

PATHOGENESIS OF *COCCIDIOIDES IMMITIS* IN MONKEYS

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ABSTRACT

CONVERSE, J. L. (U. S. Army Chemical Corps, Fort Detrick, Frederick, Md.), E. P. LOWE, M. W. CASTLEBERRY, G. P. BLUNDELL, AND A. R. BESEMER. Pathogenesis of *Coccidioides immitis* in monkeys. *J. Bacteriol.* **83**:871-878. 1962.—Respiratory exposure to arthrospores from the submerged growth of *Coccidioides immitis*, strain Cash, in liquid medium resulted in similar pathogenesis in monkeys to that of strain Silveira arthrospores harvested from solid medium. Infectivity of 100% was noted with doses of 50 to 10,000 arthrospores. The disease was characterized by loss of appetite and weight, malaise, and extreme respiratory distress accompanied by coughing, with the immediate cause of death being acute coccidioidal pneumonia. The pathological picture was one of extensive, progressive, destructive pulmonary disease in the higher dose levels and few, small, self-contained, fibrous lesions, with little destruction of lung tissue, in the low doses. This was correlated in general with the findings of serial X rays and serological tests. The presence of the parasitic phase (spherule and endospore) of the organism was noted in large numbers within the pulmonary lesions and bronchial exudates and was substantiated by cultural methods. Occasionally, hyphal elements of the saprophytic growth phase were noted around the periphery of residual cavitated areas of the lungs.

Ahlfeldt (1926), using enforced aspiration of fungus cultures into the lungs of guinea pigs, first clearly demonstrated the pulmonary route of infection of *Coccidioides immitis* after an earlier attempt by Brown (1906). Subsequently, similar studies in guinea pigs or mice were reported by

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Cronkite and Lack (1940), Tager and Liebow (1942), and Vogel et al. (1954).

Experimental pulmonary coccidioidomycosis also has been studied in monkeys, dogs, and cattle, by Biddle et al. (1953), Reed (1957), and Maddy and Reed (1958), respectively. In these studies, however, little attention was paid to dose level or dose range.

The work being reported here is part of a study to relate size of exposure dose to the pathogenesis of respiratory coccidioidomycotic infections in the monkey and to compare the effects of the two spore types (arthrospore and endospore) grown on solid medium or in liquid culture. Two phases of this study have been reported previously; the work on experimental pulmonary infections with strain Silveira arthrospores harvested from agar cultures (Lowe et al., 1959), and the pathology of coccidioidomycosis in the monkey (Blundell et al., 1961). This report concerns the pathogenesis of strain Cash arthrospores grown in liquid synthetic medium, with particular emphasis on serial X-ray studies and serology in the monkey.

MATERIALS AND METHODS

Cultures. *C. immitis*, strain Cash, isolated from a patient having a nonfatal disseminated coccidioidomycotic infection, was used because of its high virulence for animals and its unipartulate arthrospore growth in liquid medium. Cultures were incubated with shaking for 14 days at 34 C in the synthetic medium of Roessler et al. (1946) with 50 ml per 250-ml Erlenmeyer flask. The inoculum consisted of 0.1 ml of a suspension of arthrospores (approximately 3×10^7) grown in the same medium. At the completion of incubation the cultures contained approximately 3×10^8 arthrospores per ml of medium.

Aerosol exposure. *Macaca mulatta* monkeys were exposed via the respiratory route to calculated inhaled doses of approximately 10,000, 300, or 50 arthrospores (five monkeys per dose level) aerosolized from an 8% aqueous glucose

solution, with a Chicago-type atomizer, in a 4,800-liter exposure chamber. The exposed animals were housed in groups of two per cage in ventilated cages for 14 days and in open cages, thereafter. Additional unexposed monkeys were housed in each of two cages containing animals exposed to the highest dose, as cross-infection controls. These control animals were rotated to other cages of exposed monkeys, as their cage mates died off.

Observation. Progress of the disease was followed by coccidioidin skin tests; body weight-loss determinations; chest X rays made at 4, 11, 19, 34, and 59 weeks; and complement-fixation tests at 5, 7, 9, 11, 14, 19, 34, and 59 weeks. Complete necropsies were performed on all animals that died spontaneously during the holding period or upon sacrifice of those surviving for 14 months. Tissues for histopathological study were fixed in 10% formalin or in Zenker's solution; then they were impregnated with paraffin, sectioned, and stained with Giemsa's stain. The

Gomori silver methenamine and periodic acid-Schiff stains also were used for easier identification of fungal structures. Necropsy material was cultured for recovery of the organism.

RESULTS

The clinical signs of infection most noticeable in exposed monkeys were loss of appetite, malaise, and a very marked state of constitutional disorder, indicated by loss of weight and extreme respiratory distress. Monkeys with the most severe infections collapsed upon the least exertion. Violent coughing was quite commonly associated with these symptoms. The clinical response and characteristic pathology of these monkeys is given in Table 1. Four of five monkeys receiving approximately 10,000 spores died in 14 to 51 days, and three of five monkeys in the 300-spore dose group died by the 253rd day post-challenge with acute primary pulmonary coccidioidomycosis, whereas those receiving approximately 50 spores developed a mild, chronic type

TABLE 1. *Pathogenesis of respiratory coccidioidomycosis in monkeys*

Calculated inhaled dose	Dead/ total	Day of death (post-challenge)	Skin test (30 days)	Lung culture	Pathology
12,000		14	0*	+	Confluent necrotizing pneumonia
11,100		22	0*	+	Confluent necrotizing pneumonia
10,500	4/5	36	±	+	Confluent necrotizing pneumonia
12,670		51	±	+	Confluent necrotizing pneumonia with cavitary foci
10,212		S†	-, ±	+	Focal chronic pulmonary lesions, mild
330		8	0*	+	Pulmonary microabscesses‡
414		36	++	+	Confluent necrotizing pneumonia
300	3/5	253	++	+	Confluent cavitary necrotizing pneumonia
346		S	++	+	Focal chronic pulmonary lesions, mild
288		S	+	-	Focal chronic pulmonary lesions, mild
57		S	+	+	Focal chronic pulmonary lesions, mild
55		S	+	-	Focal chronic pulmonary lesions, mild
55	0/5	S	+	+	Focal chronic pulmonary lesions, mild
52		S	++	+	Focal chronic pulmonary lesions, mild
60		S	++	-	Focal chronic pulmonary lesions, mild
0 (cage control)		S	-	-	No significant pathology present
0 (cage control)	0/2	S	-	-	No significant pathology present

* Dead before test.

† Sacrificed at 14 months postchallenge.

‡ Immediate cause of death probably intestinal intussusception.

of pulmonary disease. These results indicated that the severity of the disease was dose-dependent. Results of skin tests or lung cultures upon necropsy indicated that all exposed monkeys were infected, even those receiving the lowest dose. The unexposed cage controls were negative to all tests and exhibited no histopathological evidence of the disease.

In general, the pathological findings in the infected monkeys could be placed into two categories: those resulting from the higher inhaled dose levels (10,000 or 300 spores), exhibiting an extensive progressive, destructive pulmonary disease (Fig. 1), and those infected with the lowest dose level (50 spores) in which the reaction was one of containment, manifested by small fibrous lesions with little destruction of the lung or spreading to the lymph nodes (Fig. 2). In none of these groups was there any evidence of extrapulmonary systemic spread of the infection. Microscopic examination of stained tissue sections prepared from the infected monkeys revealed the presence of the organism (spherules or endospores) in large numbers (Fig. 3) within the lesions, particularly in acute

infections. Occasionally, in the more chronic infections, the saprophytic phase or hyphal elements of the organism were noted within avascular, cavitory areas of the lung (Fig. 4). These findings were substantiated by obtaining positive cultures from the tissues.

Although there was nothing specifically diagnostic for coccidioidomycosis in the chest X rays, except possibly an occasional residual thin-walled cavity, they were a valuable adjunct to the other tests and symptoms in diagnosing the disease. The comparative X-ray findings for the three doses are given in Table 2. At 4 weeks, monkeys infected with 10,000 spores showed maximal involvement (widespread, diffuse, wispy infiltrations throughout the entire visible lung area), intermediate involvement (50 to 60% of the visible lung area) was shown by those receiving the 300 spore dose, and minimal involvement (15 to 25% of the visible lung area) by those inhaling the 50-spore dose. Gradations of lung involvement, as indicated by the terms maximum, intermediate, and minimum, are illustrated in Fig. 5. No extensive changes were noted in any of the X rays taken at 11

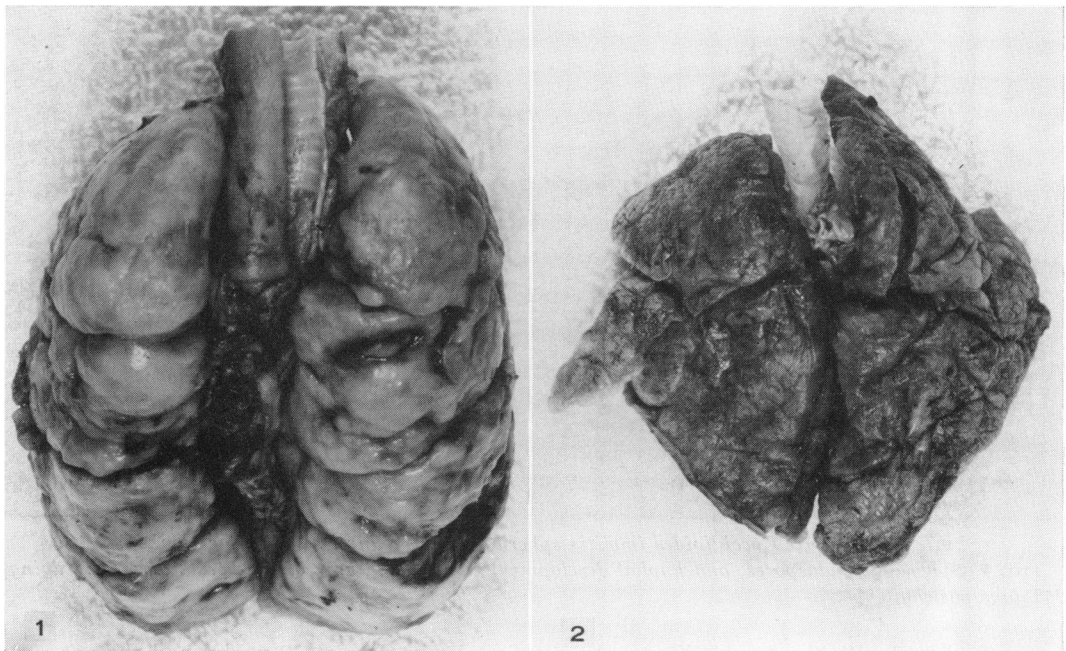


FIG. 1. Lung of monkey receiving an inhaled dose of 10,000 *Coccidioides immitis*, strain Cash, arthrospores. Note bosselated surfaces and distended "meaty" appearance.

FIG. 2. Lung of monkey receiving an inhaled dose of 300 arthrospores.

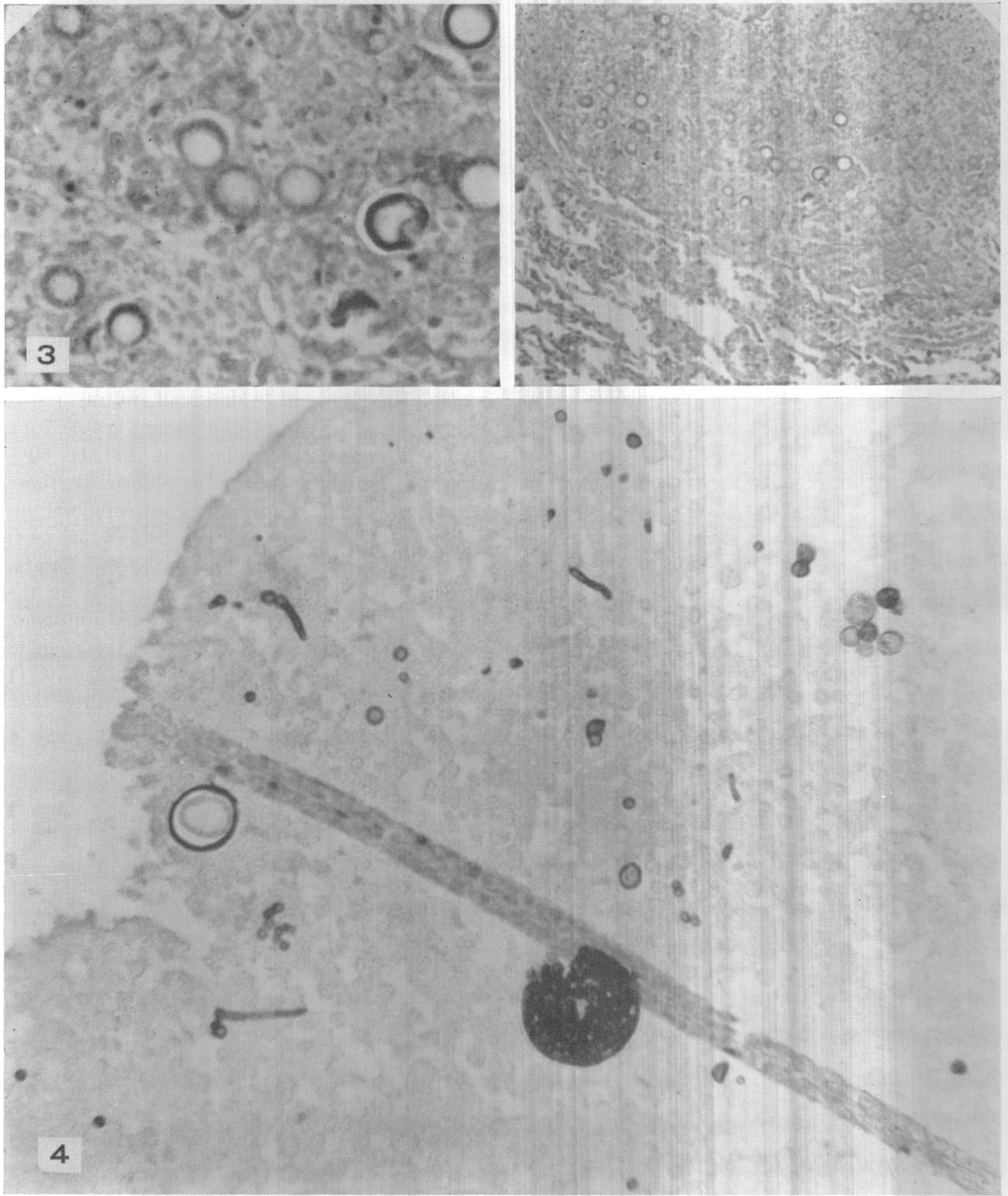


FIG. 3. Immature *Coccidioides immitis* spherules within lung lesions of the monkey.

FIG. 4. Spherules, endospores, and hyphal development in cavitated area of lung from a monkey dying 253 days postchallenge.

weeks compared with the 4-week X rays; however, a gradual resolution of the consolidated areas was noted after the 19th week. The lungs of monkeys surviving the two lower doses were

completely clear at 59 weeks, and the one survivor of the 10,000 dose exhibited involvement of only about 5% of the visible lung area (Fig. 6). A similar picture was noted in the 19-week

TABLE 2. *Roentgenology of respiratory coccidioidomycosis in monkeys*

Calculated inhaled dose	Consolidation in X-ray fields at				
	4 weeks	11 weeks	19 weeks	34 weeks	59 weeks
10,000	D*				
	D				
	Maximum Maximum Maximum	D Maximum Maximum	D Resolving	Resolving	Bare minimum
300	D				
	Intermediate	D			
	Intermediate	Intermediate	Resolving	D	
	Intermediate	Intermediate	Resolving	Resolving	Clear
50	Minimum	Minimum	Resolving	Resolving	Clear
	Minimum	Minimum	Resolving	Resolving	Clear
	Minimum	Minimum	Resolving	Resolving	Clear
	Minimum	Minimum	Resolving	Resolving	Clear
	Minimum	Minimum	Resolving	Resolving	Clear
0	Clear	Clear	Clear	Clear	Clear

* D = dead before X ray; maximum = total involvement of visible lung area; intermediate = 50 to 60% involvement of visible lung area; minimum = 15 to 25% involvement of visible lung area; bare minimum = almost clear just before death; clear = equal to pre-exposure films.

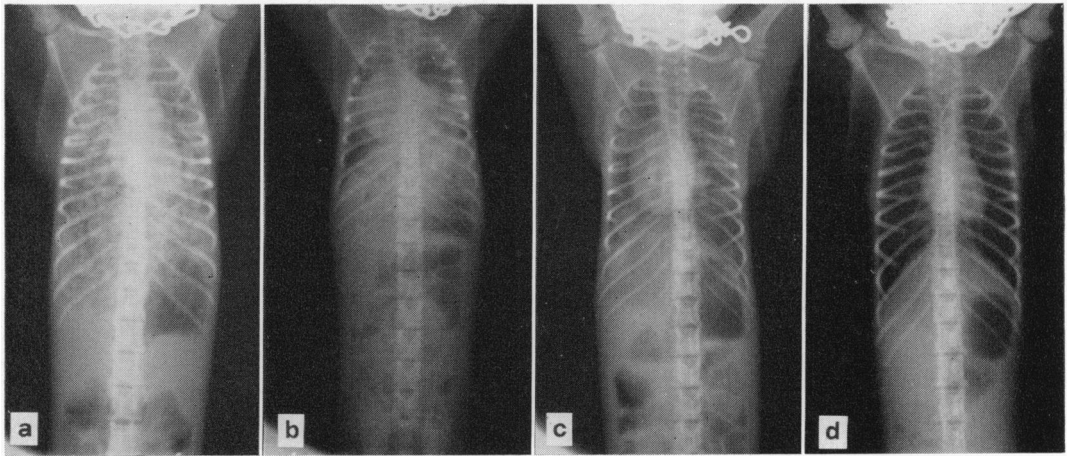


FIG. 5. Maximal (a), intermediate (b), minimum (c), and clear (d), degrees of lung involvement of the monkey. See Table 2 and text.

X ray of the animal dying at 253 days from the 300-spore dose.

A correlation between the complement-fixation titer and exposure dose was noted (Table 3). All animals receiving 10,000 spores exhibited titers greater than 1 to 512. Titers for the 300-spore

dose ranged from 128 to >512, and those for the 50-spore dose ranged from 128 to 256. Monkeys with fatal infections maintained titers greater than 512 until death; those surviving for 14 months exhibited gradual decreases to low titers, paralleling the diminution of the extent

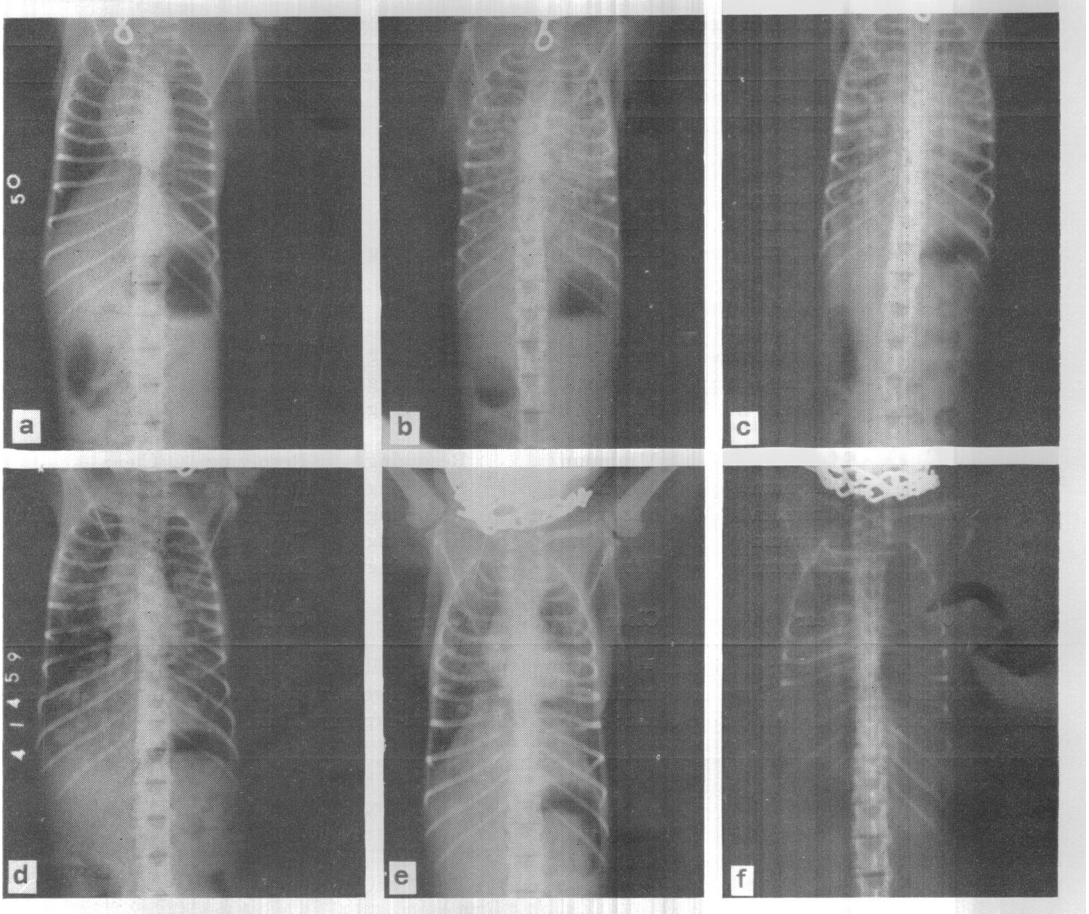


FIG. 6. Serial X rays of the one monkey who survived an inhaled dose of 10,000 arthrospores made before exposure (a) and at 4 (b), 11 (c), 19 (d), 34 (e), and 59 weeks (f) postexposure.

of the infection. The comparatively low titers of the control animals were believed to be non-specific.

DISCUSSION

The data reported here indicate very similar pathogenesis of respiratory infections in the monkey caused by *C. immitis*, strain Cash, arthrospores grown in liquid medium and that reported for strain Silveira arthrospores grown on solid medium (Lowe et al., 1959). All exposed animals were infected, even at the low dose of 50 arthrospores. Death (probably as a result of anoxia) resulted from acute coccidioidal pneumonia, usually accompanied by malaise, loss of appetite, loss of weight, and extreme respiratory distress.

The gross and microscopic lung pathology

was identical for the two strains, being characterized by extensive pulmonary destruction at the higher dose levels, and by very few chronic self-contained, fibrous lesions at the low dose

Serial chest X rays furnished an excellent indication of the extent of infection and the progress of the disease. There was good correlation between the roentgenology and pathological findings in the lungs and, in general, with the complement-fixation titer.

The ability of the serum of a patient with coccidioidomycosis to fix complement, first demonstrated by Davis (1924), and developed and refined by Smith et al. (1950) into an efficient complement-fixation test, is widely used in diagnosis and prognosis of the disease. The complement-fixing antibodies appear gradually in human infections from about the second week

TABLE 3. Complement-fixation titers in respiratory coccidioidomycosis of monkeys*

Calculated inhaled dose	Time in weeks								Type of pulmonary infection
	5	7	9	11	14	19	34	59	
12,000	D†								Acute-fatal
11,100	D								Acute-fatal
10,500	>512	D							Acute-fatal
12,670	>512	>512	D						Acute-fatal
10,212	>512	>512	>512	>512	>512	>512	128	Neg	Mild chronic
330	D								Acute-fatal
414	128	D							Acute-fatal
300	>512	>512	>512	>512	>512	>512	D		Acute-fatal
346	64	NT	128	NT	256	64	32	32	Mild chronic
288	128	NT	>512	>512	>512	>512	256	64	Mild chronic
57	256	NT	256	128	128	128	64	32	Mild chronic focal
55	128	NT	256	128	128	128	64	16	Focal-healed
55	64	NT	128	64	128	128	64	32	Mild chronic mediastinal
52	64	NT	128	64	64	32	16	16	Mild chronic focal
60	64	NT	256	64	64	32	16	8	Very mild chronic
0 (cage control)	16	NT	32	Neg	32	8	8	Neg	No infection
0 (cage control)	8	NT	Neg	NT	NT	16	8	8	No infection

* Expressed as reciprocal of serum dilution.

† Dead before test (D) and not tested at this interval (NT).

after onset of symptoms and reach a peak at approximately 3 months. In uncomplicated primary infections, regression takes place from 6 to 12 months, the titer persisting at a very low level for several years if benign residual pulmonary lesions are present. If extrapulmonary dissemination occurs, a high titer usually persists until death or recovery from the infection; a progressively rising titer indicates dissemination and very grave prognosis.

In general, complement-fixation titers in the monkey followed closely those reported for human infections, although at a somewhat higher level. They correlated with the severity of the infection, remained high in serious infections until death, and gradually decreased to very low levels in animals surviving for 14 months. The principal differences in the complement-fixation response in simian and human infections appeared to be: (i) the abnormally high level of the titers in monkeys, when compared with human cases (1 to 2 diagnostic; 1 to 16 or above indicating dissemination in human infections); and (ii)

the level and behavior of the titers in the monkey, although associated with death and severity of the infection, were not indicative of extrapulmonary dissemination as in human infections. Either of these differences might be explained by the fact that the dose levels received by the monkeys were probably of a much higher order than those initiating natural infections in humans. Death from the acute, primary pulmonary infections seen in these monkeys rarely, if ever, occurs in natural, human infections.

The low level of complement-fixation titers in the unexposed control animals (1 to 8 to 1 to 32), although higher than that considered diagnostic for humans (1 to 2), was thought to be nonspecific, since none of these animals exhibited any evidence of disease (i.e., skin tests, histopathology, X rays, and cultures all negative upon sacrifice at 14 months). These findings should indicate, again, the probable noncommunicability of the disease, since the controls were housed for long periods in cages with mon-

keys exhibiting severe pulmonary infections accompanied by coughing.

Although, normally, the saprophytic growth phase or mycelium of *Coccidioides* is limited to growth in laboratory media, the occasional finding of hyphal elements of the organism in cavitated areas in the lungs of some of these monkeys was not unexpected. Quite recently, Puckett (1954), Baker and Braud (1956), and Fiese (1958) have reported numerous instances of mycelium found in residual cavities and benign granulomas removed from the lung during surgical intervention in the disease, in avascular meningeal abscesses from fatal cases of the infection, and in the sputum of patients with cavitated lungs.

Typical in vivo growth conditions do not occur in these types of lesions. The residual lung cavities if open to the outside air would be subject to external environmental conditions; and avascular foci, walled-off from the body processes, would not be supplied with the usual nutrients from the tissues, nor would the metabolic end products resulting from growth of the organism be carried off by the vascular system. For these reasons, the finding of hyphal elements in the tissues of these monkeys does not represent a radical departure from the normal growth pattern of the organism.

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