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Experimental Sporotrichosis in Syrian Hamsters

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Syrian hamsters were infected with *Sporothrix schenckii* by subcutaneous footpad inoculation. Two types of infection could be uniformly induced: a self-limited, lymphatic infection resembling the classical disease in humans, and a generalized nonfatal infection. An infecting dose of approximately 5,300 yeast cells produced the localized subcutaneous-lymphatic disease which was limited to a single limb. In contrast, a 1,000-fold increase in the inoculum temporarily overwhelmed the animals' defense mechanisms, producing a systemic infection involving the liver and spleen. These models were used to demonstrate the development of increased resistance to subsequent infection following either infection or active immunization with ribosomal fractions or trypsinized cell wall antigens of *S. schenckii* incorporated in Freund complete adjuvant. Agglutination titers were detectable in all animals that were either infected or immunized. In one group of infected animals, the titers persisted for at least 1 year after three booster doses of Formalin-killed *S. schenckii*. The ability to produce an infection in hamsters which closely resembles the disease seen in humans makes this animal a good model with which to study experimental sporotrichosis.

Sporotrichosis in humans, as well as the naturally acquired infection in animals, is classically a chronic subcutaneous lymphatic mycosis usually restricted to a single limb. Rarely will the disease progress beyond the regional lymph nodes to produce a systemic disease.

Experimental infections with *Sporothrix schenckii* have been induced in a variety of laboratory and domestic animals, including rats (4-6, 10, 11, 15, 17, 19, 21, 24, 25, 28, 33, 39, 40), mice (1, 2, 14, 15, 22, 29, 30, 32, 34, 36-38, 41), rabbits (15, 31, 34, 43), guinea pigs (11, 13, 15, 32, 34, 40, 41, 43), dogs (7, 12, 15, 20, 34), monkeys (3, 16, 17), hamsters (26, 27, 35), and cats (8, 9). Unfortunately, most of these infections were induced by intraperitoneal inoculations which initiated a systemic disease, which is a manifestation rarely seen in humans.

In this report we will describe a hamster model for self-limited subcutaneous sporotrichosis and a model for a systemic disease which disseminates from an initial subcutaneous infection. These experimental infections were used to examine the serological response in localized and systemic sporotrichosis, and the influence of active immunization on subsequent infection with *S. schenckii*.

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MATERIALS AND METHODS

Organism. The strain of *S. schenckii*, F 33, used throughout this study was isolated from a patient in 1960 at Andrews Air Force Base, Camp Springs, Md. The organism was converted to the yeast phase by culturing on brain-heart infusion (BHI) agar (Difco Laboratories) at 37°C. The yeast phase cultures were maintained throughout the study by weekly subculture. Five-day yeast cultures grown on BHI agar slants were used in all experiments requiring viable *S. schenckii*. The fungus incubated at 37°C, under increased CO_2 tension, was washed from the slants with sterile phosphate-buffered saline (PBS) and filtered through several layers of sterile gauze and cotton to remove mycelial fragments. The filtrate was centrifuged, and the pelleted yeast cells were washed three times and resuspended to the required concentration in sterile PBS. The yeasts were quantitated by using a hemacytometer, and the viability was ascertained by culture to determine the colony-forming units (CFU) per milliliter.

Animals. Outbred, 100-g male Syrian hamsters purchased from Charles River Breeding Laboratories, Inc., Wilmington, Mass., were used throughout these investigations.

Preparation of *Sporothrix* antigens. The yeast phase of *S. schenckii* used in preparation of ribosomal and cell wall antigens was grown in BHI dialysate (BHID) medium prepared as follows: 37 g of dehydrated BHI broth medium was dissolved in 100 ml of warm distilled water. The solution was dialyzed overnight at 4°C against ca. 900 ml of distilled water. The dialysate was collected, and K_2HPO_4, 3 g; KH_2PO_4, 1 g; NH_4NO_3, 3 g; KCl, 0.5 g; and $MgSO_4 \cdot 7H_2O$, 0.5 g

were added. The final volume was adjusted to 1,000 ml, the salts were dissolved, and the pH was adjusted to 7.0. Volumes of medium in 500-ml amounts were dispensed into 2-liter flasks and autoclaved. Sterile concentrated glucose solution was added to a final concentration of 1%. Growth from 10 BHI agar slants suspended in 50 ml of PBS was used to inoculate four 2-liter flasks, each containing 500 ml of medium. The yeast phase organisms were grown for 96 h in a gyratory incubator-shaker at 35°C and 50 rpm. The culture medium was then filtered through several layers of gauze and cotton to remove the few mycelial elements, and the filtrate was centrifuged at 500 × *g* for 10 min. The pelleted yeast cells were stored at −70°C for later preparation of ribosomal and cell wall antigens.

Crude ribosomal antigen. Ribosomal antigens were prepared by using procedures employed in this laboratory to prepare *Histoplasma* ribosomal antigens, based on modifications of the methods of Youmans and Youmans (44, 45). The yeast cells were thawed, washed three times in sterile PBS, and resuspended in a solution containing 0.44 M ribonuclease-free sucrose (Schwarz/Mann, Orangeburg, N.Y.), 3×10^{-2} M $MgCl_2$, and 2×10^{-4} M phosphate buffer (pH 7.0). The cells were ruptured in a Ribi cell fractionator at 45,000 psi while maintaining the temperature at the Ribi valve between 5 and 20°C throughout the cracking procedure. The homogenate from the fractionator was centrifuged at 26,390 × *g* for 15 min to pellet the larger cell fragments. These pellets were stored at −70°C for subsequent preparation of cell wall antigen. The top 4/5 of the supernatant was centrifuged again at 46,900 × *g* for 10 min, and the pellet was discarded. The top 4/5 of the supernatant was finally centrifuged for 3 h at 144,700 × *g* to pellet the crude ribosomal fraction. The supernatant was discarded, and the wet weight of the pellet was determined. The pellets were resuspended in a small amount of cold 0.01 M phosphate buffer (pH 7.0) containing 10^{-4} $MgCl_2$. The antigen was dispensed in small amounts and stored at −70°C for later use. The protein concentration of this stock antigen (320 mg, wet weight, per ml) was 6 mg/ml, as determined by the method of Lowry et al. (23), and the ribonucleic acid content was 27% (270 mg/ml), as assayed by the method of Warburg and Christian (42).

Cell wall antigen. Cell wall antigen was prepared from the large cell wall fragments obtained during the preparation of the ribosomal antigen. The cellular fragments were washed three times in sterile PBS to remove contaminating cytoplasmic constituents, and incubated with trypsin (100 μg/ml in 0.01 M phosphate buffer, pH 7.2) at 37°C for 3 h. The trypsinized cell walls were washed three times in PBS and incubated overnight in 10% Formalin, and the mixture was centrifuged to pellet the cell walls. The pellet was again washed three times and resuspended in PBS to a final concentration of 1:100 (vol/vol). The trypsinized cell wall preparations were used as antigen for the active immunization of hamsters.

Infection of hamsters. The type and extent of infection in hamsters inoculated subcutaneously (0.05 ml) into the footpad with various doses of *S. schenckii* were assayed by several parameters. The time required for the appearance of the initial signs of infection was determined by periodic careful examination, and the localized subcutaneous infection was confirmed by recovering the fungus from footpad lesions. The extent of systemic dissemination was determined by culturing heart blood, liver, and spleen. The hamsters in each experimental group were anesthetized with ether and exsanguinated by cardiac puncture. Two or three drops of heart blood from each hamster were spread on BHI agar plates, which were then incubated at 25°C for 2 weeks. The blood collected from each group was pooled and allowed to clot. The serum was harvested, filtered (0.45-μm Millipore filter), and stored at −70°C for subsequent serological studies. The liver and spleen were aseptically removed, minced with scissors, and homogenized in Ten Broeck tissue grinders containing 5 ml of PBS with 100 U of penicillin G and 100 μg of streptomycin per ml. Tenfold serial dilutions of the homogenates were prepared in PBS containing antibiotics, and 0.5 ml of each dilution was cultured in duplicate on BHI agar plates. After 2 weeks of incubation at room temperature (25°C), the *S. schenckii* colonies were quantitated from the appropriate dilutions, and the CFU per organ were calculated.

Immunization of hamsters. Hamsters were immunized with viable yeast phase organisms, a crude ribosomal antigen incorporated into Freund complete adjuvant (CFA), and a trypsinized cell wall antigen in CFA. Immunization with viable yeasts was accomplished by infecting the hamsters with a dose of organisms previously determined to produce only a localized subcutaneous infection. This inoculum of 5.3 × 10^3 to 6.5 × 10^3 organisms in 0.05 ml was injected subcutaneously into the left hind footpad and was designated the immunizing dose. Four weeks later, the test group(s) received a challenge dose (7.0 × 10^6 to 8.0 × 10^6 cells) that had previously been determined to uniformly produce disseminated disease in normal animals. The challenge dose was injected subcutaneously into the right hind footpad. At 3 days, 1, 2, 3, 4, and 5 weeks postchallenge, groups of animals from the test and control groups were exsanguinated, and the liver of each was quantitatively cultured. The extent of dissemination was ascertained by determining the CFU of *S. schenckii* in the liver.

A crude ribosomal antigen and a trypsinized cell wall antigen were prepared as described above. Protein in the crude ribosomal antigen was determined by using the method of Lowry et al. (23), and the antigen was diluted in PBS to 3 mg of protein/ml (160 mg, wet weight, per ml). Each of the antigens was mixed in equal parts with CFA containing $H_{37}Ra$ (Difco Laboratories), and an inoculum of 0.05 ml of the antigen-adjuvant mixture was injected subcutaneously into the left hind footpad. Four weeks later the animals were challenged with viable organisms inoculated subcutaneously into the right hind footpad. The extent of dissemination in immunized animals challenged with viable organisms was assayed by determining the CFU of *S. schenckii* in the liver.

Sporotrichosis serology. The agglutination test used to quantitate the antibody response was a slight modification of the procedure reported by Norden (30) and Karlin and Nielsen (18). The yeast phase of *S. schenckii* was grown and processed as described

above. A 1:100 (vol/vol) suspension of yeast cells in PBS was heated for 2 h at 60°C. The same heat-killed stock antigen was used for all tests and diluted 1:1,000 (vol/vol) immediately before use. The test was conducted in glass test tubes (12 by 75 mm), each containing 0.25 ml of a twofold serum dilution and 0.25 ml of the yeast antigen. The mixture was incubated in a water bath at 37°C for 1 h and finally at 4°C overnight. The tubes were centrifuged at 500 × *g* for 10 min and individually swirled. The titer of each serum was recorded as the reciprocal of the highest dilution in which the agitated sediment remained in discrete clumps in a clear supernatant.

RESULTS

Preliminary experimental infections. The virulence of *S. schenckii* F 33 was determined by inoculating hamsters intraperitoneally with 5×10^5 or 5×10^6 organisms. All animals developed a disseminated disease with few or no overt signs of infection until week 3, when some animals developed orchitis. Organisms were recovered from both liver and spleen during the 4-week period of observation. Next, following the lead of Mariat and Drouhet (26), we conducted a series of experiments to determine the type of infection resulting from the introduction of various numbers of organisms into the footpad. Animals were inoculated with 3×10^4, 5×10^4, 3×10^5, or 5×10^5 yeast cells. Every animal developed cutaneous lesions at the site of inoculation and a disseminated infection in the liver which cleared between 4 and 8 weeks postinoculation.

Localized subcutaneous sporotrichosis. After the preliminary experiments, the footpad inoculum was reduced to 5.3×10^3 viable *S. schenckii* in an attempt to produce an infection that would remain localized to a single limb. All 20 animals in this study developed a localized infection at the site of injection with slight swelling and redness beginning at day 7. The lesion progressed and was open and draining approximately 2 weeks after inoculation. The etiology of the localized infection was confirmed by culture, and groups of five animals were sacrificed at 3 days and 1, 2, and 3 weeks for quantitative culture of liver and spleen. Nineteen of the 20 animals with localized sporotrichosis did not have recoverable organisms in the liver or spleen. In one hamster, five colonies of *S. schenckii* were recovered from the liver at day 3. Because the subcutaneous inoculation of 5.3×10^3 viable yeast cells produced a localized infection, with very limited dissemination to the liver or spleen, it was later used as the immunizing dose in the active immunization studies.

Production of uniformly disseminated sporotrichosis by footpad inoculation. A total of 52 hamsters was divided into two groups. The first group of 40 animals received footpad injections of 6.0×10^5 viable *S. schenckii* organisms, and the remaining 12 animals were inoculated with 5.3×10^6 viable organisms. At the end of week 1, all animals developed a localized infection beginning with redness and swelling at the site of inoculation. The lesions progressed with abscess formation, followed by drainage of purulent material. Initial signs of infection were observed earlier in animals receiving the larger dose (5.3×10^6), and the infection appeared to be more severe than in the animals injected with 6.0×10^5 organisms. The draining footpad lesions closed at about 4 weeks postinfection; however, the paw remained markedly swollen and deformed. Hamsters were sacrificed at 3 days and 1, 2, 3, 4, 5, 6, and 7 weeks postinoculation, and the livers and spleens were cultured for recovery of *S. schenckii*. The lower number of organisms (6.0×10^5), administered into the footpad, was not sufficient to produce a uniformly disseminated infection. In fact, the maximum number of animals with liver involvement was only 60% (3/5) at 2 weeks postinoculation.

In contrast, 100% of the animals that received 5.3×10^5 organisms developed an infection in the liver, but not in the spleen by week 1 after injection. These data are presented in Table 1.

Active immunization using viable yeast phase *S. schenckii*. Ninety hamsters were divided into three groups of 30 animals each. One group of animals served as the non-manipulated controls, and the remaining 60 received an immunizing dose of 5.3×10^3 viable *S. schenckii* injected into the left hind footpad. Four weeks later the non-manipulated group and one of the immunized groups received a challenge dose (7.0×10^6) known to produce a generalized infection in nonimmunized hamsters. Cultures from footpad lesions, liver, and heart blood were done on groups of five animals sacrificed at 3 days and 1, 2, 3, 4, and 5 weeks. The liver of each animal

TABLE 1. *Number of S. schenckii (CFU) recovered from the livers and spleens of hamsters after footpad inoculation with 5.3×10^6 viable yeast cells*[a]

Time postinoculation (days)	Avg CFU of *S. schenckii*/organ	
	Liver	Spleen
7	160 (3/3)[b]	7
14	20 (1/3)	0
21	5 (1/3)	0
28	0	0

[a] A total of 12 animals was used, and the results were presented as the average number of organisms recovered from the three animals cultured at each time interval.

[b] Number of animals from which organisms were recovered/number of animals cultured.

was weighed individually before mincing for culture in an attempt to correlate liver mass with disease. Cultures from the immunized-only and immunized-challenged groups were discontinued after 4 weeks because all cultures were negative after day 3. The footpad cultures always yielded a heavy growth of *S. schenckii*, and the cultures of heart blood were consistently negative. The results of the quantitative liver cultures are presented in Table 2. It is apparent from the data that the immunized animals exhibited an increased resistance to generalized disease when subsequently challenged. *S. schenckii* was recovered from the liver of only 1 of the 25 animals in the immunized-only control group. This occurred at day 3, and the colony counts were very low. Therefore, the number of organisms recovered from the immunized-challenged animals was considered to be indicative of the extent of dissemination without contribution from the prior immunizing infection.

These data were analyzed by using Student's *t* test, and a comparison of the immunized-challenged groups and non-immunized-challenged groups showed a significant difference in resistance between the two groups ($P \sim 0.001$ at 3 days and < 0.001 at 7 days). Analyses of liver weights showed no significant difference among the three groups ($P > 0.05$). A second experiment was conducted following the protocol outlined above. Twenty hamsters were divided into two equal groups. Ten were immunized, and ten nonimmunized controls were maintained without manipulation. Liver cultures were performed at 3 and 7 days postchallenge. The results are shown in Table 3. These results confirm the efficacy of active immunization to increase the resistance of hamsters to subsequent reinfection with *S. schenckii*. Comparison of the immunized-challenged and nonimmunized-challenged groups resulted in a *P* value of 0.001 at both 3 and 7 days postchallenge.

TABLE 2. *Number of S. schenckii (CFU) recovered from the livers of actively immunized (5.3 × 10³) and nonimmunized hamsters after a footpad challenge (7.0 × 10⁶) 4 weeks later*[a]

Time post-challenge (days)	Avg CFU of *S. schenckii*/liver		
	Non-immunized	Immunized	Immunized, but not challenged
3	92 (5/5)[b]	10 (5/5)	2 (1/5)
7	130 (5/5)	0	0
14	13 (4/5)	0	0
21	4 (1/5)	0	0
28	7 (3/5)	0	0
35	0	0	0

[a] Results are presented as the average number of organisms recovered from five animals cultured at each time interval.

[b] Number of animals from which organisms were recovered/number of animals cultured.

TABLE 3. *Number of S. schenckii recovered from the livers of actively immunized (6.5 × 10³) and nonimmunized hamsters after a challenge (8 × 10⁶ organisms into the footpad) 4 weeks later*[a]

Time post-challenge (days)	Avg CFU of *S. schenckii*/liver	
	Nonimmunized	Immunized
3	115 (5/5)[b]	16 (3/5)
7	530 (5/5)	0

[a] The results are presented as the average number of organisms recovered from five animals cultured at each time interval.

[b] Number of animals from which organisms were recovered/number of animals cultured.

Active immunization using *Sporothrix* ribosomal or cell wall antigens. A total of 96 hamsters, divided into four groups of 24 animals each, were used in these experiments. Two groups were immunized with either crude ribosomal fraction or cell wall fraction incorporated into CFA; the third group served as an adjuvant control (CFA-PBS) and the fourth as a nonimmunized control. Four weeks later all animals were challenged with 6.8×10^6 viable *S. schenckii*. The data obtained from the quantitative liver cultures at 7 days postchallenge showed an average of 280 CFU from nonimmunized animals; 140 CFU from animals receiving adjuvant only; 55 CFU from animals immunized with ribosomes, and 26 CFU from cell wall-immunized animals. The CFU among all groups dropped rapidly by day 14 (none more than 17 CFU), and no organisms could be recovered from their livers at day 28. Animals immunized with either ribosomal or cell wall antigen had an increased resistance to subsequent infection as indicated by the decreased numbers of *S. schenckii* found in the liver cultures. Immunization with adjuvant alone produced a nonspecific increase in resistance; however, this effect was not statistically different from the nonimmunized controls ($P > 0.05$). The additive effect of immunization with either ribosomes or cell wall antigens was statistically different from animals receiving adjuvant alone ($P < 0.05$).

***Sporothrix* serology in experimentally infected and/or immunized hamsters.** Agglutination tests were performed on most of the sera from hamsters experimentally infected or immunized with *S. schenckii*. The same heat-killed stock antigen of yeast phase *S. schenckii* was used for all tests and diluted 1:1,000 (vol/vol) immediately before use. A summary of the agglutination tests indicates: (i) hamsters receiving a small inoculum (5.3×10^3) mount a

modest humoral response to the self-limited localized infection (Table 4); (ii) as the infecting dose increases, resulting in a more generalized disease, the agglutinin response also increases (Table 4); (iii) agglutinins persist for more than 1 year in animals infected and later boosted with killed organisms (Table 5); and (iv) hamsters immunized with ribosomal or cell wall antigens in CFA and subsequently challenged with viable *S. schenckii* respond with very high agglutinin titers (Table 6).

TABLE 4. *Agglutination titers of pooled hamster sera*

Time postinfection[a]	Agglutination titers of indicated infecting dose of *S. schenckii*[b]:		
	5.3×10^3	6.8×10^6	7.0×10^6
2 days		<2	
3 days		<2	<2
5 days		<2	
1 week		8	16
2 weeks			64
3 weeks		16	64
4 weeks	32	128	128
5 weeks	32		
6 weeks	32		
7 weeks	32		

[a] The animals were infected by footpad inoculation with viable *S. schenckii.*

[b] Normal hamster serum agglutination titer was always <2.

TABLE 5. *Agglutination titers of pooled hamster sera*

Time post-infection (weeks)[a]	Agglutination titers of indicated infecting dose of *S. schenckii*[b]:		
	8.8×10^{6c}	1.1×10^{7d}	5.3×10^{3e}
5	64		128
6			128
7	128	128	128
8		128	128
9		128	
10			
11		256	
12		256	
13		256	
52	64		
54	32		

[a] The animals were infected by footpad inoculation and subsequently boosted with viable or killed *S. schenckii.*

[b] Normal hamster serum agglutination titer was always <2.

[c] At weeks 1, 2, and 3, a booster of 1.1×10^7 Formalin-killed *S. schenckii* was given intraperitoneally.

[d] At weeks 1, 2, and 3, a booster of 1.25×10^7 heat-killed *S. schenckii* was given intraperitoneally.

[e] At week 4, a booster of 7.0×10^6 viable *S. schenckii* organisms was given in the footpad.

TABLE 6. *Agglutination titers of pooled hamster sera*

Time post-immunization (weeks)[a]	Agglutination titers of indicated immunizing agents[b]:		
	CFA only	Ribosomes[c] + CFA	Cell wall[d] + CFA
5	64	256	>1,024
6	64	256	>1,024
7	64	256	>1,024
8	128	256	>1,024

[a] The animals were immunized with viable *S. schenckii, Sporothrix* crude ribosomes or cell wall preparations incorporated into CFA, and boosted at week 4 with a footpad injection of 6.8×10^6 viable *S. schenckii* organisms.

[b] Normal hamster serum agglutination titer was always <2.

[c] Four milligrams (wet weight) of *Sporothrix* ribosomal fraction per hamster.

[d] A total of 0.05 ml of 1:200 (vol/vol) trypsinized *S. schenckii* cell wall preparation per hamster.

DISCUSSION

Models of experimental infection were developed in the Syrian hamster for inducing both a self-limited, subcutaneous form of sporotrichosis and a disseminated nonfatal systemic infection. The subcutaneous inoculation of 5,300 viable *Sporothrix schenckii* yeast cells into the footpad produced a self-limited, subcutaneous lymphatic disease similar to the classical disease seen in humans; whereas a 1,000-fold increase in the inoculum (5.3×10^6) overwhelmed the hamsters' native resistance, and the initial subcutaneous disease progressed beyond the involved limb to both the liver and spleen. These models provided the systems in which we studied various aspects of the pathogenesis and the host response to infection with *S. schenckii.*

The development of increased resistance to reinfection after an active subcutaneous infection was convincingly demonstrated in two separate experiments. Hamsters with localized disease were challenged 4 weeks postinfection, with an inoculum known to uniformly produce disseminated disease in non-manipulated hamsters. In the first experiment, which involved 90 animals, *S. schenckii* was recovered from the livers of the test group, but only in small numbers and only at day 3. Liver cultures at 1, 2, 3, and 4 weeks were all negative for *S. schenckii.* However, the organism was recovered from the livers of control animals, at each of the data points, and not until week 5 were the animals able to clear the organisms. Similar results were obtained in a second experiment involving 20 hamsters. Again, the previously infected animals cultured 3 days postchallenge showed low numbers of *S. schenckii* in the liver; however, when cul-

tured at 1 week, the animals had apparently cleared the liver of viable organisms. In contrast, the cultures from control animals at day 3 revealed large numbers of yeast which were even more numerous on day 7. The manifestations of increased resistance to reinfection were apparent in the lower numbers of *S. schenckii* recoverable from the livers of previously infected animals, and the increased ability of the animals to clear organisms from the liver. Immunization with cellular components of *S. schenckii*, incorporated into CFA, also increased the level of resistance to disseminated infection when animals were challenged 4 weeks post-immunization. The subcutaneous inoculation of crude ribosomal preparations or trypsinized cell walls of the yeast phase of *S. schenckii* was effective in reducing the number of yeast cells recovered from liver cultures. In the dosages used, the cell wall preparation appeared to be slightly more effective than the ribosomal vaccine. A nonspecific increase in resistance was observed in the adjuvant control animals, but the animals receiving vaccines in CFA were statistically different from the control animals.

The specific antibody response to localized and generalized sporotrichosis was examined, as was the effect of stimulation with nonviable antigens and the combination of infection and antigen stimulation. In general, an agglutination titer was detectable 7 to 14 days after infection. Titers of 32 were seen in localized disease, and those as high as 128 were seen in generalized infections. Animals immunized with components of yeast cells in adjuvant and subsequently infected responded with titers of 256 for those receiving ribosomal antigens and with titers exceeding 1,024 for those immunized with trypsinized cell wall antigens. One group of animals with generalized sporotrichosis received 3 weekly boosters with Formalin-killed *S. schenckii*. These animals maintained an anti-*Sporothrix* titer of at least 32 for an entire year. At the termination of this experiment the cutaneous lesions had resolved, but the animals were not cultured to determine the presence of an active lymphatic infection. Therefore we can only speculate on the reasons for the prolonged antibody response in these animals, the most likely of which would be a smouldering infection or the inability of the animals to clear the massive antigenic load.

The ability to produce an experimental infection resembling the classical subcutaneous disease seen in humans makes the hamster a good animal in which to study sporotrichosis. Studies are in progress to investigate the role of cell-mediated immune responses in experimental sporotrichosis.

LITERATURE CITED

1. **Baker, R. D.** 1942. Chronic progressive sporotrichosis in mice. Fed. Proc. **1**:173–174.
2. **Baker, R. D.** 1947. Experimental sporotrichosis in mice. Am. J. Trop. Med. **27**:749–757.
3. **Benham, R. W., and B. Kesten.** 1932. Sporotrichosis. Its transmission to plants and animals. J. Infect. Dis. **50**:437–458.
4. **De Beurmann, L., H. Gougerot, and Vaucher.** 1908. La sporotrichose du rat. Bull. Mem. Soc. Med. Hop. Paris **25**:718–732.
5. **De Beurmann, L., H. Gougerot, and Vaucher.** 1908. La sporotrichose experimentale du rat. Etude histologique de quelques localisations (1). Bull. Mem. Soc. Med. Hop. Paris **25**:800–837.
6. **De Beurmann, L., H. Gougerot, and Vaucher.** 1908. Orchite sporotrichosique du rat (Epreuve diagnostique). Bull. Mem. Soc. Med. Hop. Paris **25**:837–840.
7. **De Beurmann, L., H. Gougerot, and Vaucher.** 1909. Sporotrichoses experimentales; sporotrichoses torpides chroniques; sporotrichoses curables. C. R. Seances Soc. Biol. Paris **66**:597–599.
8. **De Beurmann, L., H. Gougerot, and Vaucher.** 1909. Sporotrichose experimentale du chat. C. R. Seances Soc. Biol. Paris **66**:338–340.
9. **De Beurmann, L., H. Gougerot, and Vaucher.** 1909. Sporotrichoses cutanees du chat. C. R. Seances Soc. Biol. Paris **66**:370–372.
10. **Du Toit, C. J.** 1942. Sporotrichosis on the Witwatersrand. Proc. Transvaal Mine Med. Off. Assoc. **22**:111–127.
11. **Foulerton, A. G. R.** 1901. On the morphology and pathogenic action of *Sporothrix schenckii*. Trans. Pathol. Soc. London **52**:259–270.
12. **Gougerot, H., and J. Caraven.** 1908. Sporotrichose spontanee du chien. Gommes hypodermiques, peritonite granuleuse et gommes hepatiques. Presse Med. **16**: 337–341.
13. **Hamburger, W. W.** 1912. Sporotrichosis in man. With a summary of the cases reported in the United States and a consideration of the clinical varieties and the important factors in the differential diagnosis. J. Am. Med. Assoc. **59**:1590–1595.
14. **Hasenclever, H. F., and W. Mitchell.** 1959. Attempts to immunize mice against sporotrichosis. J. Invest. Dermatol. **33**:145–149.
15. **Hektoen, L., and C. F. Perkins.** 1900. Refractory subcutaneous abscesses caused by *Sporothrix schenckii*, a new pathogenic fungus. J. Exp. Med. **5**:77–89.
16. **Hopkins, J. G., and R. W. Benham.** 1932. Sporotrichosis in New York State. N.Y. Med. J. **32**:595–601.
17. **Hyde, J. N., and D. J. Davis.** 1910. Sporotrichosis in man. With incidental consideration of its relation to mycotic lymphangitis in horses. J. Cutan. Dis. **28**: 321–352.
18. **Karlin, J. V., and H. S. Nielsen, Jr.** 1970. Serologic aspects of sporotrichosis. J. Infect. Dis. **121**:316–327.
19. **Kesten, B., and H. Martenstein.** 1929. Experimental sporotrichosis; cutaneous and intracardial inoculation. A preliminary report. Arch. Dermatol. **20**:441–444.
20. **Kren, O., and M. Schramek.** 1909. Ueber Sporotrichose. Wien. Klin. Wochenschr. **22**:1519–1522.
21. **Lewis, G. M., and J. H. Cudmore.** 1934. Sporotrichosis. Report of a case originating in New York. Ann. Intern. Med. **7**:991–999.
22. **Liu, C. L.** 1955. Sporotrichosis. Report of a case. Chin. Med. J. **73**:330–338.
23. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
24. **Lutz, A., and A. Splendore.** 1908. Ueber eine an menschen und ratten beobachtete mykose. Beitrag zur kenntnis der sogenannten sporotrichosen. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig.

46:97-104.
25. **Mackinnon, J. E., and I. A. Conti-Diaz.** 1962. The effect of temperature on sporotrichosis. Sabouraudia **2**: 56-59.
26. **Mariat, F., and E. Drouhet.** 1954. Sporotrichose experimentale du hamster. Observation de formes asteroides de *Sporotrichum*. Ann. Inst. Pasteur Paris **86**:485-492.
27. **Mariat, F., P. Lavalle, and P. Destombes.** 1962. Recherches sur la sporotrichose. Etude mycologique et pouvoir pathogene de souches Mexicaines de *Sporotrichum schenckii*. Sabouraudia **2**:60-79.
28. **Moore, J. J., and D. J. Davis.** 1918. Sporotrichosis following mouse bite, with certain immunologic data. J. Infect. Dis. **23**:252-266.
29. **Moore, M., and L. V. Ackerman.** 1946. Sporotrichosis with radiate formation in tissue. Report of a case. Arch. Dermatol. **53**:253-264.
30. **Norden, A.** 1951. Sporotrichosis. Clinical and laboratory features and a serologic study in experimental animals and humans. Acta Pathol. Microbiol. Scand. **89**(Suppl.):1-119.
31. **Page, C. G., L. Frothingham, and J. B. Paige.** 1910. *Sporothrix* and epizootic lymphangitis. J. Med. Res. **23**: 137-150.
32. **Panja, D., M. C. Dey, and L. M. Ghosh.** 1947. Sporotrichosis of the skin in India (a new species described). Indian Med. Gaz. **82**:200-202.
33. **Pijper, A., and B. D. Pullinger.** 1927. An outbreak of sporotrichosis among South African native miners. Lancet **ii**:914-915.
34. **Schenck, B. R.** 1898. On refractory subcutaneous abscesses caused by a fungus possibly related to the *Sporotricha*. Bull. Johns Hopkins Hosp. **9**:286-290.
35. **Sethi, K. K.** 1967. Attempts to produce experimental intestinal cryptococcosis and sporotrichosis. Mycopathologia **31**:245-250.
36. **Sethi, K. K.** 1972. Experimental sporotrichosis in the normal and modified host. Sabouraudia **10**:66-73.
37. **Sethi, K. K., V. L. Kneipp, and J. Schwarz.** 1966. Pulmonary sporotrichosis in mice following intranasal infection. Am. Rev. Respir. Dis. **93**:463-464.
38. **Sethi, K. K., and J. Schwarz.** 1965. Experimental cutaneous sporotrichosis. Mykosen **8**:128-135.
39. **Simpson, F. W., M. A. F. Helm, J. W. Bowen, and B. A. Brandt.** 1947. The pathology of sporotrichosis in man and experimental animals. Sporotrichosis infection in mines of the Witwatersrand. Proc. Mine Med. Off. Assoc. **27**:34-58.
40. **Taylor, K.** 1913. Sporotrichum schenckii. J. Am. Med. Assoc. **60**:1142-1145.
41. **Walker, N., and J. Ritchie.** 1911. Remarks on a case of sporotrichosis. Br. Med. J. **2**:1-5.
42. **Warburg, O., and W. Christian.** 1942. Islierug aun kristallisation des garungsferments enolase. Biochem. Z. **310**:384-421.
43. **Warfield, L. M.** 1922. Report of a case of disseminated gummatous sporotrichosis, with lung metastasis. Am. J. Med. Sci. **164**:72-82.
44. **Youmans, A. S., and G. P. Youmans.** 1964. Effect of mitochondrial stabilizers on the immunogenicity of the particulate fraction isolated from *Mycobacterium tuberculosis*. J. Bacteriol. **87**:1346-1354.
45. **Youmans, A. S., and G. P. Youmans.** 1969. Factors affecting immunogenic activity of mycobacterial ribosomal and ribonucleic acid preparations. J. Bacteriol. **99**:42-50.