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TECHNICAL MANUSCRIPT 306

INFECTION OF NORMAL MONKEYS  
BY INOCULATED CAGEMATES

Richard H. Kruse  
Theron D. Green  
Wayne D. Leader

July 1966

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U.S. ARMY BIOLOGICAL CENTER  
Fort Detrick, Frederick, Maryland

TECHNICAL MANUSCRIPT 306

INFECTION OF NORMAL MONKEYS BY INOCULATED CAGEMATES

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Industrial Health and Safety Division  
INDUSTRIAL HEALTH AND SAFETY OFFICE

Project IC622401A072

July 1966

In conducting the research reported here, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

ABSTRACT

Monkeys were exposed either to a whole-body or to head-only microbial aerosol of Bacillus anthracis, Coxiella burnetii, Pasteurella tularensis, Rickettsia rickettsii, and the causative agents of Venezuelan equine encephalitis, yellow fever, Rift Valley fever, and psittacosis. When they were placed with unexposed control monkeys in ventilated cages they caused infection among the control animals. Infection was caused by the control monkey's inhaling microorganisms shed from the fur of the aerosol-challenged monkey. A new air-washing procedure prevented cross transmission.

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## I. INTRODUCTION

The need for monkeys in medical research is increasing. Primate centers have been established to provide monkeys for many purposes, including research on cancer, malaria, tuberculosis, and other diseases. When experimental animals are held for several months or even for a few years, there is always danger of losing these increasingly valuable animals from nonspecific infection. During short-term experiments with infectious microorganisms there sometimes is danger of transmission of infection to normal control animals or to the animal caretaker. In either case, special caging methods are desirable. One of these methods is the use of the closed ventilated cage.<sup>1</sup>

The time and expense associated with the use of closed ventilated monkey cages has caused us to examine their use in detail. The first objective was to determine whether ventilated cages are necessary to prevent infection of normal cagemates and implied danger to the animal caretaker after aerobiological challenge with highly infectious microorganisms and whether infection could be prevented by variations in the post-challenge air-washing technique. The second objective was to determine, for the same microorganisms, whether infection of normal cagemates would occur when the monkeys were kept in open wire cages after injection intraperitoneally (IP), subcutaneously (SC), intramuscularly (IM), or intravenously (IV).

The first of these studies has been reported<sup>2</sup> in which monkeys, whose bodies were exposed to aerosols of Coccidioides immitis arthrospores, infected cagemates despite various air-washing procedures, but in which forceful ruffling of the fur by manipulation of an air hose did clean the fur to a point where no cagemates were infected. These experiments define a clear differentiation between cross infection, in which diseased animals infect cagemates by urine, feces, saliva, or droplets as in simian pneumonic plague,<sup>3</sup> and cross contamination, in which cagemates are infected by organisms released from the fur or skin before the exposed animals become ill.

The present report extends the work, begun with C. immitis, to other infectious microorganisms.

## II. MATERIALS AND METHODS

### A. METHODS

The following bacteria, rickettsiae, and viruses were used: Pasteurella tularensis, Bacillus anthracis, Rickettsia rickettsii, Coxiella burnetii, and the causative agents of Venezuelan equine encephalitis (VEE), yellow fever, Rift Valley fever, and psittacosis.

Monkeys (Macaca mulatta) of both sexes weighing 1 to 4 kg were inoculated either by the respiratory route or by IP, SC, IM, or IV injection. Monkeys were tranquilized by IM injection (0.1 mg/kg body weight) of Serynl (Parke-Davis Co., Detroit, Michigan). All respiratory challenge was done in an aerosol chamber within a gas-tight ventilated cabinet (Fig. 1) with aerosols created by a Vaponefrin nebulizer (Vaponefrin Co., Metuchen, New Jersey). The inhaled dose was estimated from data of Jemski and Phillips.<sup>4</sup> Monkeys were observed daily and fed Purina Monkey Chow and water ad libitum. Daily rectal temperatures were taken with a thermistor probe (Tri-R Instrument Co., Jamaica, New York).

Air-sampling ports were located (i) in the air duct that connected a first and second cage, and either (ii) in the exhaust air duct from the second cage housing a control monkey, or (iii) in the exhaust air duct from the first cage, when a cagemate control was placed only in the cage housing an aerosol-challenged monkey. After exposure at various time intervals, 10 cubic feet of air was sampled from each port either by a funneled sieve sampler<sup>5</sup> containing an agar plate that would support growth of the bacteria, or by an AGI-30 glass impinger.<sup>6</sup> Appropriate liquid media were used for rickettsiae and viruses.

#### 1. Experiment 1

Each tranquilized monkey in turn was put in the aerosol chamber where it inhaled a calculated dose of microorganisms, during which time the whole body was exposed to the microbial aerosol. Then the monkey was moved into an attached transfer cabinet (Fig. 2) through which filtered air flowed at 150 liters per minute. After 15 minutes of this air-wash, the monkey was shifted from the transfer cabinet to an attached, closed, ventilated first cage that contained an unexposed monkey (cagemate control). After the closed space connecting the transfer cabinet to the cage was disinfected this cage with the two monkeys was detached from the transfer cabinet and transported to the animal room. There it was connected by a 3-foot-long air duct to a second closed, ventilated cage that housed another unexposed control monkey (Fig. 3).



Figure 1. Exposing Monkey to Microbial Aerosol.  
(18064-32-AMC-65)



Figure 2. Air-Washing Monkey in Transfer Cabinet.  
(18064-34-AMC-65)



Figure 3. Ventilated Cages for Aerosol-Exposed Monkeys.  
(18064-35-AMC-65)

Air passed through these cages at 65 liters per minute. Airflow was from the room, through a high-efficiency filter<sup>7</sup> into the first cage housing the aerosol-exposed monkey and his cagemate control, then out of this cage through a short rubber air duct into and through the second cage housing the second control monkey, into a manifold that contained an ultra-high-efficiency filter, and finally to the exhaust plenum. Air samples were taken at the air sampling ports at 2, 4, 6, or 8 hours after challenge.

Before another monkey was air-washed, the transfer cabinet was rinsed with a neutralizer specific for the disinfectant. After 5 minutes' contact time the cabinet was washed with water.

## 2. Experiment 2

This was a duplication of Experiment 1 except that only the head of each challenged monkey was exposed to the microbial aerosol.

## 3. Experiment 3

Each monkey was exposed to a microbial aerosol as in Experiment 1, but then was air-washed for only 10 minutes instead of for 15 minutes. After the air-wash, a towel moistened with 2% quaternary ammonium compound was moved into the transfer cabinet and the monkey was wiped with this towel. The monkeys were housed with cagemate and adjoining cage controls as in Experiments 1 and 2.

## 4. Experiment 4

Each of six monkeys was exposed (whole body) to a microbial aerosol, air-washed, and transferred to a ventilated cage as in Experiment 1. However, only three cages had a cagemate control. The three cages housing an exposed monkey and a cagemate control, and the three cages holding only an exposed monkey, were transferred to the animal room, where an air duct was connected to the manifold. No adjoining cages were used. Forty-eight hours after the microbial exposure, a non-exposed cagemate control monkey was placed in the cages housing only an exposed monkey.

Air samples were taken from the sampling port connecting the cage to the manifold at 2, 4, 6, and 8 hours after challenge and then at 4-hour intervals until several air samples yielded none of the specific microorganisms.

## 5. Experiment 5

This was a duplication of Experiment 4 except that only the head of each challenged monkey was exposed to the microbial aerosol, and non-exposed cagemate control monkeys were placed in cages 24 hours after challenge.

## 6. Experiment 6

Experiment 4 was repeated except that the air-washing time was 25 minutes, and non-exposed monkeys were placed with exposed monkeys at 24, 48, 72, and 96 hours after challenge.

## 7. Experiment 7

This was a repetition of Experiment 6 except that only the head of each challenged monkey was exposed to the microbial aerosol.

#### 8. Experiment 8

Each monkey was exposed to a microbial aerosol as in Experiment 1 and then placed in the transfer cabinet. At that time a removable flexible line and nozzle was attached to the air intake line within the transfer cabinet. Air flow was maintained at 150 liters per minute of filtered air, but was directed through the nozzle at the monkey to ruffle the fur. The monkey was manipulated so that all parts of the body were air-washed for 10 minutes by this forceful jet of air. Then the line was removed and the usual cabinet air flow was continued for 5 more minutes. These monkeys were housed with cagemate controls and adjoining controls as in Experiments 1, 2, and 3. Air samples were taken hourly for 8 hours and then at 2-hour intervals up to 24 hours after challenge.

#### 9. Experiment 9

This replicated Experiment 8 except that only the head of each challenged monkey was exposed to the microbial aerosol.

#### 10. Experiment 10

Tranquilized monkeys were placed in the gas-tight, ventilated cabinet for IP, SC, IM, and IV injections. Three monkeys were injected by each route. A cotton pledget moistened with disinfectant appropriate for the particular microorganism was used to disinfect each injection site before and after inoculation and to surround the needle of the hypodermic syringe, so as to prevent contamination by infectious fluid or accidental microbial aerosol. The animals were individually air-washed in the transfer cabinet for 15 minutes as in Experiment 1 and then moved to an animal-carrying container by which they were transported to the animal room and placed in open wire cages with an uninoculated cagemate control (Fig. 4).

### B. MATERIALS

Different techniques were used to determine clinical infection and to isolate and identify the specific microorganisms tested.

#### 1. P. tularensis

SCHU S4 strain was the test organism. Monkeys received a respiratory or injected inoculum of 75 viable organisms. Before inoculation blood was withdrawn from all monkeys for baseline serum agglutination titers. Monkeys exposed to microbial aerosols and their controls were bled 6, 8, 10, 13, 15, and 20 days post-challenge. Injected monkeys and their controls were bled 7, 9, 15, and 22 days after inoculation. A sample of this blood was heparinized and inoculated into tubes containing a liquid glucose cystine blood medium,

and the remaining blood was used for serological examination for agglutinins. Complete necropsies were performed on animals that died during the experiment, and upon survivors sacrificed at the conclusion of the experiment. Samples of the heart, lungs, liver, and spleen were triturated in 5 ml of Difco heart infusion broth (HIB) and suspensions were plated on glucose cystine blood agar.

## 2. b. anthracis

V1b strain was the test organism. Monkeys inhaled 1100 viable spores. For each of the four injection routes one monkey received 25 viable spores and two monkeys received 2500 viable spores. Necropsies were performed on monkeys that died during the experiment. Blood from the heart was plated on 2% blood agar plates, as were suspensions prepared from triturated samples of heart, lungs, and spleen.

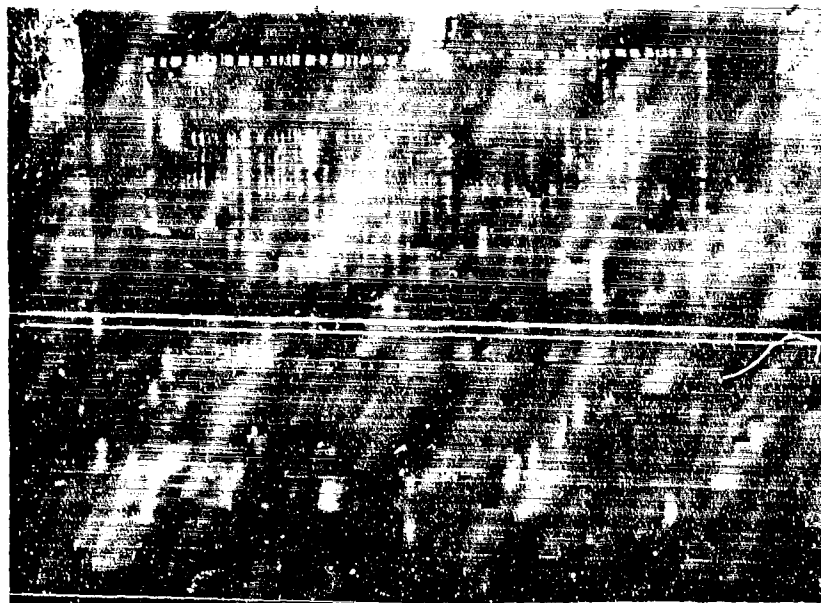


Figure 4. Cages for Parenterally Inoculated Monkeys.  
(18064-33-AMC-65)

### 3. R. rickettsi

Bitterroot strain was the test organism. Monkeys were bled before inoculation and the serum of each animal was assayed quantitatively for CF antibody.\* Monkeys exposed to aerosol inhaled 1000 monkey IPID<sub>50</sub> units. Injected animals received 100 monkey IPID<sub>50</sub> units. This dose was based on assays performed with eggs and monkeys inoculated with serial dilutions of R. rickettsi. Interpolation of data produced an estimate that 0.67 yolk-sac LD<sub>50</sub> equaled one monkey LD<sub>50</sub>. All monkeys were bled 3, 4, 5, 7, 8, 9, and 11 days after challenge. Each of three male guinea pigs (Hartley strain), weighing 350 to 400 grams, was inoculated IP with 2.5 ml of heparinized blood from each monkey. Rickettsemia in the monkeys was determined by lesions and necrosis of scrotal skin of the guinea pigs. Twenty-four days after inoculation the monkeys were bled for CF antibody.

### 4. C. burneti

AD strain was the test organism. Monkeys received a respiratory or injected inoculum of 200 guinea pig IPID<sub>50</sub> units determined by CF antibody response of convalescent guinea pigs. All monkeys were bled before inoculation and 5, 7, 9, 11, 13, and 15 days after inoculation. Heparinized blood from each monkey was diluted 1:2 in HIB and 0.5 ml was injected IP into each of three guinea pigs. After 24 days the guinea pigs were exsanguinated intracardially and agglutinins were determined from the sera by the method of Luoto.<sup>8</sup>

### 5. Venezuelan Equine Encephalitis Virus

Trinidad strain was the test organism. Monkeys exposed to aerosol inhaled 2500 mouse ICLD<sub>50</sub> units. Injected animals received 1000 mouse ICLD<sub>50</sub> units. These doses were determined by inoculating intracerebrally (IC) 0.03-ml samples of serial dilutions into mice and calculating the mouse ICLD<sub>50</sub> by the method of Reed and Muench.<sup>9</sup> All monkeys were bled before inoculation and 2, 4, 6, 8, 10, and 12 days after inoculation. Heparinized blood was diluted 1:2 in HIB and 0.2 ml injected IP and 0.03 ml injected IC into each of five mice. Viremia was established by mouse mortality. Thirty days after inoculation monkeys were bled and sera collected. Neutralization tests were performed by preparing serial tenfold dilutions of virus-infected mouse brain suspensions that were mixed with equal parts of undiluted serum from the monkeys. From each dilution of this serum-virus mixture, 0.05 ml was injected IP into each of seven mice. The mice were observed for 14 days, at which time the neutralization index was calculated by the Reed-Muench formula.<sup>10</sup>

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\* CF tests were performed by Dr. Bennett L. Ellisberg, Department of Rickettsial Diseases, Walter Reed Army Institute of Research, Washington, D.C.

## 6. Yellow Fever Virus

Asibi strain was the test organism. Monkeys received a respiratory or injected inoculum of 1000 mouse ICLD<sub>50</sub> units. Monkeys receiving a respiratory inoculum and their controls were bled before inoculation and daily on days 3 through 10 after challenge. Injected monkeys and their controls were bled before inoculation and daily for 10 days. Viremia was determined by diluting heparinized blood 1:10 in HIB containing 20% egg yolk and injecting 0.03 ml IC and 0.2 ml IP into each of five mice. Monkeys that died during the experiment were necropsied and samples of the liver were triturated in 5 ml of HIB; 0.03-ml samples of the resulting suspension were inoculated IC into each of 10 mice. Thirty days after inoculation the remaining monkeys were bled and sera collected for neutralization tests as described for Venezuelan equine encephalitis virus.

## 7. Rift Valley Fever Virus

Van Wyck strain was the test organism. Monkeys either inhaled 500 mouse ICLD<sub>50</sub> units or were injected with 100 mouse ICLD<sub>50</sub> units. Viremia was determined by bleeding the monkeys and their controls (0, 2, 3, 4, 5, 6, 7, and 8 days after injection; 0, 3, 4, 5, 6, 7, 8, and 9 days after respiratory inoculation), diluting heparinized blood 1:10 in HIB and injecting 0.05 ml IC and 0.5 ml IP into each of five mice. Twenty-one days after inoculation the monkeys were bled and sera collected. Neutralization tests were performed by the method described by Easterday.<sup>11</sup>

## 8. Psittacosis

Borg strain was the test organism. Before inoculation, chest X-ray,\* CF antibody, and blood sedimentation rate procedures were performed. Monkeys received either a respiratory or an injected inoculum of 5000 mouse ICLD<sub>50</sub> units.<sup>12</sup> At 6, 7, 8, 9, 10, 12, 13 and 14 days after respiratory inoculation or at 4, 5, 6, 7, 8, and 10 days after injection all monkeys were X-rayed and bled. Blood sedimentation rates were recorded, and viremia was established by diluting blood 1:5 in Sorenson's buffer containing 10% egg yolk and injecting 0.05 ml IC and 0.5 ml IP into each of five mice. Twenty-eight days after inoculation monkeys were bled for serum CF antibody response.

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\* Taken by Sp4 Arthur L. Self and interpreted by Lt. Col. Nelson R. Blemly, U.S. Army Medical Unit, Fort Detrick.

### III. RESULTS

The results of these studies are summarized in Table 1.

#### A. P. TULARENSIS

When monkeys were given a respiratory inoculum by exposing either the whole body or only the head to an aerosol, and then were air-washed for 15 minutes before caging, both they and their cagemate controls contracted tularemia. The control monkeys in the adjoining air-duct-connected cages did not become infected. However, when the aerosol-challenged monkeys were air-washed by ruffling the fur, although they contracted tularemia, there was no transmission of tularemia to their cagemates or to the monkeys in the air-duct-connected cages. In Experiments 1 and 2, air sampling recovered P. tularensis in the air duct from the first cage during the first 2 hours after caging of the aerosol-challenged monkeys, but did not recover P. tularensis in the air duct from the second (air-duct-connected) cage. P. tularensis was not recovered in either cage air duct by air sampling after air-ruffling the fur.

Monkeys injected IP, SC, IM, or IV did not transmit the disease to cagemate control monkeys.

#### B. B. ANTHRACIS

When monkeys were given a respiratory inoculum by exposing either the whole body or only the head to an aerosol and were air-washed for 25 minutes immediately before caging with control monkeys, both they and their cagemates contracted anthrax. However, in Experiment 6 anthrax was not contracted by cagemate controls placed with the whole-body exposed monkeys 96 hours after the aerosol challenge, nor in Experiment 7 was anthrax contracted by cagemate controls placed with the head-only-exposed monkeys 72 hours after aerosol challenge. Infection did occur when the cagemate control monkeys were placed with the aerosol-exposed monkeys sooner than 96 and 72 hours after challenge. Air samples recovered B. anthracis from the cages housing whole-body-exposed monkeys for as long as 88 hours after exposure (Experiment 6), and from the cages housing head-only-exposed monkeys for as long as 64 hours after exposure (Experiment 7). When the aerosol-challenged monkeys were air-washed by ruffling of the fur, although they contracted anthrax, there was no transmission to either of the controls. Air samples recovered B. anthracis from the first cage for 4 hours, and from the second cage for 2 hours after monkeys had received whole-body aerosol exposure, and only from the first cage for 2 hours after head-only aerosol exposure.

Monkeys injected IP, SC, IM, or IV did not transmit the disease to cagemate control monkeys.

TABLE 1. INFECTION OF NORMAL MONKEYS BY INOCULATED CAGEMATES

Microorganism	Whole-Body Respiratory Experiments										Head-Only Respiratory Experiments											
	Aerosol Challenge					Monkeys in Cage 1					Aerosol Challenge					Monkeys in Cage 1						
	Exp.	Exposed	Infected	Infected challenge; controls placed with exposed monkeys <sup>a</sup>	Cagemate Controls Infected/Exposed to Challenged Monkeys. Hours after challenge; controls placed with exposed monkeys <sup>a</sup>	Exp.	Exposed	Infected	Infected challenge; controls placed with exposed monkeys <sup>a</sup>	Cagemate Controls Infected/Exposed to Challenged Monkeys. Hours after challenge; controls placed with exposed monkeys <sup>a</sup>	Exp.	Exposed	Infected	Infected challenge; controls placed with exposed monkeys <sup>a</sup>	Cagemate Controls Infected/Exposed to Challenged Monkeys. Hours after challenge; controls placed with exposed monkeys <sup>a</sup>	Exp.	Exposed	Infected	Infected challenge; controls placed with exposed monkeys <sup>a</sup>	Cagemate Controls Infected/Exposed to Challenged Monkeys. Hours after challenge; controls placed with exposed monkeys <sup>a</sup>		
<i>B. anthracis</i> <sup>a/</sup>	6	10/10	2/2	2/2	2/2	2/2	2/2	2/2	2/2	1/2	0/2	7	10/10	2/2	2/2	2/2	2/2	2/2	2/2	2/2	0/2	0/2
<i>B. anthracis</i> <sup>b/</sup>	8	3/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3			9	3/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
<i>C. burnetii</i> <sup>a/</sup>	1	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4			2	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
<i>C. burnetii</i> <sup>b/</sup>	8	3/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3			9	3/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
<i>E. tularemiae</i> <sup>c/</sup>	1	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3			2	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
<i>E. tularemiae</i> <sup>b/</sup>	8	3/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3			9	3/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Psittacosis agent <sup>c/</sup>	1	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4			2	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Psittacosis agent <sup>b/</sup>	8	3/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3			9	3/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
<i>R. rickettsii</i> <sup>c/</sup>	4	6/6	3/3	3/3	3/3	3/3	3/3	3/3	3/3	0/3	0/3	5	6/6	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
<i>R. rickettsii</i> <sup>b/</sup>	8	3/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3			9	3/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Rift Valley fever virus <sup>c/</sup>	4	6/6	2/3	2/3	2/3	2/3	2/3	2/3	2/3	0/3	0/3	5	6/6	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3	2/3
Rift Valley fever virus <sup>b/</sup>	8	3/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3			9	3/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
VZV <sup>c/</sup>	1	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6			2	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3
VZV <sup>b/</sup>	3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3													
VZV <sup>a/</sup>	8	3/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3			9	3/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Yellow fever virus <sup>c/</sup>	1	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4			2	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Yellow fever virus <sup>b/</sup>	8	3/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3			9	3/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3

a. Air-washed 25 minutes.

b. Air-washed 10 minutes with ruffling the fur plus 5-minute normal air-wash.

c. Air-washed 15 minutes.

d. Air-washed 10 minutes and towed with 2% quaternary ammonium compound.

C. R. RICKETTSI

Monkeys infected by either whole-body or head-only aerosol exposure, and then air-washed for 15 minutes, transmitted the disease to cagemate control monkeys. However, no infection occurred in the cagemate controls if they were placed with the 15-minute air-washed infected monkeys as late as 48 hours after infection by whole-body exposure or 24 hours after infection by head-only exposure. Air sampling recovered R. rickettsi from cages housing whole-body-challenged monkeys for as long as 24 hours, and from cages housing head-only-challenged monkeys for as long as 16 hours after aerosol exposure.

Ruffling the fur of the aerosol-challenged monkeys prevented transmission of the disease to control monkeys in the first and second (air-duct-connected) cages. R. rickettsi was recovered by air sampling for 2 hours from the first cage and for only 1 hour from the second cage.

Monkeys injected IP, SC, IM, or IV did not transmit the disease to cagemate control monkeys.

D. C. BURNETI

Monkeys infected by either whole-body or head-only aerosol exposure, and then air-washed for 15 minutes, transmitted the disease to cagemate control monkeys and to the controls in the air-duct-connected second cage. Air sampling recovered C. burneti from the first cage housing whole-body-exposed monkeys and from the second cage for as long as 88 and 72 hours respectively, and from the first cage housing head-only-exposed monkeys and its associated second cage for as long as 80 and 56 hours respectively.

Ruffling the fur of the aerosol-challenged monkeys prevented transmission of the disease to control monkeys in the first and second cages. Air sampling recovered C. burneti only from the first cage for 2 hours after whole-body exposure and ruffling the fur of the monkeys; there was no recovery after head-only exposure.

Monkeys injected IP, SC, IM, or IV did not transmit the disease to cagemate control monkeys.

## E. VENEZUELAN EQUINE ENCEPHALITIS VIRUS

Monkeys infected by whole-body aerosol exposure, and then air-washed for 15 minutes, transmitted the disease to cagemate controls and to the controls in the air-duct-connected second cage. When the monkeys received a head-only challenge and a 15-minute air-wash, or received a whole-body challenge and then a 10-minute air-wash followed by a wipe of the entire

body by a towel moistened with 2% quaternary ammonium compound, only the cagemate control became infected; the monkey in the air-duct-connected second cage did not. As in previous experiments, air-ruffling the fur of aerosol-exposed monkeys prevented transmission of the disease to cagemates or monkeys in air-duct-connected cages.

Air sampling recovered VEE virus at the following hours after aerosol exposure:

Experiment	1st Cage	2nd Cage
1	48	40
2	36	32
3	24	12
8	2	1
9	1	0

Monkeys injected IP, SC, IM, or IV did not transmit the disease to cagemate control monkeys.

#### F. YELLOW FEVER VIRUS

Monkeys infected by either whole-body or head-only aerosol exposure, and then air-washed for 15 minutes, transmitted the disease to cagemate control monkeys and to the controls in the air-duct-connected cages. As in previous experiments, air-ruffling the fur of the aerosol-exposed monkeys prevented transmission of the disease to cagemates or monkeys in air-duct-connected second cages.

Air sampling recovered yellow fever virus in Experiment 1 from the first cage for as long as 32 hours after exposure of the monkeys and from the second cage for as long as 24 hours after exposure. In Experiment 2, the virus was recovered from the first cage for as long as 16 hours and from the second cage for as long as 12 hours after exposure of the monkeys. In Experiments 8 and 9, yellow fever virus could not be recovered by air sampling.

Monkeys injected IP, SC, IM, or IV did not transmit the disease to cagemate control monkeys.

#### G. RIFT VALLEY FEVER VIRUS

Monkeys infected by either whole-body or head-only aerosol exposure, and then air-washed for 15 minutes, transmitted the disease to cagemate control monkeys. Cagemate controls were not infected when they were placed with head-only challenged monkeys 24 hours after challenge, nor when they were placed with whole-body challenged monkeys 48 hours after challenge.

Air sampling recovered Rift Valley fever virus from the cages for as long as 8 hours in both experiments.

Air-ruffling the fur of the aerosol-exposed monkeys prevented transmission of the disease to cagemates or monkeys in air-duct-connected cages. Air sampling did not recover Rift Valley fever virus from the cages.

Monkeys injected IP, SC, IM, or IV did not transmit the disease to cagemate control monkeys.

#### H. PSITTACOSIS

Monkeys infected by either whole-body or head-only aerosol exposure, and then air-washed for 15 minutes, transmitted the disease to cagemate control monkeys and to the controls in the air-duct-connected cages. In Experiment 1, air sampling recovered the microorganism for as long as 40 hours from the first cage and for 36 hours from the second cage; in Experiment 2, for 24 hours from the first cage and for 24 hours from the second cage.

Air-ruffling the fur of the aerosol-exposed monkeys prevented transmission of the disease to the control monkeys and resulted in recovery of microorganisms for only 1 hour from the first cage only.

Monkeys injected IP, SC, IM, or IV did not transmit the disease to cagemate control monkeys.

#### IV. DISCUSSION

Experiments during 1944 and 1945 showed that air-washing animals for 5 or 10 minutes after whole-body aerosol exposure prevented infection of cagemate control animals in the cases of Brucella suis with mice and guinea pigs, Malleomyces pseudomallei with hamsters, meningopneumonitis virus (in 9 of 10 mice), and in psittacosis (Borg strain) with mice and P. tularensis with mice.<sup>13</sup> However, later work demonstrated infection of normal cagemates for the following combinations: B. anthracis with guinea pigs and monkeys;<sup>14</sup> Brucella suis with guinea pigs;<sup>15</sup> C. immitis with monkeys;<sup>16</sup> C. burnetii with guinea pigs;<sup>17</sup> influenza virus with swine<sup>18</sup> and mice;<sup>19</sup> Newcastle virus with chickens;<sup>20</sup> Pasteurella pestis with guinea pigs;<sup>21</sup> marmots,<sup>22</sup> and monkeys;<sup>17</sup> Rift Valley fever virus with monkeys;<sup>17</sup> and Mycobacterium tuberculosis with guinea pigs.<sup>23</sup>

Air-washing the monkeys and guinea pigs for 24 minutes after whole-body exposure to an aerosol of B. anthracis spores did not prevent infection of the cagemate controls.<sup>14</sup> When guinea pigs received whole-body exposure to a B. suis aerosol and were then air-washed in a ventilated cage for 1 hour or for 6 days, infection was transmitted to cagemates.<sup>15</sup> This and other experiences led us to a practice of placing each animal in a separate ventilated cage, or caging together only those animals that received the same dosage.

Except after respiratory challenge, parenterally inoculated (as differentiated from naturally infected) monkeys seldom transmit infection to cagemates. A literature survey revealed no instances of this sort, although it seems probable that infection of cagemates would occur after inoculation with M. tuberculosis or monkey B virus.

Cross infection might have occurred during these experiments if the cage had been constructed so that urine and feces did not drop out of reach of the monkeys. From other reports and unpublished work at these laboratories, it is known that urine and feces of infected monkeys contain B. anthracis and VEE virus. There are reports of failure to recover the microorganisms of yellow fever and psittacosis in urine and feces, but no information was found on this point in regard to monkeys infected with R. rickettsii, C. burnetii, or P. tularensis. However, it is surprising how many species of parenterally inoculated animals excrete the organisms in their urine and feces without any resultant infection of normal animals that are held in the same solid-bottomed cage.

It is a good policy to use closed ventilated cages unless experiments have shown that uninoculated cagemates are not infected by aerosol-challenged animals. After 7 days in the ventilated cage, it is usually safe to transfer the animals to ultraviolet-irradiated cages or to open cages. In the absence of cagemate infection, caging of animals inoculated with microorganisms pathogenic for man is permissible on ultraviolet-irradiated cage racks. These have been shown to be effective in reducing organisms released from within the cages.<sup>24</sup> This reduced but continued precaution is deemed necessary because, with few exceptions, all infectious microorganisms that produce illness in experimental animals are excreted in the urine or feces or both.<sup>16</sup> From these, secondary aerosols may arise to infect the animal caretaker. In the absence of cannibalism, infection of cagemates by parenterally injected animals kept in solid-bottomed cages so that urine and feces contaminate food and water is, in our experience, a clear warning of danger for the animal caretaker.

One method of eliminating the need for ventilated cages is ruffling the fur of all monkeys exposed to an aerosol. The animals can be placed in cages that permit urine and feces to drop out of reach. Ultraviolet-irradiation should be used to insure that secondary aerosols that may come from excreta will not penetrate the barrier and infect laboratory personnel.

#### V. SUMMARY

Monkeys were infected by individually exposing the entire body or only the head to an aerosol of *B. anthracis*, psittacosis agent, *C. burnetii*, Rift Valley fever virus, *R. rickettsii*, *P. tularensis*, Venezuelan equine encephalitis virus, or yellow fever virus. When each of these monkeys was placed with an uninoculated control monkey in a ventilated cage constructed so that urine and feces would drop out of reach of the monkeys, the control monkey became infected as a result of inhaling the specific microorganisms shed from the fur of the aerosol challenged monkey. In some instances air from this cage infected a second control monkey in an adjoining air-duct-connected ventilated cage. Air-washing the aerosol-exposed monkey for as long as 25 minutes or wiping it with a disinfectant-moistened towel, or a combination of these two methods, did not prevent infection of the control cagemates. However, in all instances forceful air-ruffling of the fur of the aerosol-exposed monkey for 10 minutes by manipulation of an air jet, followed by a normal 5-minute air-wash, did prevent infection of the control cagemates. This finding opens the way for eliminating the requirement for ventilated cages, so that when the fur is ruffled after aerosol exposure, the monkeys can then be kept in open-front ventilated cages or equivalent, or on ultraviolet-irradiated cage racks, either of which would permit substantial savings in time and equipment.

Monkeys injected with the above microorganisms intraperitoneally, subcutaneously, intramuscularly, or intravenously became infected but did not infect uninoculated cagemates when each injected monkey was housed with one control monkey in an open wire mesh cage constructed so that urine and feces dropped out of reach of the monkeys.

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Unclassified  
Security Classification

DOCUMENT CONTROL DATA - R&D		
<i>(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)</i>		
1. ORIGINATING ACTIVITY (Corporate author)		2a. REPORT SECURITY CLASSIFICATION
U. S. Army Biological Center Fort Detrick, Frederick, Maryland, 21701		Unclassified
		2b. GROUP
3. REPORT TITLE		
INFECTION OF NORMAL MONKEYS BY INOCULATED CAGEMATES		
4. DESCRIPTIVE NOTES (Type of report and inclusive dates)		
5. AUTHOR(S) (Last name, first name, initial)		
Kruse, Richard H.                      Leeder, Wayne D. Green, Theron D.		
6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
	24	24
8a. CONTRACT OR GRANT NO.		8c. ORIGINATOR'S REPORT NUMBER(S)
A. PROJECT NO. IC622401A072		Technical Manuscript 306
c.		8d. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d.		
10. AVAILABILITY/LIMITATION NOTICES		
Qualified requesters may obtain copies of this publication from DDC. Foreign announcement and dissemination of this publication by DDC is not authorized. Release or announcement to the public is not authorized.		
11. SUPPLEMENTARY NOTES		12. SPONSORING MILITARY ACTIVITY
		U.S. Army Biological Center Fort Detrick, Frederick, Maryland, 21701
13. ABSTRACT		
Monkeys were exposed either to a whole-body or to head-only microbial aerosol of <u>Bacillus anthracis</u> , <u>Coxiella burnetii</u> , <u>Pasteurella tularensis</u> , <u>Rickettsia rickettsii</u> , and the causative agents of Venezuelan equine encephalitis, yellow fever, Rift Valley fever, and psittacosis. When they were placed with unexposed control monkeys in ventilated cages they caused infection among the control animals. Infection was caused by the control monkey's inhaling microorganisms shed from the fur of the aerosol-challenged monkey. A new air-washing procedure prevented cross transmission.		

DD FORM 1473

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Security Classification