INFECTION AND IMMUNITY, Nov. 1970, p. 574-582

Vol. 2, No. 5
Printed in U.S.A.

Aerosol-Induced Tuberculosis in Subhuman Primates and the Course of the Disease After Intravenous BCG Vaccination

W. R. BARCLAY,[1] R. L. ANACKER, W. BREHMER,[2] W. LEIF, AND E. RIBI

Rocky Mountain Laboratory, National Institute of Allergy and Infectious Diseases, Hamilton, Montana 59840, and Naval Biological Laboratory, Oakland, California 94625

Received for publication 6 July 1970

Suitability of the rhesus monkey (*Macaca mulatta*) as an experimental host for evaluation of vaccines against airborne infection with *Mycobacterium tuberculosis* strain H37Rv was investigated. Nonvaccinated monkeys were exposed to estimated doses of 12, 25, or 49 units of H37Rv in a modified Henderson apparatus, and the course of the disease was followed by chest X rays, skin testing with purified protein derivative, body-weight determinations, and autopsy 8 weeks postinfection. These animals developed progressive and extensive tuberculosis with pathological changes proportional to the infecting dose. Four of seven monkeys vaccinated intravenously with 1 mg of live BCG 8 weeks prior to challenge with 40 units of H37Rv had no gross evidence of disease at autopsy 13 weeks postinfection; the other three monkeys had minimal disease. These data demonstrated that (i) reproducible and progressive infection could be induced in rhesus monkeys infected in a manner which simulated natural infection of man and (ii) a high level of resistance to infection could be induced by BCG vaccine in the rhesus monkey, which in nature is highly susceptible to tuberculous infection.

Many animal models have been employed to study immunity against tuberculosis. However, as Smith et al. (16) have pointed out, there is a distressing lack of agreement in results obtained with different species of animals used to evaluate viable and nonviable vaccines. Just as animals differ in their responses to vaccination and to infection with virulent tubercle bacilli, the responses of all other species differ from those of man.

The high susceptibility of monkeys to pulmonary tuberculosis is well known. Epidemics of this disease frequently occur in primate colonies maintained for biological research, with extensive transmission throughout the colony and a high mortality rate (2, 9, 15). Tuberculosis has been experimentally induced by instilling suspensions of virulent tubercle bacilli in the tracheae of anesthetized monkeys (14). This experimental model proved useful for the study of antituberculosis drugs and demonstrated that caseation necrosis with cavity formation was common in untreated control animals.

[1]Permanent address: American Medical Association, 535 No. Dearborn St., Chicago, Ill. 60610.

[2]Permanent address: Robert Koch Institute, West Berlin, Germany.

The rhesus monkey was recently used in vaccine-potency tests (6), but in these tests the animals were challenged with large numbers (approximately 1,000) of virulent tubercle bacilli by the intratracheal route. Massive infection by endotracheal instillation does not simulate natural infection in which a few organisms are inhaled and deposited in the extreme periphery of the lung.

Although no one animal model may be entirely suitable for estimating potency of a particular vaccine for man, probably any vaccine which appreciably protects all species of laboratory animals against experimental infection would also have a significant protective effect in man. Oil-treated cell walls of several mycobacteria enhanced resistance of mice to both airborne and intravenous challenge with *Mycobacterium tuberculosis* strain H37Rv (3, 12, 13). Mycobacterial cell walls also afforded protection to rabbits (11). These successes encouraged us to determine the protective potency of cell walls in the rhesus monkey (*Macaca mulatta*) against airborne challenge with a few organisms of H37Rv.

Before we could properly test mycobacterial cell wall vaccine in monkeys, we had to determine (i) the progression and extent of disease in mon-

keys challenged by aerosol with a few organisms of H37Rv in a manner which simulated natural infection of man and (ii) whether monkeys could be protected against airborne infection by the standard antituberculosis vaccine, live BCG. This experiment was designed to show the maximum possible difference between immunized and nonimmunized animals. The live BCG was administered intravenously (iv) to monkeys because earlier results with mice indicated that BCG was most effective by this route (1), and it was anticipated that the same would be true for the rhesus monkey. This expectation was substantiated in subsequent experiments which will be reported later. The results reported here demonstrated that consistent and progressive tuberculosis could be produced in monkeys challenged with a few virulent organisms and that, after iv vaccination with BCG, the monkeys developed highly significant resistance to infection.

MATERIALS AND METHODS

Animals. Rhesus monkeys from Woodard Asiatic Corp., Herndon, Va., were quarantined for a minimum of 4 weeks at the Naval Biological Laboratory (NBL) in Oakland, Calif., where all experiments were conducted. At the beginning and end of the quarantine period, the animals were skin tested with 10 tuberculin units (TU) of purified protein derivative (PPD) and X-rayed. All animals were free of disease before use, as judged by these criteria. Throughout the experiments, the monkeys were fed Purina Monkey Food and water ad libitum.

The animals were infected by exposure to aerosol containing virulent tubercle bacilli in a Henderson apparatus as modified by Wolochow et al. (17). It consisted of a rectangular duct through which the aerosol flowed and into which the heads of eight monkeys projected. A frozen preparation of H37Rv cells preserved according to the method described by Grover et al. (7) was thawed and diluted in Dubos Tween-albumin medium and atomized in an NBL Mk I atomizer. The aerosol was diluted by mixture with clean air and conducted through the exposure chamber at a rate of 4 ft^3/min.

While the animals were being exposed, samples of the aerosol were collected on Dubos Tween-albumin medium at three slit samplers (17). An estimate of the dosage received by the monkeys was obtained by calculating the total volume of air they inhaled during an 8-min period. This volume (0.32 ml per min per g) was calculated from data of Guyton (8). The volume of aerosol collected by each sampler was about 10 times that inhaled by a monkey, giving a statistical advantage desirable for dealing with low dosages.

Once infected, the animals were housed individually in isolation cages maintained at a small negative pressure. Air was filtered through two layers of fiberglass (Owens Corning Fiberglass Co.) before being drawn into the cages. The room was constantly flooded with ultraviolet light. All effluent air was passed through a Flanders Co. no. 7H70-L-D-N2N2 filter before being exhausted outdoors. Animal caretakers were dressed in protective suits which were individually ventilated by a hose attached to an independent air supply.

Beginning 4 to 6 weeks after challenge, infected animals were skin tested at weekly intervals with 10 TU of PPD on the abdomen or intrapalpebrally until a positive test was observed 48 hr later or the experiment was terminated. Chest X rays were made every 2 weeks. In the preliminary experiment to study the effect of the size of infecting dose, all animals were autopsied 8 weeks postinfection. In the other experiment, animals were tested for an immune response to BCG given iv and autopsied 13 weeks postinfection.

At autopsy, lungs and heart were removed in toto, and the lungs were inflated with a 10% solution of buffered Formalin instilled through the trachea. The trachea was then tied off and the specimen was submerged in 10% Formalin. After 1 week of fixation, the Formalin was replaced with 70% alcohol. Specimens were then suitable for photographing, counting lesions, and sectioning.

Tuberculosis at autopsy was scored by the following arbitrary scheme designed, on the basis of our experience with rhesus monkeys infected by the pulmonary route in these and subsequent experiments, to provide us with a convenient measure to compare the resistance of experimental monkeys treated in various ways. This scheme differs somewhat from that devised by Feldman (5) to evaluate disease in guinea pigs challenged with tubercle bacilli by the subcutaneous route.

Disease in lung. Disease in the lung was scored as follows: 0, no disease; 10, number of tubercles $\leq$ 25% of number of bacilli inhaled; 20, number of tubercles = 26 to 50% of number of bacilli inhaled; 30, number of tubercles = 51 to 75% of number of bacilli inhaled; 40, number of tubercles $\geq$ 76% of number of bacilli inhaled; 50, tuberculosis pneumonia, cavitation, or consolidation; and 60, lung destroyed.

Disease in tracheobronchial nodes (lymphatic spread). Tracheobronchial disease was scored as follows: 0, no disease; 10, moderate enlargement; and 20, extensive enlargement.

Disease in liver, spleen, or kidney (hematogenous spread). Hematogenous spread was scored as follows: 0, no disease in any of these organs and 20, at least one tubercle in any of these organs.

According to this scheme, 60% of the maximum possible score of 100 is assigned to the lung, the organ primarily invaded by the challenge organisms. The score of the lung is based in part on the percentage of inhaled bacilli which produced pulmonary tubercles so that the size of the infecting dose can be changed when desired. An additional 20 points may be scored according to the extent of spread of the bacteria by the lympatic route from initial foci of infection to regional tracheobronchial nodes. Under the conditions of these experiments, spread of tubercle bacilli by the hematogenous route, as evidenced by the presence of grossly observable tubercles in spleen, liver, or kidney, was usually erratic and very minor in degree. Therefore, any monkey which had at least one tubercle in any of these three organs received an additional score of 20. In summary, this scheme gives a numerical score to

those features of tuberculosis which are regarded as highly significant in establishing and perpetuating the disease in humans.

RESULTS

Response of nonvaccinated monkeys to graded doses of H37Rv. Twelve monkeys with a mean weight of 2.35 kg were divided into three groups of four animals and exposed to a calculated dose of 12, 25, or 49 infectious units of H37Rv. During an 8-week period, the animals did not lose weight or appear ill; the mean body weight for animals in the three groups increased approximately 0.4 kg during the experiment.

Their responses to 10 TU of PPD injected intradermally on the abdomen is shown in Table 1. Conversion from a negative to a positive Mantoux test was related to the infecting dose; three of four animals in the high-dose group became positive and none of the four animals in the low-dose group definitely converted. However, the number of animals in this experiment was too small for meaningful conclusions to be drawn, and contradictory relationships appeared between severity of infection and delayed hypersensitivity. The most severe tuberculosis was in monkey 10 which was tuberculin-negative; the least disease was in monkey 6 which became tuberculin-positive in the fifth week.

Chest X rays were made prior to infection and 4, 6, and 8 weeks thereafter. The earliest and most consistent finding was enlargement of the mediastinal lymph glands. A dose response was clearly evident: monkeys in the low-dose group had only questionable X-ray evidence of disease, even at 8 weeks; recipients of the intermediate dose had X-ray evidence of disease at 6 and 8 weeks; monkeys in the high-dose group had lesions detectable with X rays at 4 weeks and a progression of the disease between the 4-, 6- and 8-week films. One monkey had a pleural effusion detectable by X-ray, and this was confirmed as an extensive, caseating pleuritis on autopsy.

TABLE 1. *Abdominal skin reactions to 10 tuberculin units of purified protein derivative in rhesus monkeys infected with Mycobacterium tuberculosis H37Rv by aerosol*[a]

Estimated challenge dose	Monkey	Time (weeks)						No. positive/no. tested
		0	4	5	6	7	8	
12 IU[b]	1	−	−	±	−	−	±	0/4
	2	−	−	−	−	−	−	
	3	−	−	−	−	−	−	
	4	−	−	−	−	±	±	
25 IU	5	−	−	−	−	−	−	2/4
	6	−	−	1+[c]				
	7	−	−	1+				
	8	−	−	−	−	−	−	
49 IU	9	−	−	−	1+			3/4
	10	−	−	±	±	−	−	
	11	−	−	1+				
	12	−	−	1+				

[a] Reactions were scored as follows: − = negative, ± = doubtful, 1+ = 5 mm induration, 2+ = 5 mm induration and erythema, 3+ = 10 mm induration and erythema, and 4+ = 15 mm induration and erythema.

[b] IU, infectious units.

[c] Positive animals not retested.

TABLE 2. *Number of grossly visible or palpable lesions in lungs of rhesus monkeys 8 weeks after infection with Mycobacterium tuberculosis H37Rv by aerosol*[a]

Animal	Estimated challenge dose	Right upper lobe	Right middle lobe	Right accessory lobe	Right lower lobe	Left upper lobe	Left lower lobe	Total	Mean
1	12 IU[b]	0	0	1	5	0	2	8	8.5
2		2	1	0	2	0	1	6	
3		0	0	0	0	1	1	2	
4		3	0	1	4	4	6	18	
5	25 IU	3	0	1	6	6	6	22	16.5
6		0	1	0	0	0	0	1	
7		2	4	0	7	3	9	25	
8		4	2	1	?	6	5	18	
9	49 IU	6	2	1	8	9	11	37	38.7
10		6	7	2	11	7	9	42	
11		4	4	2	10	8	7	35	
12		6	5	2	12	7	9	41	

[a] Nine lesions in a lobe is maximum that can be counted accurately. Animals 9 to 12 had many confluent lesions.

[b] IU, infectious units.

Table 2 shows the distribution of tuberculous lesions, either grossly visible or palpable, in the lungs. Because of the similarity of mite lesions to tubercles (10), each tubercle was verified by dissection. The mean number of lesions for each group of animals corresponds very closely to that predicted and suggests that each inhaled infectious unit produced a focus that developed into a tubercle detectable by gross examination.

BCG-induced resistance. Each of seven rhesus monkeys was vaccinated iv with 1 mg (wet weight) of the Tice strain of BCG, kindly supplied by S. Rosenthal, Research Foundation, University of Illinois, Chicago. After 8 weeks, the vaccinated animals and seven nonvaccinated control animals were exposed by aerosol to approximately 40 infectious units of H37Rv. All 14 animals were autopsied 13 weeks later.

PPD was injected intradermally on either the abdomen or the eyelid at various times during the experiment (Table 3). All vaccinated animals reacted to 10 TU of PPD by the eyelid test prior to challenge, and control animals were all PPD-negative before challenge. By the sixth week after infection, all control monkeys were tuberculin-positive by the eyelid test although three of seven failed to respond to PPD injected into the skin of the abdomen. As the experiment progressed, responses of vaccinated monkeys to PPD became weaker; approximately half of the reactions at 7 and 9 weeks postinfection were less than those immediately before challenge.

The marked differences in gross pathological changes between the two groups of animals 13 weeks after infection are summarized in Table 4. Four of the seven BCG-vaccinated animals had no gross evidence of tuberculosis, three had minimal disease, and one or two had pulmonary tubercles. One of these animals had, in addition, one hepatic tubercle. The absence of caseation in the hilar nodes in all vaccinated animals particularly signified the high level of immunity. All o

TABLE 3. *Skin reactions to 10 tuberculin units of purified protein derivative in rhesus monkeys vaccinated with live BCG and challenged with Mycobacterium tuberculosis H37Rv by aerosol*

Treatment	Animal no.	Location and reaction of skin test at			
		2 days before challenge	6 wk post-challenge	7 wk post-challenge	9 wk post-challenge
Vaccinated[a]	27	Eye 2+[b]	Abd 1+		
	28	Eye 1+	Abd −	Abd − Eye −	Abd − Eye 1+
	29	Eye 3+	Abd −	Abd 1+ Eye 3+	
	30	Eye 2+	Abd −	Abd − Eye ±	Abd − Eye 1+
	31	Eye 2+	Abd −	Abd − Eye 1+	Abd − Eye 1+
	32	Eye 2+	Abd −	Abd − Eye ±	Abd − Eye 1+
	33	Eye 1+	Abd −	Abd − Eye ±	Abd − Eye 1+
Nonvaccinated	34	Eye −	Abd − Eye 3+		Abd ± Eye 2+
	35	Eye −	Abd 2+ Eye 3+		
	38	Eye −	Abd 2+ Eye 3+		
	39	Eye −	Abd − Eye 2+		Abd 2+ Eye 3+
	40	Eye −	Abd ± Eye 3+		Abd − Eye 2+
	41	Eye −	Abd − Eye 1+		Abd − Eye 3+
	42	Eye −	Abd 1+ Eye 1+		

[a] Vaccinated with 1 mg of live BCG 8 weeks before challenge.

[b] Abd = intradermally on abdomen; eye = intradermally in eyelid; see Table 1, footnote *a*, for legend of skin reactions.

TABLE 4. *Numerical scores[a] of tuberculosis in BCG-vaccinated and nonvaccinated rhesus monkeys 13 weeks after airborne infection with Mycobacterium tuberculosis H37Rv[b]*

Treatment	Animal no.	Category				
		Lung involvement	Lymphadenopathy	Hematogenous spread	Total	Mean
Vaccinated[c]	27	0	0	0	0	7.1
	28	0	0	0	0	
	29	10	0	0	10	
	30	0	0	0	0	
	31	0	0	0	0	
	32	10	0	0	10	
	33	10	0	20	30	
Nonvaccinated	34	60	20	20	100	71.4
	35	10	20	20	50	
	38	30	20	20	70	
	39	50	20	20	90	
	40	10	0	20	30	
	41	50	20	20	90	
	42	30	20	20	70	

[a] See text for description of numerical scoring procedure.

[b] Estimated dose of H37Rv per animal was 40 viable units.

[c] Vaccinated with 1 mg of BCG intravenously.

the nonvaccinated animals were extensively diseased.

In agreement with the gross pathological findings, chest X-ray films of the vaccinated animals taken 10 weeks after challenge were either normal or indicated questionable lesions. All nonvaccinated animals had roentgenographic abnormalities which are summarized in the following description: monkey 34 had massive consolidation of upper half of right lung; 35, moderately advanced nodular disease of left lung; 38, moderately advanced right upper lobe infiltrate; 39, massive mediastinal gland enlargement and consolidation of upper half of right lung (Fig. 1A, B); 40, bilateral diffuse disease; 41, massive mediastinal gland enlargement and consolidation of left lower lung (Fig. 3); and 42, large mediastinal nodes, nodule in right base, and diffuse hazy infiltrate throughout both lungs.

Figures 2 and 3 illustrate appearance of lungs in vaccinated and nonvaccinated animals, respectively, and demonstrate the marked difference in the hilar nodes of the two groups.

DISCUSSION

These experiments demonstrated the reliability of the aerosol method for infecting subhuman primates with small numbers of tubercle bacilli. Animals infected by this technique developed progressive pulmonary disease with roentgenographical changes, skin-test reactivity to PPD, and tissue pathology similar to that seen in primary tuberculosis in humans. The numerical distribution of primary tubercles in Table 2 suggests that a calculated dose of 12 infectious units may be too low for consistent, reliable infection of all animals exposed. Therefore, in subsequent experiments we attempted to expose each animal to at least 20 tubercle bacilli.

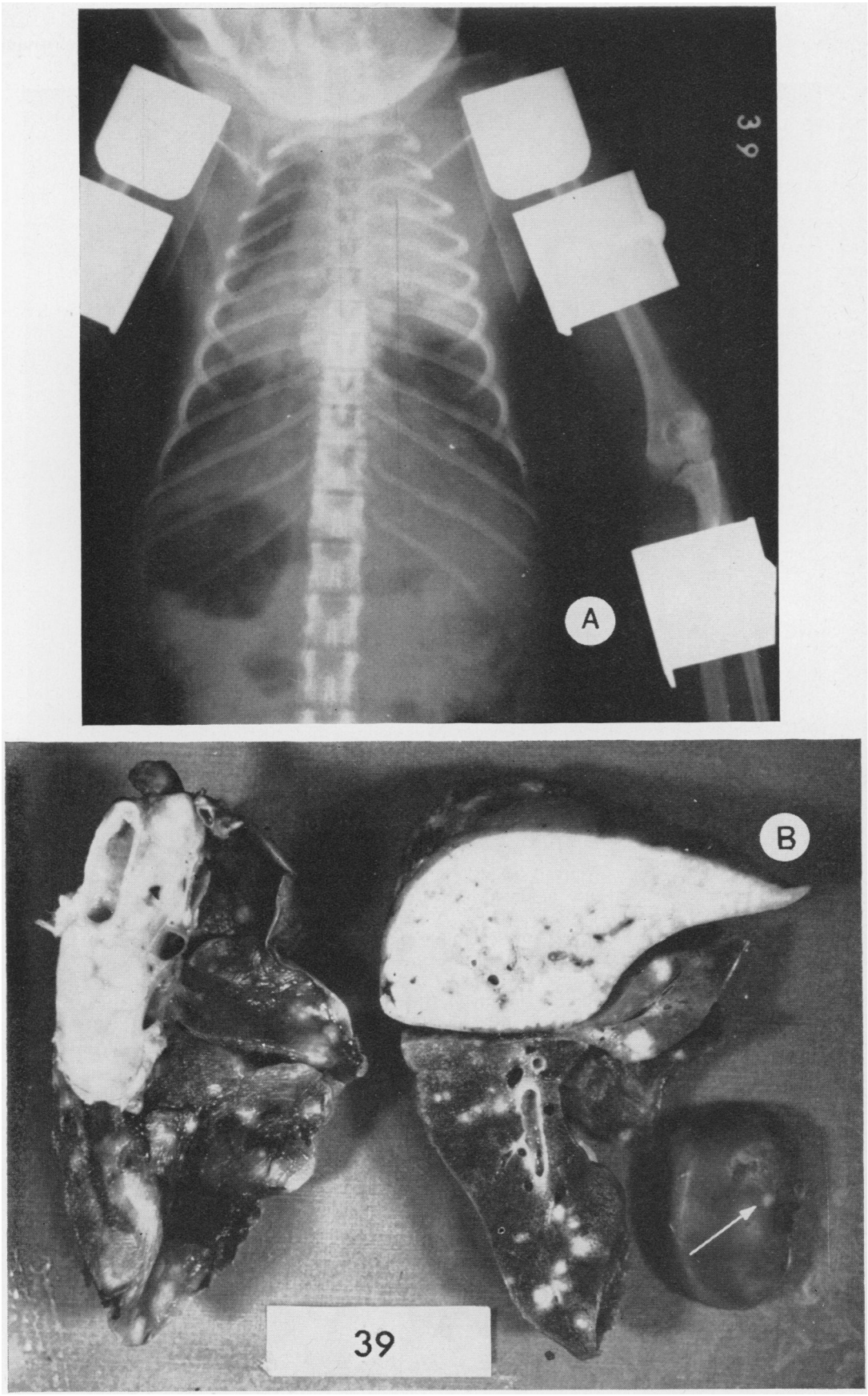
The location of primary lung lesions (Table 2) indicated a random distribution of inhaled organisms with a slightly larger number implanted in lower than in upper lobes. Distribution of bacilli under these experimental conditions might be different from that occurring in naturally acquired infection; because the monkeys were restrained in a prone position during exposure, the ventilation of their lungs could have been altered.

The course of experimentally induced disease, as judged by chest X rays, is related to size of the infecting dose. Animals receiving a large dose showed roentgenographical changes earlier than did those infected with smaller doses. This may be the consequence of multiple lesions expanding to become confluent, thereby casting a larger and more easily recognized shadow on X-ray film than is seen with isolated lesions.

Additional unpublished data on tuberculosis in the rhesus monkey indicate the importance of the hilar lymph nodes in assessing presence or absence of immunity. Nonvaccinated animals almost always have caseation of the hilar nodes after aerosol infection. The absence of caseation in the hilar nodes signifies a high level of immunity.

All nonimmune monkeys developed a hematogenous spread of tubercle bacilli, and all but one animal, a lymphatic spread. Since localization of an infectious process is an important criterion of the immune state of an animal, these consistent

FIG. 1. *Lung of nonvaccinated rhesus monkey (no. 39) 13 weeks after airborne infection with Mycobacterium tuberculosis H37Rv. Pneumonic consolidation of right upper lobe shown in X ray (A) and confirmed in cut surface of specimen (B). Massive caseous nodes around trachea and hilum shown on radial surface of left lung. Multiple discrete tubercles on pleural surface of left lung and on cut surface of right lung. Tubercle on surface of kidney (arrow).*


39
A
B
39

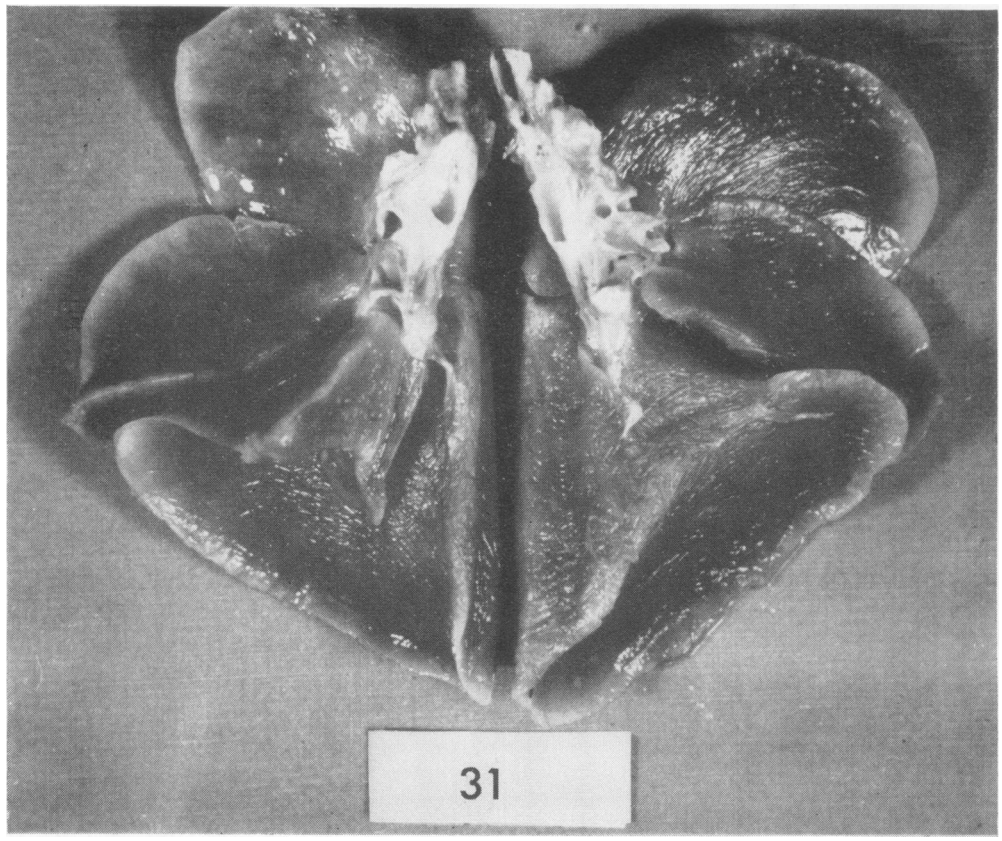


FIG. 2. *Lung of rhesus monkey (no. 31) 13 weeks after airborne infection with Mycobacterium tuberculosis H37Rv and 21 weeks after intravenous injection of viable BCG. Posterior and medial surface. Trachea and carina cut sagittally.*

and easily recognized modes of spread establish a valuable measure of the presence or absence of induced immunity.

Within the period of observation, the overall clinical picture did not diverge significantly from that in early human tuberculosis. The animals appeared well, exhibited normal physical activity, did not lose weight, and were not obviously suffering from a serious, life-threatening disease.

The response of infected monkeys to PPD varied with the type of skin test. In the first experiment, the percentage of reactors to PPD injected into the skin on the abdomen was proportional to the challenge dose of H37Rv (Table 1), but some animals which had moderate disease failed to respond to PPD. The response to PPD injected into the skin on the abdomen was similar in the second experiment. However, all control animals reacted to PPD introduced into the eyelid (Table 3), even though severity of the disease was less than that observed in the former experiment. In our studies the eyelid test was more sensitive than the abdominal skin test in detecting prior exposure to mycobacteria. However, others (4) have reported that some infected monkeys did not react positively to the eyelid test.

Although there are qualitative similarities between this study and that reported by Good (6) and, also that performed by us in collaboration with L. H. Schmidt and R. C. Good (*unpublished data*), there is one very important difference in the two kinds of experiments. In the study by Good, approximately 1,000 virulent bacilli were instilled intratracheally, principally to a limited portion of either the right or left lung. Extensive and confluent disease with much tissue destruction was often observed in one lung, whereas involvement of the remaining lung tissue was much less and perhaps due to secondary spread from the primary focus of infection. This type of infection conceivably overwhelmed local immune mechanisms and contributed to the early mortalities observed in

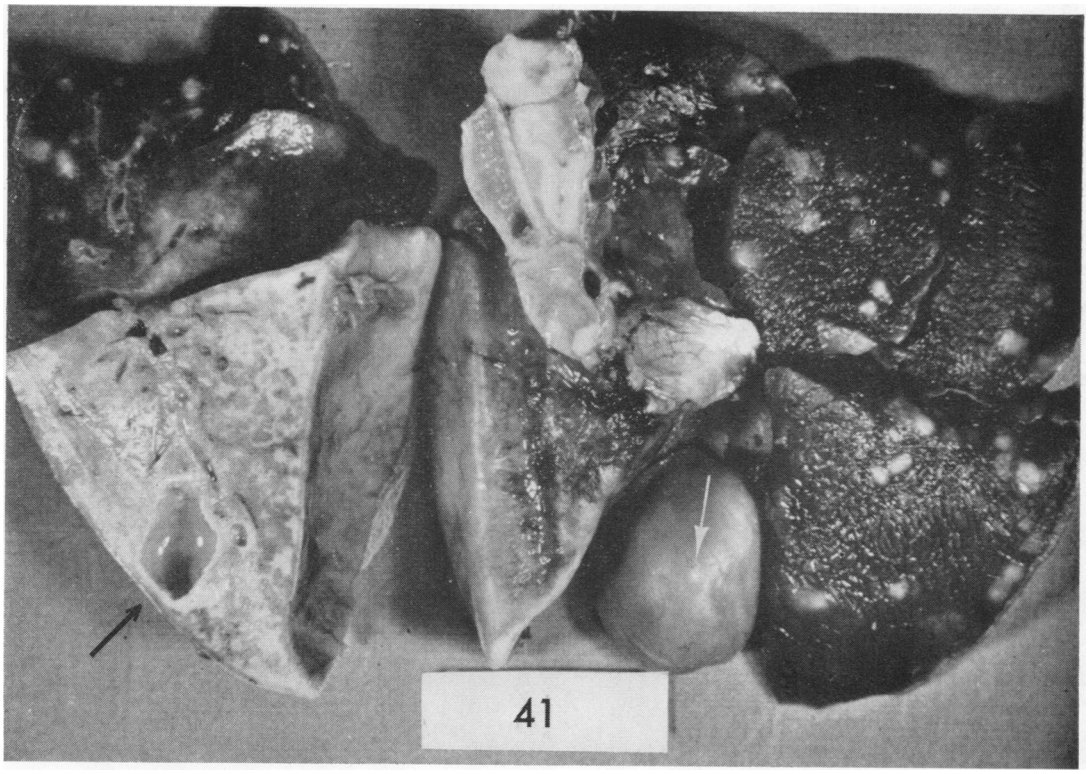


FIG. 3. *Lung of nonvaccinated rhesus monkey (no. 41) 13 weeks after airborne infection with Mycobacterium tuberculosis H37Rv. Caseous pneumonia of left lower lobe with a cavity near pleural surface (arrow). Caseous nodes surround trachea. Discrete tubercles are on surface of right and left lungs. Kidney has tubercle on surface (white arrow).*

their study. On the other hand, in our monkeys the number of discrete and widely disseminated primary tubercles closely reflected the number of bacilli inhaled. Tissue destruction was minimal, and the number of bacilli inhaled was small and approximated that number responsible for most infections of humans. In our study, no animals died during the observation period, and the disease process developed more slowly and along different lines than in the intratracheally challenged animals. Slowly developing disease could stimulate a base line of immunity that would permit resolution of the disease process before overwhelming destruction of tissue occurred. For these several reasons, we believe that our observations of animals challenged with a few airborne organisms, rather than those of animals given a massive intratracheal challenge, more accurately indicate responses which might occur in humans under natural conditions.

These results demonstrated that (i) a high level of acquired resistance can be stimulated with live BCG in the rhesus monkey, an animal which, in nature, is highly susceptible to tuberculosis, and (ii) the level of resistance can be quantitatively assayed after challenge with a few virulent mycobacteria by the pulmonary route, a method which simulates natural infection of humans. Experiments to determine whether nonviable BCG cell wall vaccines can also stimulate high levels of acquired resistance have recently been completed and will be published separately.

LITERATURE CITED

1. Anacker, R. L., W. R. Barclay, W. Brehmer, G. Goode, R. H. List, E. Ribi, and D. F. Tarmina. 1969. Effectiveness of cell walls of *Mycobacterium bovis* strain BCG administered by various routes and in different adjuvants in protecting mice against airborne infection with *Mycobacterium tuberculosis* strain H37Rv. Amer. Rev. Resp. Dis. 99:242–248.
2. Benson, R. E., B. D. Fremming, and R. J. Young. 1955. A tuberculosis outbreak in a *Macaca mulatta* colony. Amer. Rev. Tuberc. 72:204–209.
3. Brehmer, W., R. L. Anacker, and E. Ribi. 1968. Immunogenicity of cell walls from various mycobacteria against airborne tuberculosis in mice. J. Bacteriol. 95:2000–2004.
4. Bywater, J. E. C., E. G. Hartley, D. A. Rutty, and E. T. Ackerley. 1962. Observations on the intradermal palpebral

tuberculin test and subsequent autopsy findings in monkeys. Vet. Rec. 74:1414–1416.
5. Feldman, W. H. 1943. A scheme for the numerical recording of tuberculous changes in experimentally infected guinea pigs. Amer. Rev. Tuberc. Pulm. Dis. 48:248–255.
6. Good, R. C. 1968. Simian tuberculosis: immunologic aspects. Ann. N.Y. Acad. Sci. 154:200–213.
7. Grover, A. A., H. K. Kim, E. H. Wiegeshaus, and D. W. Smith. 1967. Host-parasite relationships in experimental airborne tuberculosis. II. Reproducible infection by means of an inoculum preserved at −70 C. J. Bacteriol. 94:832–835.
8. Guyton, A. C. 1947. Measurement of the respiratory volumes of laboratory animals. Amer. J. Physiol. 150:70–77.
9. Habel, K. 1947. Tuberculosis in a laboratory monkey colony. Amer. Rev. Tuberc. 55:77–92.
10. Innes, J. R. M., M. W. Colton, P. P. Yevich, and C. L. Smith. 1954. Pulmonary acariasis as an enzootic disease caused by *Pneumonyssus simicola* in imported monkeys. Amer. J. Pathol. 30:813–835.
11. Larson, C. L., R. E. Baker, and M. B. Baker. 1968. Immunization of rabbits with viable BCG and nonliving cell wall vaccines. Amer. Rev. Resp. Dis. 98:944–953.
12. Ribi, E., R. L. Anacker, W. Brehmer, G. Goode, C. L. Larson, R. H. List, K. C. Milner, and W. C. Wicht. 1966. Factors influencing protection against experimental tuberculosis in mice by heat-stable cell wall vaccines. J. Bacteriol. 92:869–879.
13. Ribi, E., W. Brehmer, and K. Milner. 1967. Specificity of resistance to tuberculosis and to salmonellosis stimulated in mice by oil-treated cell walls. Proc. Soc. Exp. Biol. Med. 124:408–413.
14. Schmidt, L. H., A. A. Grover, and R. Hoffman. 1959. The comparatve therapeutic activities of thiocarbanidin and para-aminosalicylic acid administered alone and in combination with isoniazid, p. 312–317. Trans 18th V.A.-Armed Forces Conf. Chemother. Tuberc.
15. Schroeder, C. R. 1938. Acquired tuberculosis in the primate in labcratories and zoological collections. Amer. J. Public Health Nat. Health 28:469–475.
16. Smith, D. W., A. A. Grover, and E. Wiegeshaus. 1968. Nonliving immunogenic substances of mycobacteria. Advan. Tuberc. Res. 16:191–227.
17. Wolochow, H., M. Chatigny, and R. S. Speck. 1957. Studies on the experimental epidemiology of respiratory infections. VII. Apparatus for the exposure of monkeys to infectious aerosols. J. Infec. Dis. 100:48–57.