 64. From 1956, when amphotericin began to be used, the numbers of deaths have reduced.

Extrapulmonary lesions may occur in nearly every organ. Especially frequent when there are multiple lesions are subcutaneous cold abscesses, psoas abscesses, involvement of cancellous bone, and cutaneous lesions. However, single lesions are frequent but, unless in the meninges, are seldom serious. The extrapulmonary lesions characteristically develop as a progression of the initial infection. Rare, indeed, is extrapulmonary spread after a primary infection which has been quiescent a year (19, 61, 68). However, after dissemination occurs, there may be a long course with remissions

TABLE 1. *Deaths from coccidioidomycosis: United States, 1951–1958*

Includes only deaths occurring within the continental United States. Excludes fetal deaths. Deaths are those assigned to category 133 of the Sixth and Seventh Revisions of the International Lists of Diseases, Injuries and Causes of Death, 1948 and 1955.

Sex and color	Year							
	1951	1952	1953	1954	1955	1956	1957	1958
White male	26	20	32	30	28	15	22	26
White female	7	14	9	6	8	8	10	10
Nonwhite male	33	20	39	35	20	25	14	11
Nonwhite female	2	6	5	7	6	6	7	3
Total	68	60	85	78	62	54	53	50

Data from *Vital Statistics of the United States*, each respective year. National Office of Vital Statistics, Department of Health, Education, and Welfare, U. S. Public Health Service.

for years, followed by relapse. There is usually a close association of progression with the serological titer in the complement-fixation test (59, 60). Indeed, the fixation of complement serves usefully in diagnosis and prognosis of the various components of the biological gradient. It has serious diagnostic limitation in patients with pulmonary residuals with or without cavitation, bespeaking the difference between this category and those with progressing, disseminating disease. Also, in coccidioidal meningitis, which until the advent of amphotericin was invariably fatal, complement fixation by serum may be in low titer or, rarely, even absent when the only extrapulomonary lesions are in the meninges.

FACTORS OF THE ETIOLOGICAL AGENT: *COCCIDIOIDES IMMITIS* (RIXFORD AND GILCHRIST, *1896*)

Ecology

The diphasic fungus, *Coccidioides immitis*, doubtless occurs in nature in the hyphal or so-called "saprophytic" form. Restricted to arid and semiarid climates, although occasional "index" human cases have been reported elsewhere, endemicity has been established only in the Western Hemisphere. Maddy's (39) association between the so-called Lower Sonoran Life Zone and the known coccidioidal endemic area underscores this influence of climate, but the specific factors and their interrelationships responsible for this restriction as yet are not identified. However, the saprophytism of the fungus enables it to flourish over vast areas so that eradication of it would be wholly unrealistic. Dry arthrospores survive well except when persistent low humidity is associated with persistent high temperature (24). Diurnal variations of both temperature and humidity and the protective effect of upper layers of soil ensure the survival of the fungus from season to season with sufficient spores in the soil, if blown about, to infect the air. The intensive study of Plunkett and Swatek (45) of an Indian Camp site in Inyokern, which was the origin of a point-source epidemic, indicated the limited area (8 by 10 ft) in which that particular "pocket" of *C. immitis* flourished. The experience of the Stanford University point-source epidemic in San Benito County also supports this "pocket" theory. Moreover, we have had two flurries of the infection of children who dug caves in one section of Bakersfield. Similar was the demonstration that topsoil, from an Indian burial ground, where several cases originated, caused infection of several San Diegans when the soil was used for land fills. *C. immitis* was actually recovered from these fills. Only in Plunkett's investigations have the sharp restrictions of these "pockets" been determined. However, the difficulty of recovery of the fungus from the air indicates that it is not constantly present. Indeed, considering the volume of air our lungs "impinge," infection would be much more rapid than it is if the fungus multiplied universally in endemic areas.

Studies of Elconin, Egeberg, and Lubarsky (14) in one area of the San Joaquin Valley have indicated a considerably wider geographical area, covering 12 to 14 acres, from which were recovered *Coccidioides*. Further studies will be needed to determine the restrictions of the multiplying sites, and probably only the elucidation of how the fungus grows in nature will answer these riddles. The association of a season of heavy rainfall and increased incidence of coccidioidal infections during the Army Air Field studies (55, 62) suggested that rainfall is one factor. Actual multiplication in the soil has long been an attractive theory but still is unproved. Egeberg's studies over his dozen-acre coccidioidal site support the soil theory with multiplication during the winter. There was a high proportion

(43%) of isolations from topsoil at the end of the spring rains as compared with zero isolations from soil at the end of the preceding hot summer. These comparisons also suggest that the source of infecting spores is replenished annually and, at least in the San Joaquin Valley, that this occurs in association with winter rains.

Although heat, low humidity, and possibly other factors reduce survival of dry arthrospores in the San Joaquin Valley, at temperatures of 20 to 25 C, even with varying humidities, there is no significant reduction in spore counts during 6 months (24) so that in other environments "contamination" might persist for considerable time.

The "spherules" or "sporangia" of the "parasitic phase" are much less resistant according to recent studies by L. Friedman and associates Even the act of drying on a smooth surface usually kills them. However, they survive significantly better when dried on an absorbent surface such as filter paper (35). Arthrospores show markedly superior survival to spherules with respect to temperature, humidity, drying, and high salinity ("brine effect" which would be encountered in drying of sputum or other body fluids containing coccidioidal spherules). These qualities of the fungus bespeak its adaptation for survival in its arid habitat and give some clues as to why contagion is so rare if, indeed, it does occur.

Size and Ability to Infect

On the other hand, the size of "characteristic" arthrospores is well adapted to dispersal by air currents and, if inhaled, entrance and retention in the deep pulmonary spaces. The "characteristic" arthrospores are cylindrical, ellipsoidal, or sometimes spherical. Their sizes (3) approximate 2 to 4 by 2 to 4 μ or 2 to 4 by 3 to 6 μ. Sometimes they may grow to 10 μ and on artificial media some strains grow predominantly as "hyphae" with few "spores" (25). The "spherules" or "sporangia" of the "parasitic" or "tissue" phase, though quite variable in size, are considerably larger, usually ranging from 15 to 80 μ in diameter (3). These spherules are infective when introduced parenterally or intratracheally into a susceptible animal but no instance of contagion thus far has been observed. If it does occur, contagion is rare. This is in contrast to the notorious frequency of laboratory infections when the fungus is cultured on solid media and the dramatic instances where cotton balls, wool, Indian artifacts (45), and even clothing have successfully carried infecting arthrospores.

The characteristic spherule or "tissue" phase can be grown in vitro by a variety of means (2, 6, 8–11, 13, 32, 36, 49) and the hyphal or "saprophytic" phase frequently occurs in pulmonary cavities where conditions are comparable to surface cultures. Hyphae occasionally (17, 21, 26, 46, 51, 53, 65) have been described in tissues.

Strain Differences

Differences never have been demonstrated when single strains are used to prepare antigens in dermal (57) or serological (60) testing. Even autogenous antigens have shown no difference. Strain differences have been noted in infectivity and pathogenicity when mice (25) are injected intraperitoneally. However, there has been no evident association of these murine manifestations with the human infection spectrum. Thus, strain 46, which we have long used as a relatively avirulent murine strain, was isolated from a fatal human case and even 15 direct passages failed to increase its murine virulence. In contrast, strain Silveira, which is quite lethal for mice, was recovered from a nondisseminating human case with benign erythema nodosum. Another comparably murine-virulent strain was recovered from the soil at the site of the epidemic among the Stanford students (12) which caused no human disseminations.

Human "Host" Factors

"Natural Resistance"

Thus far no one has established human host differences in susceptibility to infection except for acquired immunity. However, host variation of the infection spectrum is well documented.

Age. The effect of age has not been demonstrated although some of us suspect that pulmonary cavitation and possibly even extrapulmonary lesions are proportionately fewer in children than in adults.

Sex. In adults, coccidioidal erythema nodosum during the primary infection is from two to ten times more frequent in females than in males (54, 56). The risk of fatal dissemination is several times greater in males than in females as has long

been recognized (5, 27). Again it is evidenced in Table 1.

Race. Data also indicate the vulnerability of the nonwhites, mainly Negroes and Filipinos, as has been well recognized (5, 27, 30, 61).

During World War II, the relocation of Japanese and those of Japanese ancestry to highly endemic areas resulted in many infections but progressive disease appeared to be no more frequent than in whites. The experience with the Army Air Force during the war showed that despite the same housing, nutrition, and medical care, Negro male soldiers (56, 62) were ten times more likely to develop extrapulmonary lesions ("progressive primary" disease) than were white. A comparable phenomenon is seen among dogs in which Boxers and Doberman Pinschers (38, 47) appear comparable to Negroes and Filipinos in likelihood of dissemination in naturally acquired coccidioidal infection. We previously called attention to strain differences in mice (22).

Pregnancy. Another host factor which has been suggested in human infection spectrum is pregnancy (4, 52) if the primary infection is acquired near term. However, no controlled study has proved this possibility and any heightened risk must be small. We never have heard of any coccidioidin-negative pregnant woman being advised to leave an endemic area until after delivery nor would we urge it on the evidence thus far available.

Portal of entry. Insufficient data are available to ascertain the effect of the portal of entry on the infection spectrum of human coccidioidomycosis. As Wilson, Smith, and Plunkett (66) have discussed, most reported examples of cutaneous portal of entry actually represent the usual respiratory-acquired infections with cutaneous extrapulmonary dissemination. Their patient, who was an embalmer accidentally infected by the spherules from a cadaver, as were the patients of Trimble and Doucette (63), and two others, who were infected traumatically by arthrospores and for whom we also performed confirmatory serological tests, all recovered completely. This woefully meager "series" would have to be augmented by thousands more human percutaneous or subcutaneous coccidioidal infections before one could make valid comparisons with infections acquired "normally" through the respiratory tract and "unusually" through the skin.

However, animal studies have indicated that the subcutaneous route of infection was better withstood (42) than the intraperitoneal or respiratory (43) route. Indeed, Pappagianis (41) showed that strain 46, which for over a decade we had been using as a relatively avirulent murine strain in a dosage of 500 to 1,000 arthrospores intraperitoneally, was usually lethal when 100 arthrospores were inoculated intranasally. We now recognize that the experimental challenge for both killed and living coccidioidal vaccines should be by the respiratory rather than by the intraperitoneal route (34, 44).

"Acquired Resistance"

Of relevance to our later consideration of control is the host factor of immunity after man has undergone a coccidioidal infection. We have indicated (61) that recrudescence or relapse may occur in those who once have undergone dissemination of their primary infection. Also, very rarely, surgery may result in extrapulmonary lesions (7). However, with only one verified exception (61) a well-focalized primary infection has conferred immunity. This case was a second infection after an asymptomatic laboratory infection proved by "conversion" of his coccidioidin skin test. The patient received a tremendous respiratory challenge after he shook a desiccator in which he was drying coccidioidal arthrospores to release its adherent top. The spores scattered sufficiently for some to adhere to the top; and when the top was pulled off, the spores puffed into his face. Fifteen days later he developed a mild febrile illness with some malaise but without localizing pulmonary symptoms or even cough. His pulmonary roentgenogram showed no lesions but precipitins developed in his serum. His dermal sensitivity became much greater and he developed cross sensitivity to histoplasmin, to which he had failed to react after his inapparent infection. The complement-fixation test was negative. Although the massive dosage "broke through" his immunity, the second infection was mild. Even though the laboratory probably affords greater possibility of heavier exposure than is seen in nature, this is the only[2]

[2] At this Conference Tiggert described the case of a laboratory worker who was a coccidioidin reactor, doubtless previously infected "naturally." He accidentally inoculated himself deeply into a wrist bone with a suspension of *Coccidioides immitis*. Coccidioidal osteomyelitis developed and persisted until the lesion was excised. Viable *C.*

second infection we have observed. In the point-source epidemic of the Stanford University students previously discussed (12), two members of the group who were at the digging and whose clinical and laboratory findings and roentgenograms remained entirely normal, probably had been infected previously. Moreover, the person who investigated the site and brought the spores home on his clothes to infect his wife remained symptomless. Certainly the few spores one encounters naturally seem incapable of causing second infections. Among 432 cases of coccidioidal erythema nodosum, 2 patients gave histories of previous erythema nodosum (54) and thus might have had two attacks of coccidioidal "Valley Fever." Very probably their two attacks represented different etiologies of erythema nodosum. While at times one-third of the military personnel in the San Joaquin Valley Army Air Field were reactors (62), only those untested or known to be nonreactors to coccidioidin provided active coccidioidal infections. Moreover, we never have proved a second coccidioidal infection among over 7,000 patients in whom we established the diagnosis of a primary infection serologically. Like man-to-man transmission ("contagion"), second coccidioidal infections of man must be exceedingly rare if they ever occur.

Experimental studies provide further evidence of immunity conferred by initial infection. Rixford and Gilchrist (48) were not able to produce another dermal lesion in one of their initial disseminated cases nor, after they had successfully infected a dog, could they reinfect it. For nearly 20 years we have been using strain 46 as a relatively avirulent murine strain (25) to study immunity in mice and guinea pigs. Pappagianis et al. (42) reported the comparability of the immunity conferred to mice by subcutaneous infection produced by this strain and by murine-virulent strain Silveira originally isolated from a benign human case of coccidioidal erythema nodosum. When mice were challenged with strain Silveira, this immunity withstood intraperitoneal challenge solidly and even intranasal challenge relatively well. After the subcutaneous infection of highly susceptible cynamolgous monkeys with murine-virulent strain Silveira, Pappagianis et al. (43) found roentgenographic and histological evidence of resistance to subsequent respiratory challenge with strain Silveira. However, with only 11 monkeys in the series and in view of their "mixed" clinical responses, interpretation is uncertain. The experimental evidence in animals certainly supports the human experience that an initial "active" infection with strains recovered from nature confers a relatively good protection even to second exposure via the respiratory tract. However, it may be noted (44) that although no deaths occurred in mice subcutaneously "vaccinated" with 100 live arthrospores of strain Silveira, when challenged intranasally 1 month later with 1,000 arthrospores, over 80% of the mice showed moderate pulmonary lesions. Of course, this heavy challenge may well have 'broken through" immunity although the effect of the host response was to deprive the fungus of virulence. An analogous situation will be noted later in recent vaccine studies both with a living avirulent auxotroph and with killed spherules but in that instance the virulence of the fungus itself was changed.

immitis was recovered from the surgical specimen. Thus far his postoperative course has been uneventful. Here, again, "challenge" was in sufficiently high dosage to "break through" immunity.

Environmental Factors

Environmental control measures have immediate but limited importance. Thus, during during World War II, rigorous dust control reduced incidence of coccidioidal infection in the California and Arizona Army Air Fields (55, 62). However, in the San Joaquin Valley Field most intensively studied, they still occurred at 8% per year and thus for long-term residence, infection would be inevitable. Aronson (1) showed that over half the Indian children of endemic Arizona reacted to coccidioidin in the 2- to 4-year age group and nine-tenths reacted in the age group 5 to 9. Emmett's (15) studies of Phoenix school children showed one-third more reactors to coccidioidin among the lifetime residents attending one school in the dusty outskirts than among those attending two less dusty schools in the center of the city. Lifelong residents probably would not be benefited, but for transients with limited terms of exposure, dust control is valuable.

The reverse situation occurred at Edwards Air Force Base in 1958 (31). An epidemic occurred when the ground was prepared for new housing to the windward of the existing housing. One case of coccidioidal meningitis and one of dissemination to the spine occurred. Since turf has been re-

stored, the incidence has dropped back to the few annual cases seen previously. Certainly at sites of construction or other ground-breaking in endemic areas, especially on military installations where large numbers of new-comers ("susceptibles") are present, dust should be controlled.

EPIDEMIOLOGICAL CONSIDERATION OF CONTROL

Source Control

As we recall the discussion of the *etiological agent*, control by eradication of this pathogenic saprophyte in nature is unrealistic. It is too well adapted to its arid habitat. However, "source control" by extreme caution in handling cultures on solid media can prevent laboratory infections. Fortunately, the risk of man-to-man transmission is so remote that ordinary hygienic precautions in disposing of infected sputum or dressings should suffice so that elaborate isolation procedures are unnecessary.

Environmental Control

Environmental control by dust control will alleviate the problem of infection in those who are transiently exposed. In known endemic areas, dust-producing exposures to groups of newcomers who may well be susceptible should be prevented. However, among those who are long-time residents of endemic areas, ultimate infection is well-nigh inevitable. There may even be a disadvantage in delaying the infection beyond childhood.

Host Control

There remains a single focus for application of realistic control in man, the susceptible, potential "host." The two possible points of attack are, first, the use of an effective safe antimicrobial drug for a primary infection, comparable to penicillin in hemolytic streptococcal infections in prevention of rheumatic fever, antituberculous drugs, or to the sulfonamides for meningococcal infections and, second, immunization.

Drug treatment. Unfortunately, a safe, effective anticoccidioidal drug is not yet available. Amphotericin B is so toxic that is use virtually is restricted to disseminated progressive disease. In some cases it is being used to "cover" coccidioidal surgery. Sometimes it is used in severe primary infections when risk of dissemination seems great. However, to be effective it must be given in full dosage approximating 1 mg per kilo daily over a number of weeks (67). In two cases which were so treated, meningitis developed several weeks after the drug was stopped, underscoring the point that amphotericin is fungistatic rather than fungicidal. Even though suppressed, *C. immitis* may lurk to flare up when the drug is stopped if the immune mechanism is not capable of coping with the fungus. A safe effective drug which could terminate symptomatic primary infection, prevent dissemination, and minimize the possibility of coccidioidal cavitation would meet our major needs. Moreover, it would be expected to treat successfully the occasional case of dissemination occurring after asymptomatic primary infection. Because coccidioidomycosis is not communicable, drug resistance would not pose the public health problem seen in staphylococcosis.

Immunization. Of course, immunization would be the optimal answer to our problem. Two approaches have been used. One has been vaccination with a living avirulent strain. The other has been vaccination with killed organisms or their products.

1) Vaccination with living avirulent *C. immitis:* Twenty years ago we discovered that strain 46 was relatively avirulent for mice. Whether there had been a spontaneous mutation from its relative virulence for man, we could not determine. However, while most mice would survive 1,000 arthrospores administered intraperitoneally, occasionally a mouse would succumb from 200 organisms. We continued to search for avirulent strains and found several "natural" strains with relatively low virulence for mice (25). However, all occasionally did produce fatal disease in mice. We then sought, by irradiating spores, to develop avirulent mutant strains which would be dependent for growth upon specific added nutrients. Foley, Berman, and Smith (20) recovered two riboflavin-requiring auxotrophs of strain Silveira which had been irradiated with X-ray. Both were avirulent and 4 months after being infected with 1,000 spores intraperitoneally, all of 15 mice survived an intraperitoneal challenge with 115 strain Silveira spores, which killed 92% of the controls. Pappagianis et al. (44) have shown that even 5,000,000 arthrospores of the Foley strain 887, inoculated by the exacting intranasal route, did not cause murine deaths within 3 months. They extended the studies of the protection afforded by infecting mice first with strain 887 and challenging with strain Silveira. Eighteen weeks

after subcutaneous injection with 1,000 arthrospores of strain 887, all mice challenged even with 200 arthrospores of strain Silveira intranasally survived 3 months, although half of those challenged with 500 spores died. Most of the "protected" surviving mice were found to have pulmonary infections, even those challenged with as few as 50 organisms. Gross lesions were seen in the lungs of from 20 to 50 % of the survivors, although none were noted in liver or spleen. As we commented previously regarding the protection afforded by subcutaneous "vaccination" with virulent strain Silveira, although subsequent intranasal challenge resulted in infection, it was nonlethal.

Moreover, although even 5,000,000 arthrospores of strain 887 were nonlethal when given intranasally, severe lesions were seen in unchallenged mice and even with 100,000 spores, lesions were observed and cultural recoveries were made from the liver and spleen as well as from the lungs. Disconcertingly, 5 of 20 cultures tested had lost their dependence on the added riboflavin and all 4 of those which were tested had regained virulence. Thus, in all respects, the auxotroph had reverted to its Silveira strain prototroph. Admittedly, if immunity was established prior to reversion, the infection would continue to be focalized as it is in most of us who have been "naturally" infected and from whose pulmonary residual lesions virulent strains of *C. immitis* can be recovered. However, we cannot foresee the outcome if a human host manifested the previously discussed defective coccidioidal immunity. Although encouraging, immunization with living strain 887 retains major risks. The desirability of another mutation which might provide additional and necessary safeguards is evident.

2) Vaccination with killed *C. immitis* or its products: Vaccination with killed fungus would eliminate any risk of the mutation of an avirulent strain. Vogel et al. (64) described less extensive pulmonary lesions in guinea pigs challenged by the respiratory tract when they had been vaccinated with heat-killed coccidioidal spherules but lethality could not be used as a criterion. Friedman and Smith (23) showed that mice were protected against death from intraperitoneal challenge with 100 Silveira strain spores by preceding multiple subcutaneous vaccination with acetone- or formalin-killed mycelial vaccine. However, again nearly all survivors were found to have been infected. Subsequently, Pappagianis et al. (44) and Levine, Cobb, and Smith (34) have shown that even the protective effect of such arthrospore and hyphal vaccines against death from intraperitoneal challenge does not stand up against murine challenge by the respiratory route. McNall et al. (37) reported that subcutaneous vaccination of mice with heat-killed mycelial residue extracted by sodium hydroxide, ethanol, acetone, and ether, and termed P_1X polysaccharide, conferred a degree of immunity, but the amount of challenge, route of challenge, and absolute number of survivors were not indicated. However, Levine et al. (34) have reported that subcutaneously administered formalin-killed spherule vaccine produced a high degree of protection against murine deaths after an intranasal challenge which "broke through" the protection afforded by mycelial vaccine. Their initial report indicated that 70% of the spherule-immunized mice yielded positive cultures after challenge, most of the animals also showing pulmonary lesions. Thus, again we note that infection is not prevented, although the immunizing procedures diminished, even if they did not eliminate, pathogenicity and virulence for the host.

In testing the effectiveness of vaccines, challenge in graded dosages and via the respiratory route is essential. Tests in various susceptible mammalian species will be essential before a vaccine should be tried in man. Moreover, evaluation in "natural" infection will be difficult if, as seems probable, the vaccine, living or killed, sensitizes to coccidioidin. Then protection can be evaluated only by comparing clinically manifest disease in vaccinated and unvaccinated subjects. Moreover, unless the ardently desired safe, effective anticoccidioidal agent is discovered, human volunteer challenge would be impossible. However, immunization against *C. immitis* now holds great promise and, if achieved, will be the major answer to the problem of human coccidioidomycosis.

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