BACTERIOLOGICAL REVIEWS, Sept., 1966 Vol. 30, No. 3

Printed in U.S.A.

Industrial Inhalation Anthrax

PHILIP S. BRACHMAN, ARNOLD F. KAUFMANN, AND FREDERIC G. DALLDORF

Epidemiology Branch, Communicable Disease Center, U.S. Public Health Service, Atlanta, Georgia, and Department of Pathology, School of Medicine, The University of North Carolina, Chapel Hill, North Carolina

INTRODUCTION

"Woolsorter's disease," or inhalation anthrax, was a serious problem in Europe in the latter part of the 19th century; at least several hundred cases were reported, with an associated high fatality rate. Governmental inquiries conducted in different countries (9, 10, 15, 17) resulted in a series of effective control regulations, including the establishment in Liverpool of a disinfection station (14), where all "dangerous wool and goat hair" had to be decontaminated before being further processed in England.

In the United States, however, inhalation anthrax has never been a serious problem, although workers have routinely been exposed to naturally occurring aerosols while processing imported wool, goat hair, and hides. Since 1900, there have been fewer than 20 cases of inhalation anthrax reported in the United States (13); approximately half of these occurred among individuals with only fleeting contact with materials known to be contaminated. The only reported epidemic of the disease in this country occurred in 1957 among employees at a goat-hair processing mill in Manchester, N.H. (6, 13). Five individuals developed inhalation anthrax over a 10-week period, with four fatalities. Other cases since 1957 have involved a laboratory employee who was accidentally exposed, a secretary in a goat-hair processing mill who entered a highly contaminated area for only a brief moment, and a 27-year-old man with quiescent Boeck's sarcoidosis whose only known contact was in passing the open receiving door of a tannery on his way home from work (7).

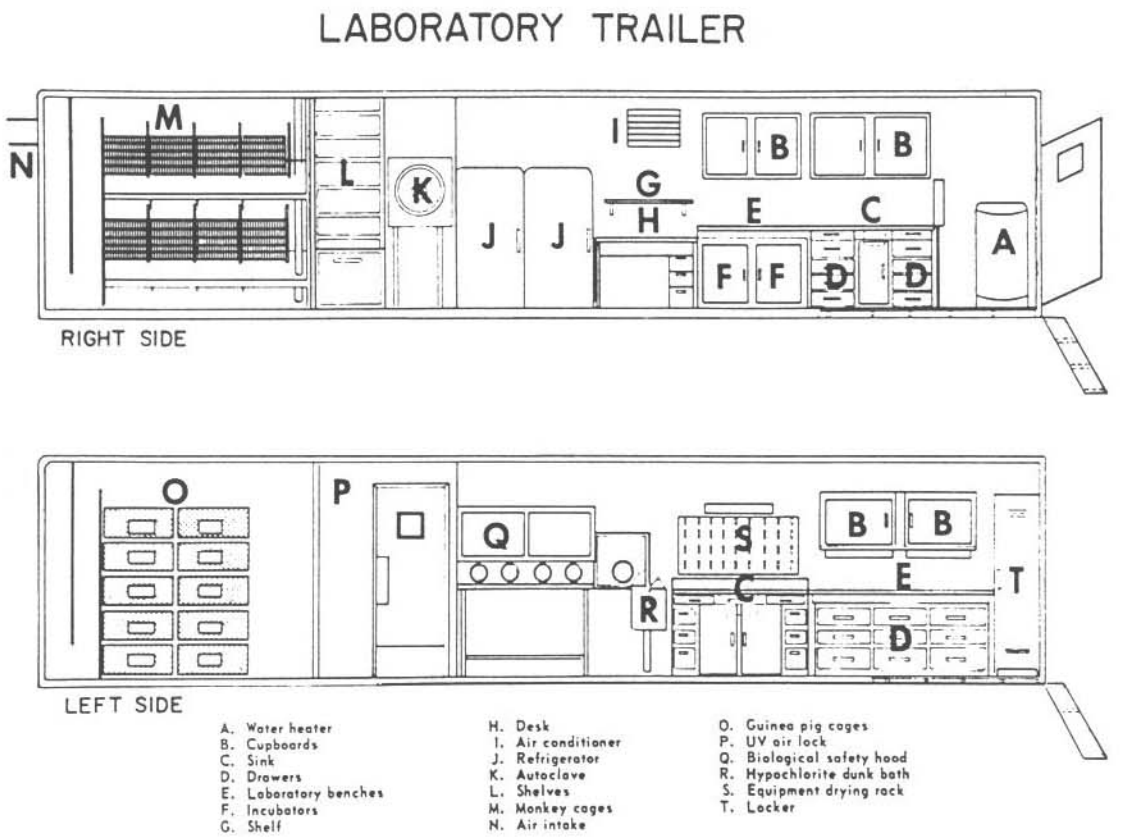
It is not clear why more cases have not occurred in goat-hair and woolen mills and in tanneries, especially among employees working in the dustiest areas where the most concentrated *Bacillus anthracis*-containing aerosols are created. It may be that the dose to which employees are exposed is below the infecting dose for man, or that employees have developed resistance from chronic exposure. It is conceivable that cases have occurred that were not properly diagnosed. Equally unusual has been the sporadic occurrence of cases in people with no industrial exposure. It may be that these individuals are unusually susceptible, as may have been the case with the individual with Boeck's sarcoidosis. More specific information about inhalation anthrax in man is currently difficult to obtain because almost all workers in the high-risk industries within the United States have been immunized (8).

Significant data related to the pathogenesis of the disease and the dose-response relationships have been reported on the basis of animal studies conducted in both this and other countries (1, 2, 4, 16, 19). However, these studies have involved exposures to pure, concentrated aerosols of *B. anthracis* over relatively short periods of time. There have been no reports on the effect of chronic exposure of animals to aerosols containing *B. anthracis*, either homogeneous aerosols, as in laboratory experiments, or heterogeneous aerosols, as in the natural situation in a factory.

At the Conference on Airborne Infection, Riley discussed his studies on airborne tuberculosis in guinea pigs (11), in which he utilized a holding chamber through which air passed from rooms housing patients with sputum-positive, cavitary tuberculosis. Using this physical arrangement as a model, the authors, with Harold Glassman and Elwood Wolfe of Fort Detrick, developed a protocol to study the clinical course, pathogenesis, and dose-response relationships of experimental animals to a naturally occurring *B. anthracis* aerosol produced in a goat-hair processing mill.

MATERIALS AND METHODS

A 40-foot trailer was outfitted at Fort Detrick (under the direction of Harold Curry) as a com-



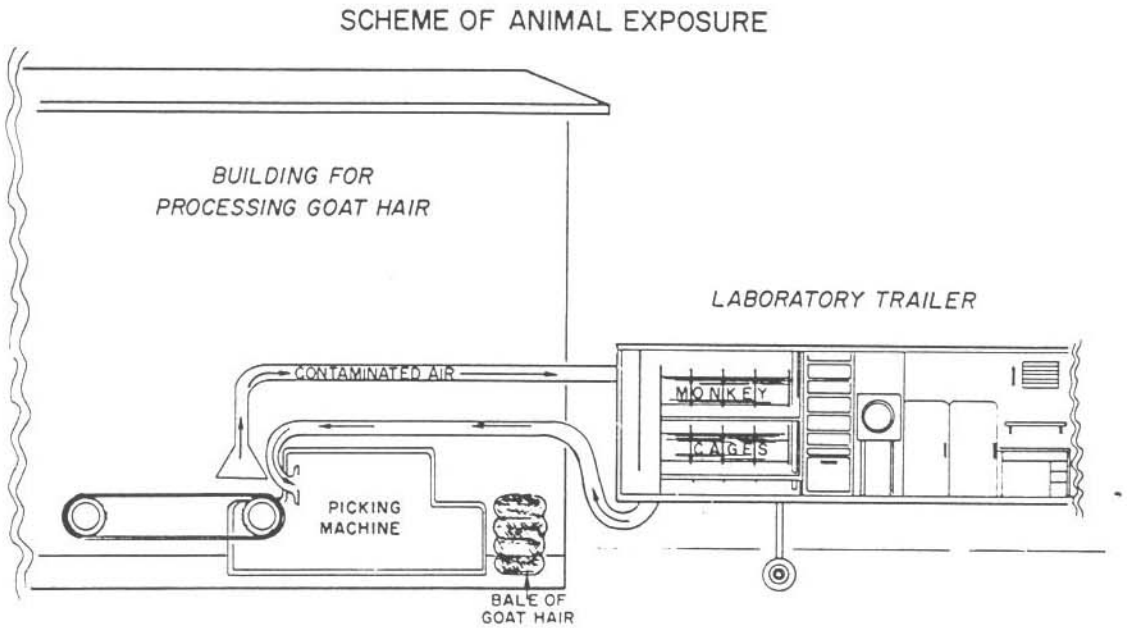


FIG. 1. *Laboratory trailer and scheme of animal exposure.*

bined animal exposure chamber and laboratory and was subsequently located at a mill in South Carolina (Fig. 1). This mill processes goat hair, imported mainly from India and Pakistan, into a woven hair-cloth interlining for suit coats. There were approximately 250 employees, all of whom had been immunized with the Wright anthrax antigen (8). Prior to immunization, 19 cases of cutaneous anthrax had been reported during the 2.5 years the mill had been in operation. Inhalation anthrax had never been reported at this mill.

The aerosol for animal exposure was created around the picking machine, the first machine in the processing cycle, where the clumps of hair were raked apart. The mill usually worked 8 hr a day and 5 days a week, but the picking machine was in operation intermittently during the working day for a total operational time of from 2 to 4 hr. Plastic conduits located in a hood over the picking machine and a suction fan were installed to carry the aerosol from the mill through the animal exposure chamber and back again to the mill (Fig. 1, bottom). A "T" connection made it possible to bring in outside air when the animals were not being exposed to mill air. The trailer was completely self-sufficient except for water and electricity.

As a result of experiences at Fort Detrick, the cynomolgus monkey was selected as the test animal. Preconditioned 3-lb monkeys imported from Asia were used in all runs. All monkeys were tuberculin-negative; if necessary, they were treated for respiratory disease and parasites but not less than 7 days prior to exposure. The monkeys were grouped two or three to a cage and fed a standard diet, fresh fruit, and water *ad libitum*. The temperature in the exposure area was controlled between 22 and 33 C. Monkeys were bled for serological studies before they entered the trailer, at intervals during the exposure period, and at the termination of exposure in the case of survivors. Monkeys were observed at least three

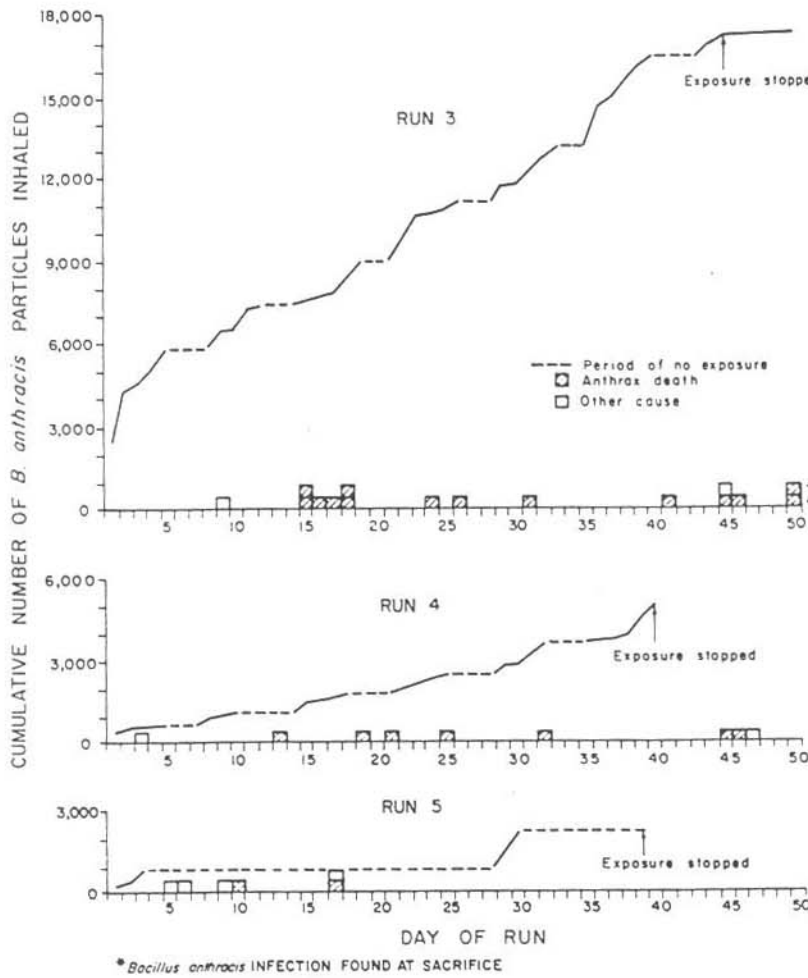


FIG. 2. *Occurrence of deaths by day, from beginning of exposure.*

times a day, and autopsies were performed as soon as possible after death. All surviving animals were sacrificed with an intravenous injection of Nembutal. Postmortem examinations were conducted in general accord with the procedures outlined in the veterinary necropsy protocol of the Armed Forces Institute of Pathology, Washington, D.C. Appropriate cultures were obtained, and the tissue blocks in 10% formaldehyde were returned to Fort Detrick, where Frederic G. Dalldorf, Pathology Division, performed the histological examinations.

With the cooperation of Harry Lefkowitz, Fort Detrick, the protocol for obtaining air samples in the exposure chamber was developed. The all-glass impinger with the British preimpinger was selected as the standard air-sampling equipment to be used (18). Each sampler was run for 20 min, and air samples were obtained throughout all periods during which the monkeys were exposed to mill air. The impinging fluid consisted of 20 ml of gelatin phosphate collecting fluid with 3 drops of a 1:10 dilution of Dow-Corning Antifoam A. The bacterial content of the collected samples was determined by streaking 0.1 ml from the reagitated collecting fluid on each of three 5% human blood-agar plates, which were then incubated at 37 C for 15 to 20 hr. All suspicious colonies were counted, and a representative number were examined by routine bacteriological methods. Calculation of the dose of *B. anthracis*-bearing particles less than 5 μ in diameter inhaled by individual monkeys was based on the various dilution factors, the average number of *B. anthracis* colonies per plate during exposure, and an estimated respiratory rate of 1 liter per min. (All further discussion of the calculated, inhaled dose of *B. anthracis*-bearing

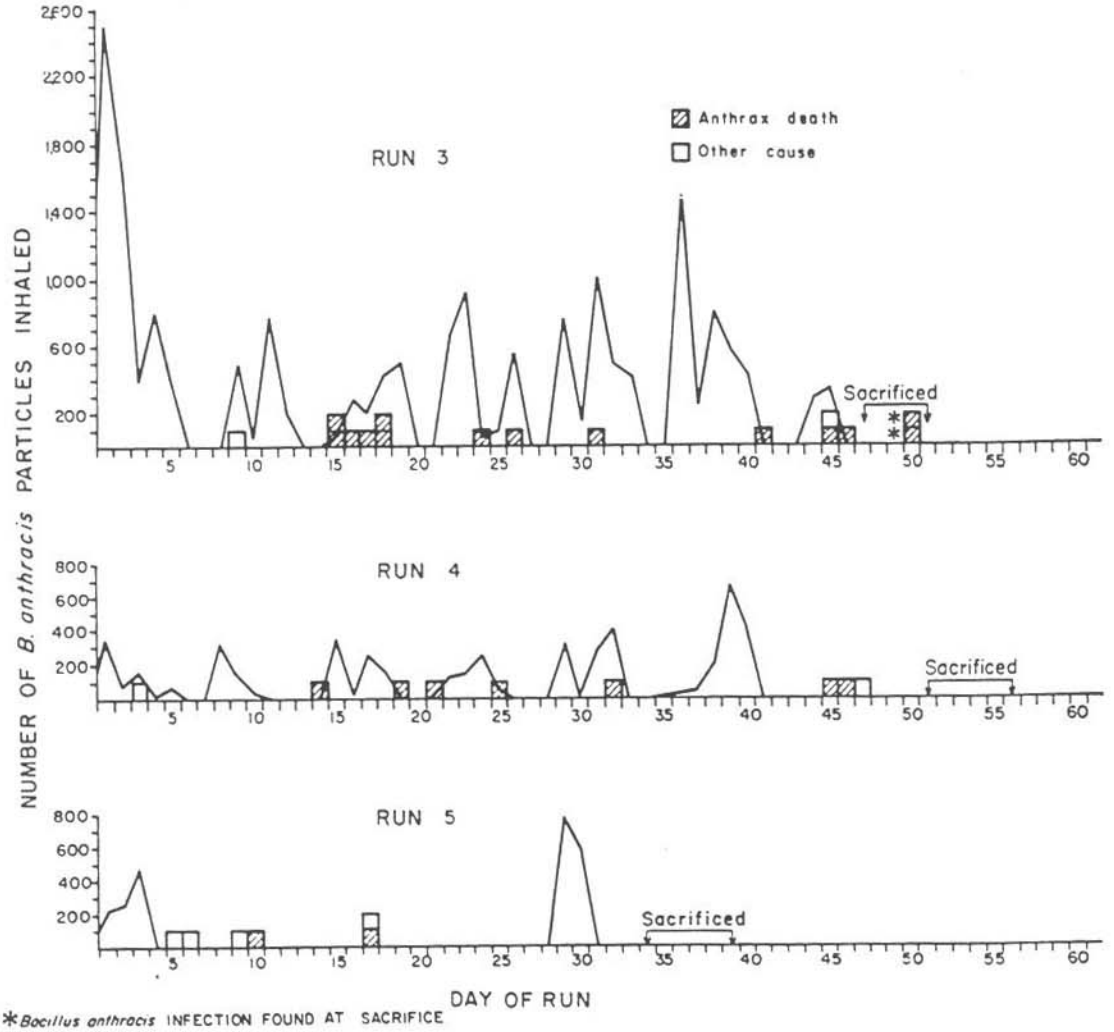


FIG. 3. *Estimated daily dosage (aerosol) per monkey of Bacillus anthracis.*

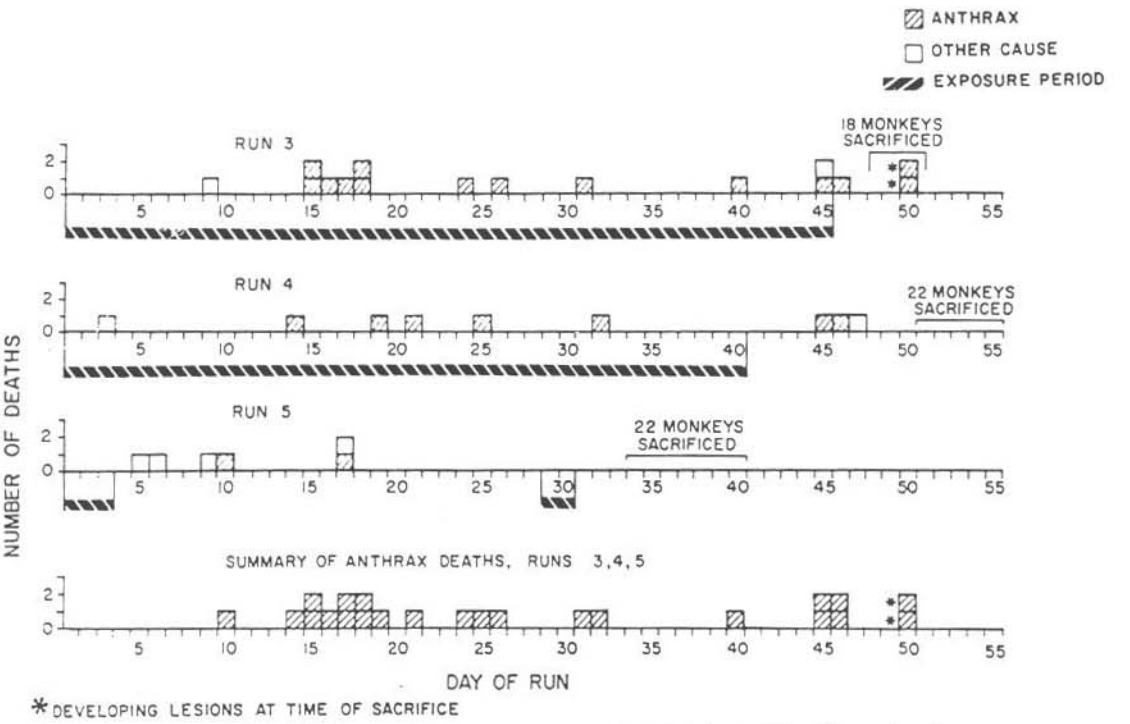


FIG. 4. *Estimated cumulative dosage (aerosol) per monkey of Bacillus anthracis.*

particles refers to those particles less than 5 μ in diameter.)

There were five separate exposure periods extending over 18 months. Because of inadequate preconditioning of the monkeys, difficulties with the environmental control within the exposure chamber, and deficiencies in the collection of air samples and serum specimens, the data from the first two runs were considered to be inadequate for analysis. These technical problems were corrected by changes in the trailer set-up and protocol. The remainder of this discussion will deal with the data developed from the third, fourth, and fifth runs. In the third and fourth runs, the animals were exposed constantly during the working day, regardless of whether the picking machine was in operation, and air samples were collected continuously during this exposure. During the fifth run, exposure was limited to the periods when selected bales of goat hair were actually being picked.

RESULTS

During the third run, 32 monkeys were exposed during 47 consecutive days; 12 of them died of anthrax between the 15th and the 46th day of exposure (Fig. 2, 3, 4, Table 1). Anthrax in 11 of these deaths was diagnosed at necropsy, and, in an additional death, when the microscopic sections were examined. Two monkeys died of other causes. The 18 survivors were sacrificed and examined; two of them evidenced anthrax infection (no. 51 and 50), one by positive blood culture (no. 51), and both by the presence of organisms resembling *B. anthracis* in mediastinal lymph node sections. Fluorescent-antibody studies of sections from these two monkeys confirmed the identification of anthrax organisms in one (no. 51) and were questionably positive in the other (no. 50). The remaining 16 monkeys appeared to be free from any anthrax infection. The final anthrax fatality rate was 43.8%.

Actual exposure occurred during only 32 of the consecutive 47 days of the third run, and the daily mean for these days was 530 *B. anthracis*-bearing particles. Exposure on the 1st day of the third run was greater than any other single day's exposure in all the runs, being calculated at an inhaled dose of 2,500 *B. anthracis*-bearing particles. A cluster of six deaths occurred between 15 and 18 days after this peak exposure. Other deaths were scattered through the remainder of the run. The total calculated inhaled dose for monkeys surviving the exposure was 16,962 *B. anthracis*-bearing particles less than 5 μ in diameter.

During the fourth run, 31 monkeys were exposed over 41 days. Survivors were held for an additional 10 days before being sacrificed. A

TABLE 1. *Summary of the five exposures*

Dates of exposure	Type of exposure	Length of exposure (cumulative days)	Observation period following termination of exposure (days)	Frequency of collection of air samples	Calculated inhaled dose of *Bacillus anthracis*[a]		No. of monkeys	Results				Total anthrax deaths	Mortality rate (%)
					Total cumulative dose	Avg 24 hr dose		Died during exposure		Survivors			
								Anthrax	Other causes	Evidence of anthrax infection	Normal[b]		
1. 28 January through 28 February 1963	Constant	32	None	Sporadic	—[c]	—[c]	17	2	13	0	2	2	11.8[d]
2. 25 March through 10 May 1963	Constant days 1–6; intermittent days 7–20; constant days 21–35	47	None	Sporadic	—[c]	—[c]	20	2	15	0	3	2	10.0[d]
3. 14 October through 29 November 1963	Only when "picking" hair	47	2 to 5	Total	16,962	530	32	12[e]	3	2	16	14	43.8[f]
4. 3 February through 13 March 1964	Only when "picking hair"	41	10 to 15	Total	4,959	198	31	7	2	0	22	7	22.6
5. 1 June through 3 June 1964	Only when "picking" hair	55 hr	25	Total	947	413	28	2	4	—[g]	—[g]	2	7.1
29 June through 30 June 1964	Only when "picking" hair	31 hr	4 to 8	Total	1,347	1,041	22	0	0	0	22	0	0

[a] Particles less than 5 μ in size.
[b] No evidence of anthrax infection.
[c] Data too fragmentary to use for calculations.
[d] Minimal figure.
[e] In one monkey (no. 40), anthrax was considered a contributing cause of death.
[f] Two animals survived and found infected with *B. anthracis* at autopsy.
[g] Survivors re-exposed.

TABLE 2. *Summary of gross findings (third, fourth, fifth runs—monkeys that died of anthrax)*[a]

Run	Animal no.	Sex	Cumulative days after exposure began	Medastinum			Pleural effusion	Parenchymal abnormalities[b]	Adrenal	Spleen	Hemorrhagic meningitis	Culture	Final histopathologic diagnosis
				Edema	Nodes	Paratracheal nodes							
3	52	F	15	+	+	−	+	+	+	+	+	+	Anthrax
	66	F	15	−	−	−	−	+	−	−	−	−[c]	Bronchopneumonia and anthrax pneumonia
	40	F	16	+	+	−	−	−	−	−	−	−	Anthrax; staphylococcal septicemia; cachexia
	63	F	17	+	+	−	+	−	+	+	−	+	Anthrax
	43	F	18	+	+	+	+	+	+	+	−	+	Anthrax
	61	M	18	+	+	+	+	−	+	+	−	+	Anthrax
	44	F	24	+	+	+	+	−	+	+	−	+	Anthrax
	69	M	26	−	±	−	+	+	+	−	+	+	Anthrax pneumonia
	55	F	31	+	+	+	+	+	+	+	−	+	Anthrax
	59	M	39	+	+	+	−	+	+	+	−	+	Anthrax
	49	F	45	+	+	+	+	+	+	+	+	+	Anthrax
	60	M	46	+	+	+	−	+	+	+	−	+	Anthrax
	50[d]	F	50	−	−	−	−	−	−	−	−	−	Anthrax in one lymph node
	51[d]	M	50	−	−	−	−	−	−	−	−	+	Anthrax in two paratracheal lymph nodes
4	80	M	14	−	+	−	−	+	−	?	+	+	Anthrax
	110	M	19	+	+	−	+	+	+	+	?	+	Anthrax
	101	M	21	−	+	−	−	+	?	+	−	+	Anthrax
	100	M	25	+	+	−	+	+	?	+	−	+	Anthrax
	87	F	32	−	+	−	−	+	−	+	−	+	Anthrax
	86	F	45	+	+	−	−	−	+	+	?	+	Anthrax
	92	M	46	−	+	+	−	−	?	+	−	+	Anthrax
5	147	F	10	−	−	−	−	−	?	+	−	+	Anthrax
	151	F	17	+	+	−	+	+	+	+	−	+	Anthrax

[a] Symbols: + = grossly pathologic; − = no significant abnormal findings; ? = questionable abnormal findings.

[b] Hemorrhagic appearance to lungs.

[c] Heavy overgrowth with *Proteus* species.

[d] Sacrificed as normal at end of run.

total of seven monkeys (22.6%) died of anthrax, five during the 41 days of exposure between the 14th and the 46th day, and two during the 10-day postexposure holding period 4 and 5 days after exposure was terminated. Two monkeys died of other causes. None of the sacrificed animals had evidence of anthrax infection. There was actual exposure on 25 days of the total of 41 consecutive days, and the daily mean for these days was 198 *B. anthracis*-bearing particles. The total calculated inhaled dose over the 41-day period was 4,959 *B. anthracis*-bearing particles.

To develop additional specific dose-response data, in the fifth run animals were exposed to as concentrated an aerosol as possible and then held for a prolonged observation period. Accordingly, arrangements were made with the mill to process a maximal number of bales of goat hair through the picking machine as rapidly as possible. Twenty-eight monkeys were initially exposed during three separate periods over three successive days to a calculated aerosol of 947 *B. anthracis*-bearing particles. The animals were then held for an additional 25 days without further exposure to mill air. Two animals died of inhalation anthrax 10 and 17 days after the first day of exposure, and four died of other causes. The limits of the incubation periods for these

TABLE 3. *Summary of microscopic findings—(monkeys that died—third, fourth, and fifth runs)*

Run	Animal no.	Diagnosis	Anthrax bacteremia	Mediastinal lymph nodes[a]	Cervical lymph nodes[a]	Liver necrosis	Splenic necrosis[b]	Anthrax meningitis	Adrenal hemorrhage and necrosis	Anthrax lobar pneumonia	Pulmonary edema[c]	Colonies of bacilli in submucosa of trachea
3	52	Anthrax	+	+++	++	−	+++	+	0	−		−
	66	Bronchopneumonia and anthrax pneumonia	+	++	−	−	+++	−	−	+		−
	40	Anthrax; staphylococcal septicemia; cachexia	−	+	−	−	++	−	−	+		−
	63	Anthrax	+	++	+	+	++	+	+	−		−
	43	Anthrax	+	+++	++	+	+++	+	−	−		−
	61	Anthrax	+	+++	+	−	+	−	0	−		−
	44	Anthrax	+	+++	0	+	+++	−	+	−		−
	69	Anthrax pneumonia	+	+++	−	+	+++	+	+	+		+
	55	Anthrax	+	+++	0	−	+++	−	−	−		−
	59	Anthrax	+	+++	+	+	+++	+	+	−		−
	49	Anthrax	+	+++	+++	+	++	−	+	−		−
	60	Anthrax	+	++	++	−	+++	−	+	−		−
	50	Anthrax in one lymph node	−	++	−	−	+	0	−	−	−	−
	51	Anthrax with two paratracheal lymph nodes	+	++	−	−	−	0	−	−	−	−
4	80	Anthrax	+	+++	+	−	++	+	−	−	+	−
	110	Anthrax	+	+++	+	−	+++	+	+	−	+	−
	101	Anthrax neumonia	+	+++	+	−	+++	−	−	+	++	−
	100	Anthrax	+	+++	+	−	+++	+	−	−	+	−
	87	Anthrax	+	+++	+	−	+++	+	−	−	−	+
	86	Anthrax	+	+++	+	−	++	−	+	−	+	−
	92	Anthrax	+	+++	+	−	+++	−	−	−	−	−
5	147	Anthrax	+	++	+	−	++	−	−	−	−	−
	151	Anthrax	+	+++	+	+	+++	−	−	−	−	−

[a] Key for lymph node morphology: + = anthrax bacilli with follicular necrosis; ++ = bacilli with necrosis and edema; +++ = bacilli with necrosis, edema, and hemorrhage.

[b] Key for splenic morphology: + = sinusoids engorged with neutrophils; ++ = neutrophils in sinusoids plus central necrosis of malpighian bodies; +++ = necrosis of red and white pulp.

[c] Key for pulmonary edema: + = minimal edema; ++ = moderate edema; +++ = marked edema; − = no edema in alveoli. (0 = no tissue submitted.)

two deaths were 7 and 17 days. The fatality rate was 7.2%. Subsequently, the remaining 22 monkeys were exposed during two separate periods over 31 hr to a calculated aerosol of 1,347 *B. anthracis*-bearing particles. No deaths occurred during the following 4-day observation period. The surviving monkeys were sacrificed over a 6-day period, and none revealed evidence of anthrax infection. During the fifth run, 47 guinea pigs were exposed to the same aerosols and held for the same period as the monkeys. None of them died of anthrax.

Dalldorf studied sections from the 91 monkeys on which autopsies had been done. All had lesions attributable to other causes, such as parasites. Twenty-three showed evidence of anthrax infection (Table 2). Twenty died of inhalation anthrax, and in one anthrax was considered a co-primary

cause of death along with staphylococcal septicemia and cachexia due to enteritis. Two monkeys sacrificed at the end of the third run had early infection in the mediastinal lymph nodes only. Nonanthrax deaths were primarily due to pneumonia and enteritis.

The most consistent pathological findings in anthrax-positive monkeys were mediastinal edema, pleural effusion, enlarged hemorrhagic mediastinal lymph nodes, and enlarged, soft spleens. In four instances, gross hemorrhagic meningitis was observed. *B. anthracis* was recovered on culture from all but three infected animals: a sacrificed animal with an early infection, the monkey in which anthrax was considered a contributory cause of death, and a monkey from which the plates prepared at autopsy were heavily overgrown with *Proteus* sp. There was no gross evidence of primary cutaneous or gastrointestinal anthrax, and there were no lesions associated with the oral cavity, including the tonsils.

Histological examination of the tissues showed that infection was largely limited to the reticuloendothelial system, though there was always widespread dissemination of the bacilli through the vascular system at the time of death (Table 3). Tissue response was primarily that of edema, hemorrhage, and necrosis. The mediastinal lymph nodes were infected in all cases, and in a few monkeys the paratracheal lymph nodes were also infected. No primary lesions were found in the trachea or bronchi. In three monkeys, there was evidence of anthrax pneumonia, but this was not considered primary.

Pathological changes noted in other organs as a result of anthrax infection included splenic and hepatic necrosis, adrenal hemorrhage and necrosis, ovarian hemorrhage, and meningitis. The renal glomeruli contained many bacilli, but the kidneys were otherwise normal.

Serological studies were conducted by George Wright, Immunology Branch, Fort Detrick, by use of a micromodification of the Ouchterlony double-diffusion technique. A total of 210 sera, 66 collected before exposure and 144 collected during or after exposure, were tested without demonstration of any antibody titers.

DISCUSSION

The overall fatality rate of 25.3% indicates the susceptibility of the cynomolgus monkey to naturally occurring, industrially produced aerosols containing *Bacillus anthracis* and attests to the feasibility of the experimental design. The objectives initially outlined have been partially attained. The clinical and pathological effects of chronic exposure are not dissimilar to those seen after acute exposure in laboratory experiments.

The pathogenesis of inhalation anthrax after chronic exposure is similar to that postulated after acute exposure of laboratory animals or of man naturally exposed, such as was seen in the Manchester, N.H., epidemic. The necropsy findings of mediastinal edema, mediastinal hemorrhagic lymphadenitis and necrosis, and pleural effusion, without tracheal or bronchial lesions and without primary anthrax pneumonia, support the concept that inhaled *B. anthracis* spores are carried to the mediastinal lymph nodes, where they germinate and produce toxin with development of toxemia and bacteremia. Additional evidence to support this concept is found in the necropsy data from the two sacrificed monkeys in the third run; tissues from these monkeys revealed *B. anthracis*-like organisms primarily in mediastinal lymph nodes. These animals were undoubtedly in the early stages of disease and presumably would have developed systemic disease and died, had the experiment not been terminated. Histological examination of the necropsy tissue from all monkeys that died of anthrax shows widespread dissemination of *B. anthracis* organisms.

The serological studies do not support the development of subclinical infection. Norman et al. (12) studied sera from 72 unvaccinated employees of a goat-hair mill and found 11 who had anthrax antibodies demonstrated by a precipitation inhibition test. Most of the positive reactions occurred among employees who worked in the dustiest part of the mill.

In discussing industrial anthrax, Brachman and Fekety (5) compared the length of employment in goat-hair processing mills of a group of employees who did not have a history of anthrax infection with the length of employment of employees with a history of previous anthrax infection; they found that the two curves were essentially parallel. This suggests that the length of employment did not influence the development of anthrax. They noted that some cases of cutaneous anthrax occurred in employees who had worked in these mills for 15 to 20 years. Their conclusion: "These workers do not develop subclinical infection or immunity to anthrax by prolonged exposure to the organism."

The studies with monkey sera may support these data; however, it is possible that the serological test employed was not sensitive enough to demonstrate antibodies actually present, that the antigen used was not specific for protective antibodies, or that the inhaled dose was too low to stimulate production of demonstrable antibodies.

Data from the fifth run indicate that, with a total exposure to 947 *B. anthracis*-bearing particles intermittently over a 55-hr period, there were two deaths at 10 and 17 days after first exposure, for a fatality rate of 7.2%. The shortest incubation period possible was 7 days, and the longest was 17 days. The second exposure during the fifth run resulted in an inhaled dose of 1,347 *B. anthracis*-bearing particles over 31 hr. The fact that there were no deaths during the 4-day holding period and the 6-day period during which survivors were sacrificed may represent either a lack of susceptible monkeys or, more likely, an inadequate observation period.

Data from the third and fourth runs are more difficult to interpret, because of the irregular, sawtooth pattern of exposures on successive days, without knowing the effect of repetitive exposures on the monkeys. For example, frequent small doses may stimulate an antibody response that increases an animal's resistance to clinical disease, or repeated exposures may build up a level of *B. anthracis* organisms in the body which causes disease when a certain threshold level is reached. Another possibility is that repetitive exposure over a period of several days increases the chances of the animal's acquiring an infecting dose.

As already discussed, the serological studies did not demonstrate the presence of circulating antibodies, which supports the concept that chronic exposure does not lead to development of resistance to infection. The lack of deaths following the second exposure in the fifth run cannot be assumed to represent protection resulting from the first exposure because of the lack of an adequate observation period after the second exposure.

The peak exposure during the first 5 days of the third run, 5,685 *B. anthracis* particles, may be causally related to the six anthrax deaths that occurred from 15 to 18 days after the first day of exposure. If related, the fatality rate was 20% (6 of 30), and the incubation period would then have ranged from 10 to 17 days, which is similar to the incubation period in the fifth run.

Another peak exposure occurred from the 36th to the 40th day, when the surviving 20 monkeys were exposed to 3,525 *B. anthracis*-bearing particles with two deaths occurring from 5 to 11 days after exposure. The two animals found to be infected at autopsy may have become infected as a result of contact with this same aerosol; the incubation period would have been from 10 to 14 days for these two monkeys. If all four deaths are related to the last peak aerosol, the fatality rate would be 20% (4 of 20). It is most likely

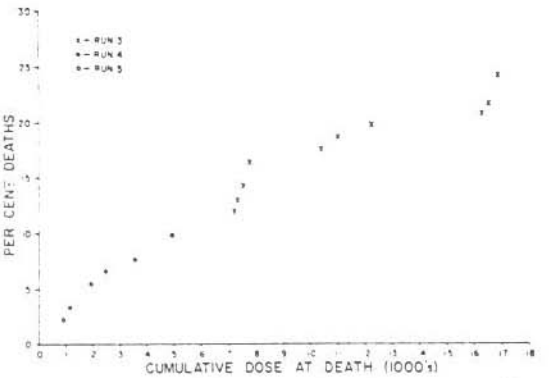


FIG. 5. *Calculated cumulative dose of Bacillus anthracis at time of death.*

that the death on the 41st day was from exposure to the preceding week's aerosol, that is, from days 28 to 33, which would then indicate an incubation period for this particular death of from 8 to 12 days.

The two deaths on successive days at the end of the 4th run (days 45 and 46) occurred 5 to 8 days after a peak exposure to 1,250 *B. anthracis*-bearing particles. If this relationship is correct, then this exposure would be associated with a fatality rate of 8%. The other deaths in the third run and all deaths in the fourth run are harder to associate with specific periods of exposure.

If repeated low-dose exposure results in accumulation of *B. anthracis* organisms until a certain level is reached, after which disease develops, the comparison of the percentage of deaths and the accumulative dose at death should show a straight-line relationship until the critical level is reached, after which there should be a sharp upsurge in the percentage of deaths. As shown in Fig. 5, this is not the case. Additionally, the data were examined to see whether the dose accumulated during specific periods preceding death, that is 7, 10, 12, or 15 days, would suggest an effect of accumulation of *B. anthracis* organisms. Such an analysis is presented in Fig. 6, for which the particles inhaled during the 7 days preceding death are plotted against the day of death of each monkey. Again, the scattering of deaths over a wide range of calculated inhaled doses would tend to be against the theory of accumulation.

Analysis of these data does suggest a dose-response relationship with exposure to approximately 1,000 *B. anthracis*-bearing particles over a 3- to 5-day period, resulting in a fatality rate of approximately 10%. When the exposure is from 3,500 to 5,500 *B. anthracis*-bearing particles over a 5-day period, the rate is from 20 to 25%.

The prolonged incubation periods are un-

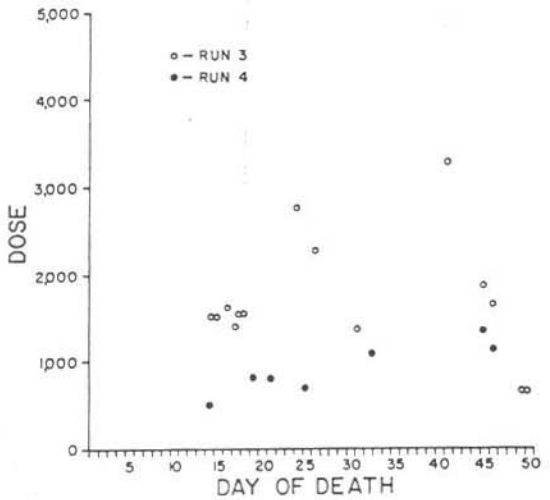


FIG. 6. *Calculated inhaled dose during 7 days preceding death.*

doubtedly related to the low-dose exposure to *B. anthracis*. Exposure to more concentrated, pure aerosols is usually associated with incubation periods of from 3 to 7 days.

Extrapolation of these data to man is difficult. One reason is that man samples a greater proportion of the contaminated mill air because his minute volume is 10 times that of the monkey. There is no reason to suspect any change in the type of goat hair processed or in the method of production from the years before immunization to the present. Thus, the aerosol produced during the three experimental runs is probably representative of the working situation in the picking area at the time when employees were not protected by the anthrax-protective antigen. The lack of cases of inhalation anthrax may therefore represent the lack of exposure to "peak" aerosols as defined above. Also, the monkeys were exposed to a maximal concentration of *B. anthracis* aerosol produced by the picking machine; people, however, are never exposed to the total aerosol produced, but only to a relatively small part of it while they work in the vicinity of the picking machine.

The 1957 epidemic of inhalation anthrax in Manchester, N.H., can possibly be explained by the exposure of the five susceptible individuals to a "peak" aerosol related to a specific batch of hair. In addition, the sporadic cases that have been reported unassociated with the goat-hair processing industry also may represent the chance exposure of susceptible individuals to a "peak" aerosol.

SUMMARY

Exposure of 91 cynomolgus monkeys to naturally produced aerosols containing *B. anthracis* resulted in an anthrax fatality rate of 25.3%. The pathological findings of mediastinal edema and hemorrhagic lymphadenitis and necrosis are similar to findings in animals after acute exposure to pure aerosols of *B. anthracis*, and also to the findings in humans who have developed fatal inhalation anthrax after industrial or sporadic exposure. With the low-dose chronic exposure to natural aerosols, the incubation period appears to range from 5 to 17 days.

Analysis of the data suggests a dose-response relationship with fatality rates ranging from 10 to 25% after exposure to from 1,000 to 5,500 organisms over 3 to 5 days. There is no specific evidence to support the development of subclinical infection, or of an accumulative effect of anthrax organisms. These studies do support previous concepts concerning the pathogenesis and dose-response relationships of anthrax in monkeys.

ACKNOWLEDGMENTS

This investigation was supported by a contract with the U.S. Army Biological Center, Fort Detrick, Frederick, Md.

We acknowledge the capable assistance of Peter Skaliy and Karl Branch, Sr., Biophysics Section, Technology Branch, Communicable Disease Center, in decontaminating the exposure chamber between runs, and of Eli Friedman and W. Eugene Sanders, Jr., former EIS Officers, Epidemiology Branch, in developing the initial protocol and conducting the initial runs.

LITERATURE CITED

1. ALBRINK, W. S., AND R. J. GOODLOW. 1959. Experimental inhalation anthrax in the chimpanzee. Am. J. Pathol. **35**:1055–1065.
2. BARNES, J. M. 1947. The development of anthrax following the administration of spores by inhalation. Brit. J. Exptl. Pathol. **28**:385–394.
3. BELL, J. H. 1880. On woolsorters disease. Lancet **1**:871.
4. BERDJIS, C. C., C. A. GLEISER, H. A. HARTMAN, R. W. KUEHNE, AND W. S. GOCHENOUR. 1962. Pathogenesis of respiratory anthrax in macaca mulatta. Brit. J. Exptl. Pathol. **43**:515–524.
5. BRACHMAN, P. S., AND F. R. FEKETY. 1958. Industrial anthrax. Ann. N.Y. Acad. Sci. **70**: 574–584.
6. BRACHMAN, P. S., S. A. PLOTKIN, F. H. BUMFORD, AND M. M. ATCHISON. 1960. An epidemic of inhalation anthrax: the first in the 20th century. II. Epidemiology. Am. J. Hyg. **72**:6–23.
7. BRACHMAN, P. S., J. S. PAGANO, AND W. S. ALBRINK. 1961. Two cases of fatal inhalation anthrax, one associated with sarcoidosis. New Engl. J. Med. **265**:203–208.

8. BRACHMAN, P. S., H. GOLD, S. A. PLOTKIN, F. R. FEKETY, M. WERRIN, AND N. R. INGRAHAM. 1962. Field evaluation of a human anthrax vaccine. Am. J. Public Health **52**:632–645.
9. EPPINGER, H. 1894. The rag pickers disease. A typical respiratory infection of man. Gustav Fischer, Jena.
10. GREENFIELD, W. S. 1882. Supplementary report on the woolsorters disease in the Bradford District. Eleventh annual report of the Medical Office of the local government board, London, p. 207–238.
11. RILEY, R. L. 1961. Airborne pulmonary tuberculosis. Bacteriol. Rev. **25**:243–248.
12. NORMAN, P. S., J. G. RAY, P. S. BRACHMAN, S. A. PLOTKIN, AND J. S. PAGANO. 1960. Serological testing for anthrax antibodies in workers in a goat hair processing mill. Am. J. Hyg. **72**:32–37.
13. PLOTKIN, S. A., P. S. BRACHMAN, M. UTELL, F. H. BUMFORD, AND M. M. ATCHISON. 1960. An epidemic of inhalation anthrax, the first in the 20th century. I. Clinical features. Am. J. Med. **29**:992–1001.
14. Prevention of anthrax among industrial workers, memorandum on the disinfecting station established in Great Britain for disinfection of wool and hair, p. 1–5. 1921. His Majesty's Stationery Office, London.
15. Report of the Departmental Committee appointed to inquire as to precautions for preventing danger of infection from anthrax in the manipulation of wool, goat hair, and camel hair, vol. 1, 2, and 3. 1918. His Majesty's Stationery Office, London.
16. Ross, J. M. 1957. The pathogenesis of anthrax following the administration of spores by the respiratory route. J. Pathol. Bacteriol. **73**:485–494.
17. SPEAR, W. 1880. Tenth annual report of the local government board. London.
18. WOLF, H. W., P. SKALIY, L. B. HALL, M. M. HARRIS, H. M. DECKER, L. M. BUCHANAN, AND C. M. DAHLGREN. 1959. Public Health Monograph No. 60, U.S. Government Printing Office, Washington, D.C.
19. YOUNG, G. A., JR., M. R. ZELLE, AND R. E. LINCOLN. 1946. Respiratory pathogenicity of *Bacillus anthracis* spores. I. Methods of study and observation on pathogenesis. J. Infect. Diseases **79**:233–246.

Discussion

HAROLD N. GLASSMAN

U.S. Army Biological Laboratories, Fort Detrick, Frederick, Maryland

Industrially acquired anthrax has been associated with transportation and processing of imported wool, hides, and goat hair. The spores of *Bacillus anthracis* have been cultivated from as many as 50% of samples of the raw products (4, 15, 17). Industrial processing creates dust, so that anthrax spores regularly contaminate the surfaces and air of the factories; up to 66% of surface samples were positive in three mills processing goat hair (5). Respiratory exposure of workers may reach 510 spores in particles 5 μ or less in diameter in an 8-hr working period (9). A significant percentage of anterior nasal swabs and pharyngeal washings from mill workers processing goat hair yielded *B. anthracis* (8).

Despite this, inhalation anthrax is rare in the United States (7). Brachman et al. (6) studied the response of the cynomolgus monkey exposed chronically to the air from the dustiest portion of a goat-hair processing mill in an effort to enlarge our understanding of industrial anthrax. It is my purpose to assist in this objective by relating their observations to selected laboratory studies. Specifically, I will consider the variable incubation period they observed, and the dose-response relationship.

Modern views of the pathogenesis of inhalation anthrax are based on the studies in laboratory animals of the experimentally induced disease by Young et al. (19), Barnes (3), Ross (16), Albrink and Goodlow (2), and Gleiser et al. (10), and the pathological findings in three fatal cases in man reported by Albrink et al. (1). The observations of these investigators agree in defining the role of the lung as a portal of entry in inhalation anthrax; primary anthrax lesions are not found in the trachea or bronchi, at least not in the absence of pre-existing lung lesions. Thus, we may visualize spore-bearing, airborne particulate matter of sufficiently small size (i.e., 5 μ in diameter, or less) after inhalation, penetrating to the deep recesses of the lungs and being deposited there as essentially inert particles. Subsequent removal of the spores is accomplished principally by alveolar macrophages that transport them via the lymphatics to the regional

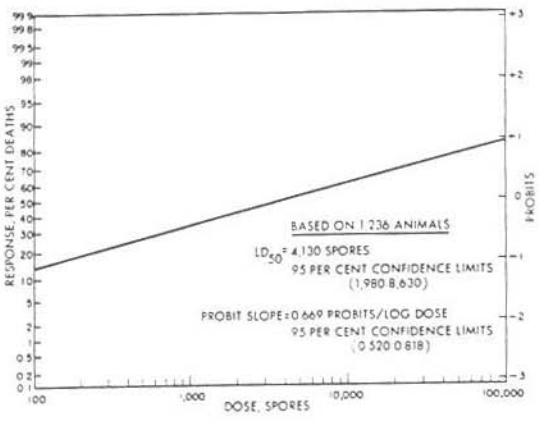


FIG. 1. *Response of the cynomolgus monkey to aerosols of Bacillus anthracis.*

lymph nodes. Neglecting, for our present discussion, the further steps in the pathogenesis of inhalation anthrax, it seems reasonable that a significant period of time will be required for clearance of a large number of spores, even if these are introduced in acute experimental exposures. The respiratory retention of inhaled spores was initially studied by Harper and Morton (12), using radioactively labeled *Bacillus subtilis*. When these were presented to guinea pigs for inhalation as particles 1 μ in diameter, the majority of the radioactivity was found in the lungs (as contrasted with the head or the trachea), and essentially no loss of radioactivity of the lungs was measurable over a 24-hr observation period. Subsequently, in studies of the prophylaxis of inhalation anthrax in the rhesus monkey, Henderson et al. (13) demonstrated that a daily regimen of procaine penicillin, initiated 24 hr subsequent to aerosol exposure, could delay the onset of disease and death, and that this protection ceased promptly on termination of the therapy. They also showed that spores of *B. anthracis* can be detected for as long as 100 days after their deposition onto the lung epithelium. In a more recent similar study, Gochenour et al. (11) provided further direct evidence of prolonged spore retention in the lungs after an acute inhalatory exposure. One of their monkeys died with anthrax meningitis 25 days after completion of an apparently successful course of therapy. Cultures of the lungs of all animals surviving 55 to 84 days after exposure to aerosols of anthrax spores were positive for this organism. Finally, one of my colleagues, Joseph V. Jemski, has made available information he obtained several years ago in making a study of the time to death of the cynomolgus monkey after inhalation of aerosolized anthrax spores. Several of the animals in that study, which was directed toward determining the minimal holding period required to assure statistically valid dose-response data, died of culturally proven anthrax after prolonged incubation periods—one animal succumbed 98 days subsequent to exposure.

The concepts involved in the pathogenesis of inhalation anthrax, as well as the experimental evidence cited above regarding the prolonged retention of spores in the lungs, are completely consonant with the variable incubation periods reported by Brachman et al. (6) in their studies of chronic exposure to varying doses over many days. An additional pertinent laboratory observation has been the dose dependency of the incubation period, with lower inhaled doses of spores resulting in longer incubation times (10).

In considering the dose-response relationship of the cynomolgus monkey in experimentally induced inhalation anthrax, I am again indebted to Dr. Jemski for placing at my disposal hitherto unpublished data. These represent a compilation of the results of several individual experiments in which large numbers of cynomolgus monkeys were acutely exposed (1 to 10 min) to heterogeneously sized aerosols of anthrax spores. The aerosol clouds were sampled with an impinger preceded by a preimpinger, the latter device screening out the majority of particles greater than 5 μ in diameter (14, 18). Thus, the dose reported, after microbiological assay of the collection fluid of the impinger, represents spores present in particles predominantly 5 μ in diameter, or less. This dose, and the mortality of the monkeys from culturally proven anthrax during a 10-day observation period subsequent to aerosol exposure, have been subjected to statistical analysis by the probit method (Fig. 1).

It will be noted that the median lethal dose (LD_{50}) based upon a total of 1,236 animals is 4,130 spores with 95% confidence limits of 1,980 to 8,630 spores. The probit slope is 0.669 probits per log dose, with 95% confidence limits of 0.520 to 0.818. As a consequence of this unusually low probit slope, large changes in the dose of inhaled spores will result in comparatively small changes in the per cent mortality. For example, a 100-fold range of dose (10-fold above and 10-fold below the calculated LD_{50}) will only change the predicted mortality from 25 to 75%.

These laboratory studies of Jemski et al. have many similarities to the experimental epidemiological investigations of Brachman et al. (6). There are, however, several important differences. The former involved a very large number of animals, acutely exposed to laboratory-grown spores, under well-controlled conditions for

experimental airborne infection. The latter represented the chronic exposure, over many days, of a small group of animals to an uncontrolled, industrially generated aerosol of spores that were present as a result of the industrial use of contaminated animal products. Nevertheless, the dose-response relationship derived from the laboratory data was predictive of the field results, if the cumulative dose of spores inhaled by the monkeys is considered the most important factor in the chronic exposures [see particularly Fig. 4 and 5 of Brachman et al. (6)].

Precise comparisons of the laboratory observations and the field studies are not possible for reasons already mentioned. For the same reasons, conclusions from consideration of both sets of investigations must be drawn with caution. With this caveat firmly in mind, it does seem that the following statements are justified. (i) The dose-response relationships determined with the cynomolgus monkey in the laboratory permitted prediction of the outcome when the same species was exposed chronically to a contaminated industrial atmosphere. (ii) The adequacy of the cynomolgus monkey as a model for predicting the quantitative aspects of the response of man in the industrial environment is open to reasonable doubt.

LITERATURE CITED

1. ALBRINK, W. S., S. M. BROOKS, R. E. BIRON, AND M. KOPEL. 1960. Human inhalation anthrax. A report of three fatal cases. Am. J. Pathol. **36**:457–471.
2. ALBRINK, W. S., AND R. J. GOODLOW. 1959. Experimental inhalation anthrax in the chimpanzee. Am. J. Pathol. **35**:1055–1065.
3. BARNES, J. M. 1947. The development of anthrax following the administration of spores by inhalation. Brit. J. Exptl. Pathol. **28**:385–394.
4. BIEGELEISEN, J. Z., JR., W. B. CHERRY, P. SKALIY, AND M. D. MOODY. 1962. The demonstration of *Bacillus anthracis* in environmental specimens by conventional and fluorescent antibody techniques. Am. J. Hyg. **75**:230–239.
5. BRACHMAN, P. S., AND F. R. FEKETY. 1958. Industrial anthrax. Ann. N.Y. Acad. Sci. **70**: 574–584.
6. BRACHMAN, P. S., A. F. KAUFMANN, AND F. G. DALLDORF. 1966. Industrial inhalation anthrax. Bacteriol. Rev. **30**:646–657.
7. BRACHMAN, P. S., S. A. PLOTKIN, F. H. BUMFORD, AND M. M. ATCHISON. 1960. An epidemic of inhalation anthrax: the first in the twentieth century. II. Epidemiology. Am. J. Hyg. **72**: 6–23.
8. CARR, E. A., JR., AND R. R. REW. 1957. Recovery of *Bacillus anthracis* from the nose and throat of apparently healthy workers. J. Infect. Diseases **100**:169–171.
9. DAHLGREN, C. M., L. M. BUCHANAN, H. M. DECKER, S. W. FREED, C. R. PHILLIPS, AND P. S. BRACHMAN. 1960. *Bacillus anthracis* aerosols in goat hair processing mills. Am. J. Hyg. **72**: 24–31.
10. GLEISER, C. A., C. C. BERDJIS, H. A. HARTMAN, AND W. S. GOCHENOUR, JR. 1963. Pathology of experimental respiratory anthrax in *Macaca mulatta*. Brit. J. Exptl. Pathol. **44**:416–426.
11. GOCHENOUR, W. S., JR., W. D. SAWYER, J. E. HENDERSON, C. A. GLEISER, R. W. KUEHNE, AND W. D. TIGERTT. 1963. On the recognition and therapy of simian woolsorter's disease. J. Hyg. **61**:317–322.
12. HARPER, G. J., AND J. D. MORTON. 1953. The respiratory retention of bacterial aerosols: experiments with radioactive spores. J. Hyg. **51**:372–385.
13. HENDERSON, D. W., S. PEACOCK, AND F. C. BELTON. 1956. Observations on the prophylaxis of experimental pulmonary anthrax in the monkey. J. Hyg. **54**:28–36.
14. MAY, K. R., AND H. A. DRUETT. 1953. The preimpinger, a selective aerosol sampler. Brit. J. Ind. Med. **10**:142–151.
15. MINISTRY OF LABOUR. 1959. Report of the committee of inquiry on anthrax. Her Majesty's Stationery Office, London.
16. ROSS, J. M. 1957. The pathogenesis of anthrax following the administration of spores by the respiratory route. J. Pathol. Bacteriol. **73**: 485–494.
17. SEMPLE, A. B., AND T. L. HOBDAY. 1959. Control of anthrax—suggestions based on survey of imported hides. Lancet **2**:507–508.
18. WOLFE, E. K., JR. 1961. Quantitative characterization of aerosols. Bacteriol. Rev. **25**:194–202.
19. YOUNG, G. A., JR., M. R. ZELLE, AND R. E. LINCOLN. 1946. Respiratory pathogenicity of *Bacillus anthracis* spores. I. Methods of study and observations on pathogenesis. J. Infect. Diseases **79**:233–246.