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COMPLEMENT-FIXATION TITERS IN EXPERIMENTAL COCCIDIOIDOMYCOSIS IN RABBITS[1]

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ABSTRACT

BROSBE, EDWIN A. (Veterans Administration Hospital, Long Beach, Calif.), JEWELL N. KIETZMAN, AND NATHANIEL B. KURNICK. Complement-fixation titers in experimental coccidioidomycosis. J. Bacteriol. **88**:233-241. 1964.—The course of experimental coccicioidomycosis in rabbits was followed with complement-fixation (CF) tests. Uniformity of complement-fixing antibody response to large infective doses was observed, and levels as high as 1:4,096 in the New Zealand albino and 1:16,384 in the Dutch rabbit were attained. Mortality reflected the size of the inoculum rather than the level of CF titer. Animals receiving additional challenges displayed no marked elevation in titer, although high titers persisted. Nitrogen mustard, for the most part, did not influence the CF titer.

The value of the complement-fixation (CF) test in the diagnosis and prognosis of human coccidioidomycosis is well established (Smith et al., 1950; Smith, Saito, and Simons, 1956). As part of a study to find compounds effective against *Coccidioides immitis*, the course of experimental coccidioidomycosis in rabbits was followed with CF tests, with the thought that complement-fixing antibody titers could be used to evaluate in vivo anticoccidioides activity of potential drugs. This appeared to offer a method of following the course of the disease and the effect of experimental agents by serological tests, rather than by survival studies alone, as applied to mice (Campbell, O'Dell, and Hill, 1955; Gordon, Smith, and Wedin, 1955; Emmons, 1961). The findings presented in this report indicate that the rabbit may be a good laboratory animal to employ in chemotherapeutic and immunological studies in coccidioidomycosis.

[1] Portions of this work were presented at the 60th Annual Meeting of the American Society for Microbiology, Philadelphia, Pa., 1-5 May 1960, and the 6th Annual Meeting of the Veterans Administration-Armed Forces Coccidioidomycosis Cooperative Study, Los Angeles, Calif., 30 November-1 December 1961.

MATERIALS AND METHODS

Strains. Two virulent strains of *C. immitis* were used. Strain CI-5 was recovered from a patient with chronic, disseminated coccidioidomycosis in this hospital. Strain Silveira, isolated from a severe primary coccidioidal infection with erythema nodosum (Friedman, Smith, and Gordon, 1955), was obtained from M. Huppert. Both strains were maintained on Mycosel Agar (BBL).

Preparation of inocula. Strain CI-5 was grown in synthetic liquid medium (Roessler et al., 1946), with the use of a "special" culture bottle [square glass bottle (120 ml) containing glass beads and fitted with a one-hole rubber stopper in which was inserted a cotton-plugged glass tube to permit aeration]. Duplicate cultures were kept stationary except for a brief interval each day, when they were swirled by hand to keep the growth submerged. After 2 weeks of incubation at 34 C, the growth was dispersed by shaking the bottles in a Kahn shaker for 15 min. Heavy particles were allowed to settle and the supernatant fluids were decanted, pooled, and diluted 1:3 with 0.9% sodium chloride solution. The suspension was then diluted with NaCl to give a reading of 85 units in a Klett-Summerson photoelectric colorimeter with a 540-mμ filter. The suspension, consisting predominantly of short mycelial fragments, was used on the day of preparation. Glucose-yeast extract-agar medium (Friedman et al., 1953) was employed to determine the number of infective units.

Strain Silveira was cultured on Mycosel Agar for 5 weeks at 34 C. The suspension was prepared essentially as described by L. Walker (*personal communication*). Distilled water (5 ml) was added to each of three slants, and the mycelial growth was harvested by gentle agitation. The suspensions were pooled by decanting into a glass bottle

containing glass beads, and were subjected to vigorous shaking for 30 min in a Kahn shaker. The suspension was then centrifuged at 60 × *g* for 15 min to remove large aggregates. The supernatant fluid was transferred to a 15-ml graduated centrifuge tube, and was centrifuged at 950 × *g* for 20 min. The supernatant fluid was discarded and the packed cells were washed twice with distilled water. The packed cells were then diluted 1:1,000 with distilled water, and a viability count was performed with glucose-yeast extract-agar medium. The suspension, consisting predominantly of arthrospores, was stored in a refrigerator until used, generally within 48 to 72 hr. The number of viable units was determined on the day of inoculation.

Animals. Two breeds of young, male rabbits were obtained from a commercial source 2 to 3 weeks before the day of infection. New Zealand albino rabbits, used in the first experiments, weighed 2.2 to 3.1 kg. The body weights of Dutch rabbits, employed later, ranged from 1.3 to 1.7 kg.

Animals were housed individually, and cages were steam-sterilized three times weekly to control sporulation of contaminated excreta.

Infection of animals. The rabbits were inoculated intravenously with 1 ml of *C. immitis* suspension. A separate syringe and needle were used for each inoculation. Infective doses varied from 6,000 to 16,800 viable units.

CF tests. The rabbits were bled from the marginal ear vein before and beginning 2 weeks after infection. In general, the blood was obtained weekly for 6 to 7 weeks, biweekly for 3 to 4 months, and then at 1-month intervals until the experiment was terminated. The sera, containing 1:10,000 Merthiolate as preservative, were stored in a refrigerator until tested.

The Kolmer CF technique with overnight ice-box incubation for fixation of complement (Smith et al., 1957*b*) was used. The lowest serum dilution was 1:4. The antigen (lot numbers XV-B-10F, XV-B-21F, and XV-B-50L) was generously supplied by the Coccidioidomycosis Research Laboratory, Veterans Administration Hospital, San Fernando, Calif. CF titers of selected sera, with an antigen (lot number 47-63) kindly furnished by C. E. Smith, were equivalent or slightly lower than those found with the coccidioidin from the Veterans Administration.

Nitrogen mustard administration. Mustargen (Merck, Sharp and Dohme & Co., Inc., Rahway, N.J.) was administered as a single, intravenous injection in a dose of 1.5 mg per kg of body weight. This dose produced a 50% or greater fall in leukocyte count 5 days after drug administration. A concentration of 1 mg/ml, used at first, caused a severe inflammatory reaction along the marginal ear vein. Reducing the concentration to 0.3 mg/ml lessened the extent of local reaction appreciably.

Animals were separated into experimental and control groups without knowledge of their actual CF titers. The initial course of nitrogen mustard was given 7 to 13 weeks after infection, depending on the time required to attain a CF of 4+ at a single serum dilution of 1:32. The rabbits received one to four courses of nitrogen mustard at 6-week intervals.

Animal necropsy. Animals were necropsied as soon after death as possible. Survivors of the experimental period were killed by exsanguination from the heart. The extent of macroscopic disease seen in the lungs, liver, spleen, and kidneys was estimated. Representative tissues were placed in sterile petri dishes for microscopic examination and culture, and were fixed in 10% formalin for histopathology.

RESULTS

Clinical manifestations and pathology. One of the first pathological signs of coccidioidal infection was the appearance of gross lesions along the inoculated ear vein within 4 to 14 days after inoculation (Fig. 1). The lesions varied in size and number; many were ulcerated and draining. Wet preparations revealed numerous organisms. The lesions gradually resolved and many disappeared completely.

The most prominent clinical symptoms were those of respiratory disease. Labored breathing and nasal discharge appeared as early as 2 weeks after inoculation, depending on the acuteness of the illness. Anorexia and weight loss were unfavorable signs, and generally prognosticated early death. Of 56 rabbits, 4 developed inflammatory signs and symptoms of involvement of the extremities. However, conclusive evidence of bone involvement by X ray (Fig. 2 and 3), histopathology (Fig. 4 and 5), and recovery of *C. immitis* on culture was found in only one animal.

At autopsy, gross disease was most often seen in the lungs and, to a lesser extent, in the kid-

neys. Spherules were readily found in periodic acid-Schiff (PAS) stained sections (Fig. 6). Definite macroscopic disease was rarely observed in spleen and liver. Demonstration of *C. immitis* in spleen and liver by culture and in PAS-stained sections was infrequent. Subcutaneous lesions, containing organisms in large numbers, were not uncommon.

CF titers after primary infection. In separate experiments (R2 and R4), 11 and 21 New Zealand albino rabbits were inoculated intravenously with strain CI-5, 16,800 (R2) and 6,000 viable units (R4), respectively. Eight rabbits in the second experiment received nitrogen mustard; results of this phase of the study will be presented later in the text.

The response of untreated New Zealand albino rabbits to primary coccidioidomycosis is summarized in Table 1. The larger infective dose induced an acute, progressive disease with high mortality associated with high CF titers. The maximal CF titer ranged from 1:1,024 to 1:4,096. Uniformity of response was indicated by the fact that the CF titer reached within one dilution of the ultimate maximum 4 to 6 weeks after inoculation. Of 11 animals, 10 died between 6 and 16 weeks after infection. The animal which survived (R2-2) reached a maximal titer of 1:4,096, and was free of demonstrable disease when killed at 52 weeks. Titers remained elevated or fell slightly after reaching the peak. In the animal which survived, the titer remained elevated (within 1 dilution of the maximum) through the 20th week, and then fell gradually to 1:128 at the time of sacrifice. It had clinical evidence of disease from 2 weeks after inoculation, when lesions appeared along the injected vein, until 3 months later when vestiges of respiratory disease subsided.

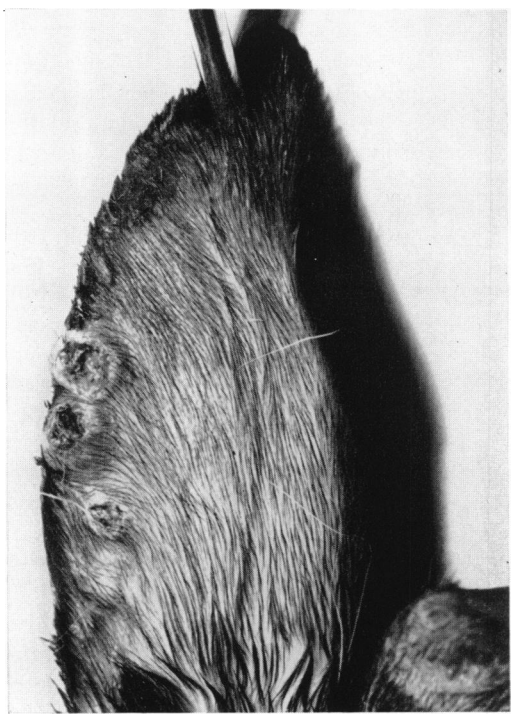

FIG. 1. *Coccidioidal skin lesions on inoculated ear, rabbit R9-27.*

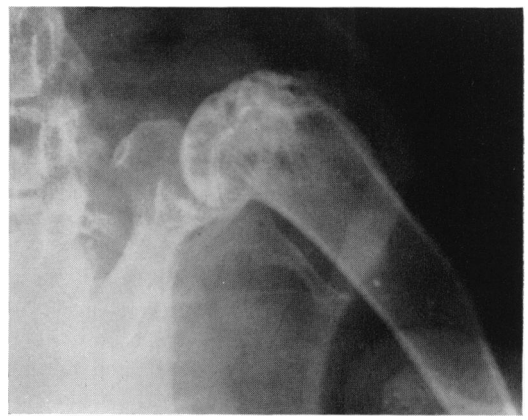

FIG. 2. *X ray, rabbit R4-9, showing "punched out" appearance of the humerus caused by coccidioidomycosis.*

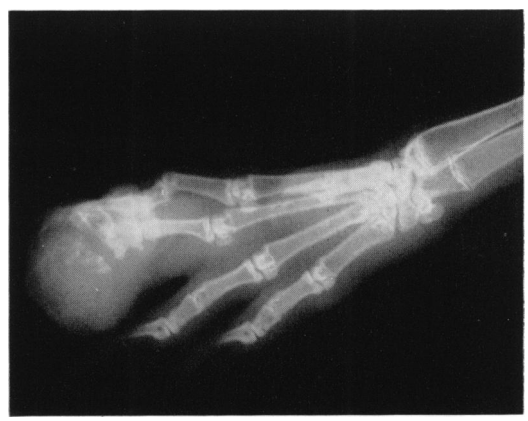

FIG. 3. *X ray, rabbit R4-9, revealing marked inflammatory area and destruction of the phalanx due to coccidioidomycosis.*

In the group receiving the 6,000-unit inoculum, the maximal CF titer varied from 1:16 to 1:1,024. The CF titer reached within 1 dilution of the maximal level between the 4th and 13th weeks. Three animals died between the 13th and 21st weeks. Their maximal titers varied between 1:32 to 1:256. One rabbit died of accidental death at 22 weeks, and nine survived beyond 6 months without evidence of residual disease. Their titers reached maxima of 1:16 to 1:1,024. Titers were maintained near maximal levels for approximately 20 weeks before falling slowly.

In two other experiments, 24 Dutch rabbits were inoculated intravenously with strain Silveira. Six animals (R9-19 through R9-24) received 12,000 viable units, and six (R9-13 through R9-18) received 6,000 units of the same stock suspension. In a later experiment, 12 rabbits (R9-25 through R9-36) were inoculated with 8,000 viable units of another culture of strain Silveira. The results of CF titers, mortality, and autopsy findings are shown in Table 2.

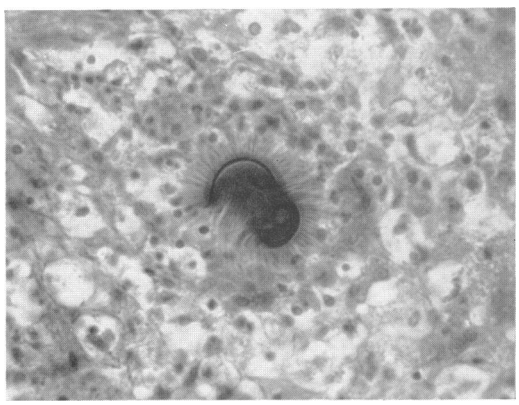

FIG. 4. *Humerus section, rabbit R4-9. Asteroid form of a mature, ruptured sporangium. Periodic acid-Schiff stain with Harris's hematoxylin counterstain. Magnification, 715 ×.*

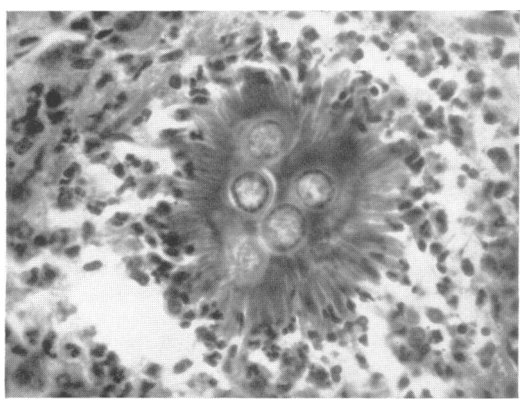

FIG. 5. *Phalanx section, rabbit R4-9. Group of spherules exhibiting asteroid reaction. Hematoxylin and eosin stain. Magnification, 715 ×.*

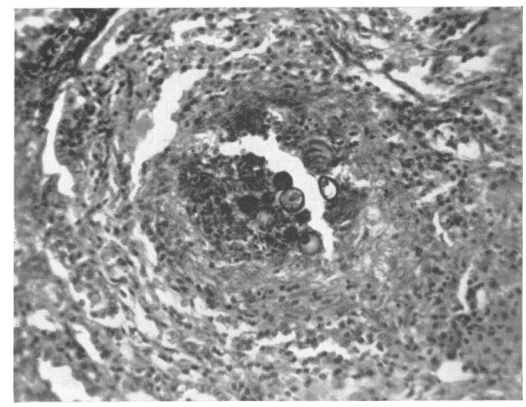

FIG. 6. *Lung section, rabbit R9-24. Cluster of spherules in coccidioidal granuloma. Periodic acid-Schiff stain with Harris's hematoxylin counterstain. Magnification, 300 ×.*

With an infective dose of 12,000 viable units, the maximal CF titer ranged from 1:4,096 to 1:16,384. The CF titer reached within 1 dilution of the maximum between the 8th and 10th weeks. All animals died in 11 to 28 weeks, with evidence of disease at autopsy.

After an infective dose of 8,000 units, the maximal CF titer of 12 animals varied from 1:2,048 to 1:4,096. The CF titer rose to within 1 dilution of the maximum in 4 to 6 weeks. Nine animals died of disease between the 8th and 17th weeks. Two rabbits survived the 30-week experimental period and were given further challenging doses of *C. immitis*. Both of the survivors reached maximal titers of 1:4,096, and showed titers of 1:256 and 1:1,024 at the termination of the experiment. They were free of clinical disease.

Six animals, inoculated with 6,000 viable units, exhibited maximal CF titers of 1:4,096 to 1:16,384. The CF titers rose to within 1 dilution of the maximum between the 6th and 10th weeks. The two survivors (R9-16 and R9-17) were free from clinical disease at 36 weeks, and were used for the reinfection experiment. Their maximal

TABLE 1. *Complement-fixation titers, mortality, and autopsy findings of New Zealand albino rabbits inoculated intravenously with Coccidioides immitis strain CI-5*

Viable unit	Rabbit no.	Complement-fixation titer[a]			Time of death[b]	Macroscopic disease[c]	
		Maximum[d]	At 6 weeks	At death[e]		Lungs	Extra-pulmonary
16,800	R2-2	4,096	2,048	128	52 (K)	±	0
	R2-4	2,048	2,048	2,048	7	++	+
	R2-6	2,048	1,024	2,048	10	++	+
	R2-8	2,048	2,048	1,024	16	++	+
	R2-10	4,096	4,096	4,096	7	++	+
	R2-11	2,048	2,048	2,048	6	++	++
	R2-12	2,048	2,048	2,048	11	++	+
	R2-14	2,048	2,048	2,048	16	+	+
	R2-16	1,024	1,024	1,024	12	++	+
	R2-18	4,096	2,048	2,048	14.5	++	+
	R2-20	2,048	2,048	512	11	+	+
6,000	R4-1	128	16	16	21	+	0
	R4-4	512	128	32	52 (K)	±	0
	R4-5	16	<4	—	—		
	R4-8	256	256	256	13	++	0
	R4-10	64	32	16	48	±	±
	R4-11	32	32	—	—		
	R4-13	256	256	8	52 (K)	0	0
	R4-16	64	8	4	52 (K)	±	±
	R4-17	1,024	16	512	22[f]	0	0
	R4-19	256	64	32	52 (K)	0	0
	R4-21	16	<4	—	—		
	R4-22	256	128	32	52 (K)	±	0
	R4-23	32	8	16	14	±[g]	0

[a] Reciprocal of the highest dilution of serum showing a 3+ or greater fixation of complement.

[b] Expressed as the number of weeks postinfection. Symbols: K, killed at 52 weeks; —, not killed, employed for another study 6 months after infection.

[c] Extent of macroscopic disease was estimated as follows: 0, no gross disease; ±, gross appearance abnormal but no definite lesions; +, one to ten discrete lesions; ++, ten or more discrete lesions; +++, confluent disease but less than one-half of the organ involved; ++++, one-half or more of the organ involved.

[d] Maximal titers were attained between the 4th and 12th week postinfection with 16,800 viable units, and between the 4th and 19th week postinfection with 6,000 viable units.

[e] Within 2 weeks or less of time of death.

[f] Accidental death.

[g] Culture of lung homogenate positive for *C. immitis*.

titer was 1:4,096 and fell to 1:1,024 and 1:512, respectively, at the termination of the experiment. Among the four animals which died of disease, three died at 13 weeks and one at 32 weeks.

CF titers after reinfection. In humans, with rare exception, the immunity acquired from coccidioidomycosis is permanent (Smith, Pappagianis, and Saito, 1957*a*). The effect of reinfection on the CF titer was studied with rabbits from previous experiments which appeared to be clinically well and showed stable CF titers (1:64 to 1:1,024) at lower levels than the previous maxima (1:512 to 1:4,096). Three rabbits received a single challenge, one was reinfected twice, and one four times; infective doses ranged from 8,000 to 12,000 viable units of strain Silveira. Simultaneously, six animals, not previously infected, were inoculated with the same challenge dose, and served as controls for the virulence of the inoculum.

TABLE 2. *Complement-fixation titers, mortality, and autopsy findings of Dutch rabbits inoculated intravenously with Coccidioides immitis strain Silveira*

Viable unit	Rabbit no.	Complement-fixation titer[a]			Time of death[b]	Macroscopic disease[c]	
		Maximum[d]	At 6 weeks	At death[e]		Lungs	Extra-pulmonary
12,000	R9-19	8,192	1,024	2,048	12[h]	++	+
	R9-20	4,096	2,048	4,096	30[h]	−	−
	R9-21	16,384	4,096	16,384	22	±[i]	+
	R9-22	8,192	2,048	8,192	11	++	+
	R9-23	4,096	2,048	512[f]	28	+	0
	R9-24	8,192	2,048	4,096	17	+++	+
8,000	R9-25	4,096	4,096	256[g]	30(S)	−	−
	R9-26	2,048	1,024	1,024	27.5[h]	+	+
	R9-27	4,096	4,096	2,048	8	+	+
	R9-28	2,048	2,048	2,048	10	++	0
	R9-29	4,096	4,096	4,096	9.5	++	+
	R9-30	2,048	2,048	1,024	17	+	0
	R9-31	4,096	4,096	4,096	11	++	+
	R9-32	4,096	2,048	512	16	+	+
	R9-33	4,096	4,096	1,024[g]	30(S)	−	−
	R9-34	2,048	2,048	2,048	6.5	+	+
	R9-35	4,096	2,048	4,096	17	++	+
	R9-36	2,048	2,048	1,024	9	+	+
6,000	R9-13	16,384	2,048	4,096	32	+	+
	R9-14	4,096	2,048	2,048	13	++	+
	R9-15	8,192	2,048	8,192	13	++	+
	R9-16	4,096	1,024	1,024[g]	36(S)	−	−
	R9-17	4,096	512	512[g]	36(S)	−	−
	R9-18	8,192	8,192	4,096	13	++	+

[a] Reciprocal of the highest dilution of serum showing a 3+ or greater fixation of complement.

[b] Expressed as weeks postinfection. Symbols: *S*, survived experimental period; animal used for reinfection experiment; −, autopsy not done.

[c] Extent of macroscopic disease was estimated as follows: 0, no gross disease; ±, gross appearance abnormal but no definite lesions; +, one to ten discrete lesions; ++, ten or more discrete lesions; +++, confluent disease but less than one-half of the organ involved; ++++, one-half or more of the organ involved.

[d] Maximal titers attained between (i) the 8th and 20th week postinfection with 12,000 viable units, (ii) the 4th and 12th week postinfection with 8,000 viable units, and (iii) the 6th and 24th week postinfection with 6,000 viable units.

[e] Within 2 weeks or less of time of death unless noted otherwise.

[f] Titer at 4 weeks before time of death.

[g] Titer at the end of experimental period.

[h] Accidental death.

[i] Culture of lung homogenate positive for *C. immitis*.

A rise of more than 1 dilution in CF titer was observed only twice. One of these increases occurred in a rabbit which was initially inoculated with strain CI-5. None of the titers rose above the maximal level attained after primary infection. Subsequent CF tests showed that titers remained level (within 1 dilution) after challenge. Four animals were killed 16 to 45 months after primary infection. Their titers at the time of sacrifice varied from 1:128 to 1:1,024. No evidence of disease was demonstrable. The controls showed a high mortality rate (67% or greater) and achieved maximal titers of 1:4,096 to 1:16,384.

CF titers after HN2 administration. Clinical

improvement associated with a fall in CF titers in human patients with disseminated coccidioidomycosis was observed after nitrogen mustard therapy (Kurnick, 1958). An attempt was made to determine whether the rabbit would serve as an experimental animal to study this effect. Of 21 animals in experiment R4, 8 were given nitrogen mustard. Six of the eight rabbits received their initial administration of nitrogen mustard 7 weeks after inoculation. Rabbits R4-3 and R4-15 were not given nitrogen mustard until 13 weeks after infection; rabbits R4-1 and R4-16 served as controls for the latter two treated animals. Lethal toxicity of nitrogen mustard was observed in one animal (R4-15).

Nitrogen mustard was without influence on the CF titer within the range of ±1 dilution in four of eight animals. Falls in titer of 2 or more dilutions occurred in four treated animals. In rabbit R4-12 there was a fall of 3 dilutions beginning 4 weeks after the first dose of nitrogen mustard; in rabbits R4-18, R4-20, and R4-24, falls of 2 dilutions occurred 6 weeks after the second course of nitrogen mustard (19 weeks after infection). Among the nine control animals, between 9 and 19 weeks after infection, titers were stable ±1 dilution in six animals and fell 2 dilutions in three animals.

The CF titers, mortality, and autopsy findings are summarized in Table 3. Six of the eight nitrogen mustard-treated animals died with disease. One treated animal survived beyond 1 year and

TABLE 3. *Complement-fixation titers, mortality, and autopsy findings of nitrogen mustard-treated and nontreated rabbits inoculated intravenously with 6,000 viable units of Coccidioides immitis strain CI-5*

Group	Rabbit no.	Complement-fixation titer[a]			Time of death (weeks postinfection)	Macroscopic disease[b]	
		Maximum[c]	At 6 weeks	At death[d]		Lungs	Extrapulmonary
HN2-treated	R4-9	256	128	256	25	++	+
	R4-12	1,024	512	128	18	++	+
	R4-14	64	64	8	52[f]	0	0
	R4-18	512	256	128	20[g]	+	±
	R4-20	128	64	32	21	++	0
	R4-24	2,048	1,024	1,024[e]	31	++	0
	R4-3	64	<4	64	15	±[h]	±
	R4-15	64	32	16	31[g]	0	0
Non-treated	R4-4	512	128	32	52[f]	±	0
	R4-8	256	256	256	13	++	0
	R4-10	64	32	16	48	±	±
	R4-13	256	256	8	52[f]	0	0
	R4-17	1,024	16	512	22[g]	0	0
	R4-19	256	64	32	52[f]	0	0
	R4-22	256	128	32	52[f]	±	0
	R4-1	128	16	16	21	+	0
	R4-16	64	8	4	52[f]	±	±

[a] Reciprocal of the highest dilution of serum showing a 3+ or greater fixation of complement.

[b] Extent of macroscopic disease was estimated as follows: 0, no gross disease; ±, gross appearance abnormal but no definite lesions; +, one to ten discrete lesions; ++, ten or more discrete lesions; +++, confluent disease but less than one-half of the organ involved; ++++, one-half or more of the organ involved.

[c] Maximal titers attained between the 6th and 13th week postinfection by the HN2-treated rabbits, and between the 4th and 19th week postinfection by the control animals.

[d] Within 2 weeks or less of time of death unless noted otherwise.

[e] Titer at 4 weeks before time of death.

[f] Sacrificed at 52 weeks.

[g] Accidental death.

[h] Culture of lung homogenate positive for *C. immitis*.

was free from disease when sacrificed, whereas among the controls five animals survived beyond 1 year and were free from demonstrable disease when killed.

DISCUSSION

Reports by Goodman, Fountaine, and Vincent (1953) and Bieberdorf and Chamblis (1955) indicated that rabbits are markedly variable in their response to an intraperitoneal inoculation of *C. immitis*. Although some individual variation was observed in our study, both the New Zealand albino and the Dutch rabbit showed reproducible susceptibility to a high dose of *C. immitis* inoculated intravenously. The higher CF titers exhibited by the Dutch rabbits, as compared with those demonstrated by the New Zealand albino breed, may be due to the use of a different (i) strain of *C. immitis;* (ii) method of culture and preparation of fungal suspension, resulting in a greater number of free arthrospores in the inocula used to infect the Dutch rabbits; or (iii) breed of rabbit.

The Dutch rabbits exhibited marked uniformity of response, with respect to time and level of the CF titer, to inoculation with *C. immitis*. However, there was no prognostic significance to the maximal titers attained. Mortality appeared, rather, to be a function of the size of the inoculum. The CF titers were independent of the size of the inoculum in the range used, suggesting that the smallest inoculum (6,000 units) produced a maximal CF response. The titers tended to fall with time after reaching the maximum, whether or not the animals ultimately succumbed to the disease. However, the titers remained greater than 1:128 even after 6 months. All animals revealed transitory ear lesions.

With the New Zealand albino breed, the CF response was uniform at the higher infective dose (16,000 viable units), whereas the titers were lower and more variable at the lower dose (6,000 units). Again, mortality was a function of infective dose rather than titer. Some animals with very high titers survived. As with the Dutch breed, titers tended to recede from the maximum with time, whether or not the animals subsequently died. All animals, except a few which received the small inoculum, had transitory ear lesions.

These results are somewhat different from those experienced with natural infections in man. In man, Smith et al. (1950, 1956) observed a direct correlation between CF titer and severity of disease. Fall in titer indicated favorable prognosis, whereas an unfavorable prognosis was associated with rising titer. A titer as low as 1:32 was indicative of dissemination. In the rabbit, some animals with titers as high as 1:4,096 survived and became free from demonstrable coccidioidal disease both clinically and pathologically, whereas others with titers never exceeding 1:64 died with demonstrable disease. The rise and fall in CF titer followed similar time courses in our animals without regard to clinical course.

Whereas nitrogen mustard produced a fall in CF titer and clinical improvement in most human patients with disseminated disease (Kurnick, 1958), only four of eight nitrogen mustard-treated rabbits showed a fall in CF titer of 2 dilutions or more. This was not associated with clinical improvement. The two animals which became free from disease did not show significant reduction in CF titers after nitrogen mustard therapy. Three of nine controls also showed similar falls in CF titers; nitrogen mustard, thus, appears to have no influence on the CF titer in rabbits, and has no favorable effect on the disease. In fact, the mortality was higher in the nitrogen mustard-treated group.

Animals which were free from clinical disease 30 weeks after primary infection appeared to resist reinfection. Only one animal showed a few ear lesions along the inoculated vein after the first reinfective dose; this rabbit had been infected initially with a different strain of *C. immitis*. No animal developed demonstrable disease, either locally or systemically with subsequent challenges. All animals, despite freedom from clinical signs of disease, had CF titers greater than 1:32 at the time of challenge. These results indicate acquired immunity in animals surviving intravenous *C. immitis* infection.

No difficulty was encountered in performing the CF tests; anticomplementary activity of rabbit serum at the lowest dilution of 1:4 was uncommon. Attempts to demonstrate precipitin antibodies, of diagnostic value in human (Smith et al., 1950) and canine (Reed, 1954) coccidioidomycosis, were rarely successful (*unpublished data*).

Our findings indicate that the rabbit is a useful experimental animal for the study of *C.*

immitis infection and treatment, because of its uniformity of complement-fixing antibody response and clinical course after infection.

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