

LIVE TULAREMIA VACCINE

I. HOST-PARASITE RELATIONSHIP IN MONKEYS VACCINATED INTRACUTANEOUSLY OR AEROGENICALLY¹

H. T. EIGELSBACH, J. J. TULIS, M. H. MCGAVRAN,² AND J. D. WHITE

U.S. Army Chemical Corps, Fort Detrick, Frederick, Maryland

Received for publication June 18, 1962

ABSTRACT

EIGELSBACH, H. T. (Fort Detrick, Frederick, Md.), J. J. TULIS, M. H. MCGAVRAN AND J. D. WHITE. Live tularemia vaccine. I. Host-parasite relationship in monkeys vaccinated intracutaneously or aerogenically. *J. Bacteriol.* **84**:1020-1027. 1962.—Bacteriological, histological, immunohistochemical, and serological studies were made on monkeys administered live tularemia vaccine strain LVS by either of two routes. Comparative data are presented on nonvaccinated monkeys exposed via the respiratory route to a highly virulent strain of *Pasteurella tularensis*. Tissue changes resulting from either aerogenic or intracutaneous vaccination were mild, and consisted primarily of the proliferation of histiocytes without the formation of granulomas. The vaccine strain was isolated from the site of vaccination of animals inoculated dermally, from the lungs of animals vaccinated aerogenically, and from the regional lymph nodes, liver, and spleen of both groups; it was not isolated from the blood or bone marrow. Proliferation of the vaccine strain at the site of dermal inoculation and in the lungs of animals exposed aerogenically was observed within 24 hr; in both groups, the maximal viable population was reached within 3 days and maintained through the 10th day. A reduction in the number of viable vaccine organisms had begun by the 14th day; isolations were obtained only from the regional lymph nodes on the 28th day, and the vaccine strain was not isolated from any of the tissues cultured on the 90th day. Because the monkey is less resistant to tularemia than is man, the benign response of this animal to live

tularemia vaccine indicates that the vaccine might also be safe for man when administered by either the dermal or respiratory route.

It has been established that live tularemia vaccine prepared at Fort Detrick (Eigelsbach and Downs, 1961) is superior to killed preparations for the immunization of animals (Eigelsbach, Downs and Herring, 1958; Eigelsbach et al., 1959) and man (Saslaw et al., 1961b; McCrumb, 1961) against virulent challenge. Eigelsbach et al. (1961) reported that guinea pigs and monkeys vaccinated aerogenically with live tularemia vaccine showed higher levels of antibody and comparable or greater protection against virulent challenge than did animals vaccinated dermally. The potential of aerogenic vaccination for effective immunization of man against tularemia and other diseases has been recognized by Aleksandrov et al. (1958) and by Eigelsbach et al. (1962). In the latter study, 30 volunteers were exposed to aerosolized live tularemia vaccine in doses ranging from 1,500 to 30,000 viable organisms. No clinically discernible reaction was observed, and good agglutinin response was obtained. Additional volunteers (24) inhaled 200 to 1,200 live vaccine organisms. Again, no clinically discernible reaction was observed. An agglutinin response was noted in 25 to 50% of the volunteers, irrespective of dose. However, in general, agglutinin titers were lower than in the previous study in which the vaccine dose ranged from 1,500 to 30,000 organisms.

No information has been reported on the host-parasite relationship resulting from administration of live tularemia vaccine to primates, by either the dermal or respiratory route. To obtain such data, relevant to better understanding of the development of immunity through vaccination with live attenuated organisms, a systematic study was designed. The monkey was chosen as

¹ Portions of this paper were presented at the 62nd Annual Meeting of the American Society for Microbiology, Kansas City, Mo., May, 1962.

² Present address: Department of Anatomy, Washington University School of Medicine, St. Louis, Mo.

the test animal because reports by Coriell, King, and Smith (1948), Eigelsbach et al. (1959), and Saslaw et al. (1961*a, b*) indicated similarities in tularemic infection and in effectiveness of vaccine prophylaxis in man and monkey. The purpose of the study was to determine the fate of live vaccine organisms in vivo, establish the serological response to vaccination, characterize the vaccine-induced infection histologically and immunohistochemically, and evaluate the role of dermal and aerogenic vaccination in host-parasite relationship. The present report concerns the bacteriological and serological studies in the simian vaccinated dermally or aerogenically, and includes comparative data on nonvaccinated animals challenged via the respiratory route with highly virulent *Pasteurella tularensis* SCHU S4. A summary of the histological and immunohistochemical observations is presented; detailed data appear elsewhere (McGavran et al., 1962; White et al., *Am. J. Pathol.*, *in press*).

MATERIALS AND METHODS

P. tularensis strains. Live vaccine strain LVS (Eigelsbach and Downs, 1961) and strain SCHU S4 (Eigelsbach, Braun, and Herring, 1951) were cultivated in a modified casein partial-hydrolysate liquid medium similar to that described by Mills et al. (1949). Cultures harvested after 14 hr of incubation at 37 C contained 35×10^9 to 40×10^9 viable organisms per ml.

Monkeys. *Macaca irus* monkeys were caged individually and conditioned for 6 months before use. Animals weighed between 1.8 and 4.9 kg and were distributed so that each experimental group included animals of comparable weight and sex.

Intracutaneous vaccination. LVS culture was diluted with gelatin-saline (0.1% gelatin, 0.85% sodium chloride) to give 10^5 viable cells per vaccine dose (0.2 ml). Inoculations were performed with a 25-gauge needle, and a bleb approximately 1 cm in diameter was formed; 24 monkeys were administered LVS intracutaneously in the midline of the interscapular area after the hair had been clipped and the region cleansed with alcohol.

Aerogenic vaccination with LVS or challenge with SCHU S4. LVS or SCHU S4 aerosols were generated with a nebulizer that produced particles primarily in a range of 1 to 5μ in diameter. Before animals were exposed, the aerosol was allowed to

equilibrate for 20 min (at 21 C and 85% relative humidity) in a large sphere of the type described by Wolfe (1961). On the basis of aerosol sampler data, 24 monkeys received 1×10^5 to 4×10^5 viable LVS, or a mean inhaled dose of 3×10^5 organisms; 12 monkeys received 4×10^5 to 1×10^6 viable SCHU S4, or a mean inhaled dose of 6×10^5 organisms.

Temperatures. Rectal temperatures of all animals were recorded twice daily for 3 days before, and 14 days after, vaccination with LVS or challenge with SCHU S4.

Bacteriological assay. Two monkeys from each of the two groups that received LVS were sacrificed 1, 6, and 12 hr, and 1, 2, 3, 5, 7, 10, 14, 28, and 90 days, after vaccination. Of the monkeys exposed aerogenically to SCHU S4, two each were sacrificed at 1 and 12 hr and 1 and 2 days. On the 3rd day, one of the four remaining animals was found dead; three were moribund and were sacrificed.

Blood was taken from the saphenous vein for culture and serology. Animals were anesthetized by intravenous injection of Pentothal, and killed by cardiac exsanguination. Samples of tissue were removed aseptically from the dermal inoculation site, the right apical and diaphragmatic lobes of the lung, the spleen, the liver, the femoral bone marrow, and the lymph nodes (right and left axillary and inguinal, cervical, tracheobronchial, and coeliac). These were weighed, placed in Ten Broeck tissue grinders with 2 ml of sterile gelatin-saline solution, and triturated. Blood and dilutions of tissue suspensions were plated on glucose cysteine blood agar containing 0.1 mg of cycloheximide and 0.2 unit of penicillin per ml, and incubated at 37 C. Colony counts were made after 72 or 96 hr; all plates were kept for 7 days before being discarded as negative. Weights of the lungs, spleen, and liver were recorded, and viable *P. tularensis* populations were reported on the basis of the entire organ.

Serology. A formalin-treated suspension of *P. tularensis* was used as antigen, and agglutination tests were performed and read according to the National Institutes of Health method (Brigham, 1950).

Conjugates. Monkey sara, with *P. tularensis* agglutinin titers of 1:1,280 to 1:2,560, were fractionated with ammonium sulfate, and the crude globulin fractions conjugated with fluorescein isothiocyanate, according to the methods of

Riggs et al. (1958). The conjugate was adsorbed twice with acetone-extracted rabbit-liver powder (100 mg/ml) and twice with spleen-marrow powder (50 mg/ml). A portion of the conjugate was further adsorbed with washed, formalin-killed strain SCHU S4 cells to remove homologous antibody. This reagent, used for a control, did not stain *P. tularensis*. A companion section was treated similarly, but stained with the conjugate adsorbed with strain SCHU S4 cells.

Histological examination of tissues. Samples of tissues were fixed in 10% formalin, processed through paraffin, cut, and stained or frozen in isopentane at -70°C , for fluorescent antibody studies. To demonstrate antitularensis γ -globulin (ATGG) in tissue, sections of frozen tissue were fixed in 95% ethyl alcohol for 15 min, dried in air, and covered with a 1:10 dilution of a sonic lysate of *P. tularensis* SCHU S4. After 1 hr, the slides were washed, and then stained for 30 min with a 1:40 dilution of conjugate. Control slides were prepared by using the conjugate adsorbed with strain SCHU S4 cells or by omitting the sonic lysate. To demonstrate *P. tularensis* antigen in tissues, sections of frozen tissues were placed in acetone for 30 min, dried in air, and stained for 30 min with a 1:50 dilution of conjugate. Slides were examined with a Zeiss fluorescence

microscope equipped with a 200-w Osram lamp, Schott UG-2 and UG-5 transmitting filters, and a Schott GG-4 barrier filter.

RESULTS

Bacteriological data. The rate of growth of LVS at the site of intracutaneous vaccination, and of LVS or SCHU S4 in the lungs of monkeys exposed aerogenically, is shown in Fig. 1. Within 1 hr after intradermal vaccination with 10^5 LVS, the mean viable population recovered at the site of inoculation was 5×10^2 organisms. Preliminary studies in which the viable population of LVS was assayed within 1 min after a comparable vaccination indicated that at least 10^4 organisms could be recovered. Therefore, a rapid disappearance of many viable organisms occurred within 1 hr. During the same period, LVS was recovered from the regional lymph nodes. Multiplication occurred at the site of dermal vaccination within 6 to 12 hr.

After aerogenic exposure (1 hr) to 3×10^5 to 6×10^5 viable LVS or SCHU S4 cells, 5×10^4 to 1×10^5 viable organisms were estimated to be present in the lungs. At this time, LVS was also recovered from the tracheobronchial lymph nodes. Maximal viable populations of approximately 10^6 LVS at the site of dermal inoculation or in the lungs occurred within 3 days after

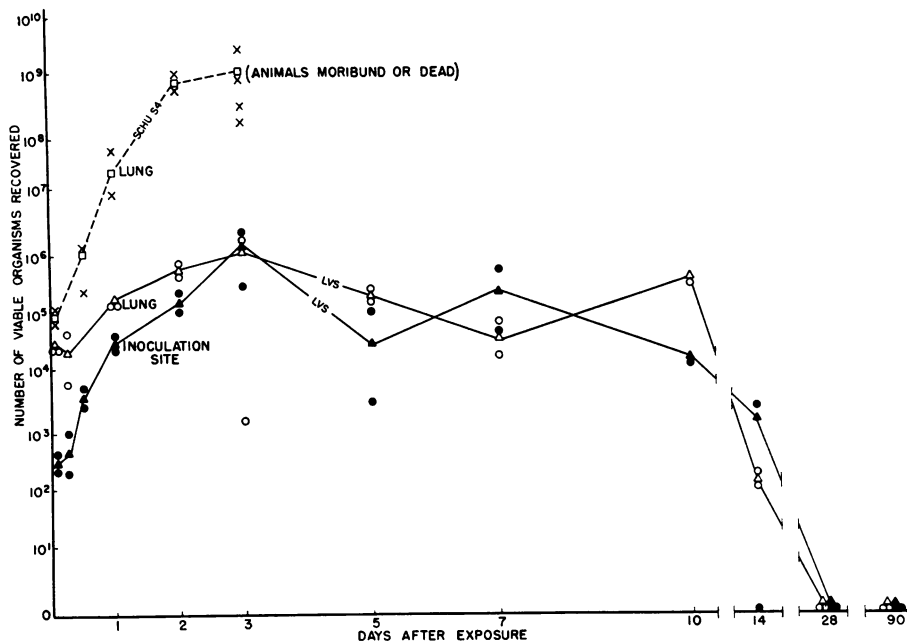


FIG. 1. Rate of growth of *Pasteurella tularensis* LVS or SCHU S4 in the monkey: respiratory exposure, ○ or ×; intracutaneous inoculation, ●; mean viable population, △, ▲, or □.

dermal or aerogenic vaccination. LVS was cultured from either the dermal site or lung of one or both of the vaccinated animals sacrificed at each interval through the 14th day, but not from animals examined 28 or 90 days after vaccination.

Growth of SCHU S4 in the lungs of monkeys exposed aerogenically was characterized by rapid and persistent multiplication; within 2 days, the viable population approached 10^9 organisms. Animals were either moribund or had died 3 days after exposure; the viable count of SCHU S4 in the lungs of the four animals examined ranged from 3×10^8 to 5×10^9 organisms.

LVS was not cultured from the blood or bone marrow nor from the inguinal or coeliac lymph nodes of monkeys after intracutaneous or aerogenic administration of live tularemia vaccine; neither was LVS isolated from the lungs of the animals vaccinated intracutaneously. Additional quantitative data on the isolation of LVS from vaccinees are presented in Table 1.

The axillary lymph nodes of animals vaccinated

intracutaneously were invaded within 1 hr. However, LVS was not again isolated from this tissue until the 7th day. Thereafter, to the 28th day, axillary nodes from one or both animals examined at each period contained 10^2 to 10^4 viable organisms per g. Sporadic isolations of LVS in approximately the same concentration were made from the cervical and tracheobronchial lymph nodes of these animals. From the 1st through the 14th day after intracutaneous vaccination, with the exception of the 2nd day, isolations were made from the spleen of one or both animals sacrificed at each period. LVS was isolated from the liver of only one of the animals sacrificed after the 5th day. When cultures were obtained, it was estimated that 10^2 to 10^4 viable organisms were present in the spleen, and 10^2 in the liver.

LVS was not cultured from the axillary lymph nodes of animals vaccinated aerogenically, and fewer isolations were made from the spleen and liver than from animals vaccinated intracutane

TABLE 1. *Viable Pasteurella tularensis (LVS) in various tissues of the monkey after intracutaneous or aerogenic administration of live tularemia vaccine*

Tissue	Vaccination*	No. of organisms at indicated interval after vaccination†											
		Hr		Day									
		1	6, 12	1	2	3	5	7	10	14	28	90	
Axillary lymph nodes	IC	10 ² , 10 ²	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 10 ²	0, 10 ⁴	10 ³ , 10 ³	0, 10 ³	0, 0
	A	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0
Cervical lymph nodes	IC	0, 0	0, 0	0, 10 ⁴	0, 0	0, 10 ³	0, 0	0, 0	0, 0	0, 10 ³	0, 0	0, 0	0, 0
	A	0, 0	0, 0	0, 0	0, 0	10 ² , 10 ³	0, 10 ⁴	0, 10 ³	0, 10 ⁴	0, 10 ⁴	0, 0	0, 0	0, 0
Tracheobronchial lymph nodes	IC	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 10 ³	0, 0	0, 10 ³	0, 10 ²	0, 0	0, 0
	A	0, 10 ³	0, 0	10 ³ , 10 ³	10 ⁵ , 10 ⁵	10 ⁵ , 10 ⁵	10 ³ , 10 ⁴	10 ² , 10 ³	10 ⁴ , 10 ³	10 ⁴ , 10 ⁵	10 ³ , 10 ⁴	0, 10 ²	0, 0
Spleen	IC	0, 0	0, 0	0, 10 ²	0, 0	10 ³ , 10 ³	0, 10 ⁴	0, 10 ³	10 ² , 10 ³	0, 10 ²	0, 0	0, 0	0, 0
	A	0, 0	0, 0	0, 0	0, 0	10 ² , 10 ³	0, 10 ²	0, 0	0, 0	0, 10 ²	0, 0	0, 0	0, 0
Liver	IC	0, 0	0, 0	0, 0	0, 10 ³	10 ³ , 10 ³	0, 10 ³	0, 0	10 ³ , 0	0, 0	0, 0	0, 0	0, 0
	A	0, 0	0, 0	0, 0	0, 0	0, 10 ³	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0

* Intracutaneous (IC) dose, 10^5 cells; aerogenic (A) dose, mean of 3×10^5 cells.

† Two animals of each group sacrificed at each time indicated. Data in upper part of table are based on results per g of lymphatic tissue; lower part of table refers to results per organ.

TABLE 2. *Viable Pasteurella tularensis* in various tissues of the monkey after aerogenic challenge with highly virulent strain SCHU S4*

Tissue	No. of organisms per ml of blood, per organ, or per g of lymphatic tissue or bone marrow				
	Hr		Day		
	1	12	1	2	3†
Blood.....	0, 0	0, 0	0, 0	0, 30	10 ² , 10 ² , 10 ² , 10 ²
Spleen.....	0, 0	0, 0	0, 10 ²	10 ⁵ , 10 ⁶	10 ⁷ , 10 ⁷ , 10 ⁷ , 10 ⁷
Liver.....	0, 0	0, 0	0, 10 ³	10 ⁵ , 10 ⁵	10 ⁶ , 10 ⁶ , 10 ⁵ , 10 ⁵
Axillary lymph nodes.....	0, 0	0, 0	0, 0	0, 0	10 ³ , 10 ⁴ , 10 ³ , 10 ²
Cervical lymph nodes.....	0, 0	0, 0	0, 0	10 ³ , 10 ⁷	10 ⁴ , C‡, 10 ⁷ , 10 ⁸
Tracheobronchial lymph nodes.....	0, 0	0, 10 ³	10 ⁵ , 10 ⁵	10 ⁷ , 10 ⁸	10 ⁸ , 10 ⁹ , 10 ⁸ , 10 ⁸
Inguinal lymph nodes.....	0, 0	0, 0	0, 0	0, 0	10 ³ , 10 ⁴ , 10 ⁴ , 0
Coeliac lymph nodes.....	0, 0	0, 0	0, 0	0, 0	10 ³ , 10 ⁶ , 10 ³ , C
Bone marrow.....	0, 0	0, 0	0, 0	10 ⁴ , 10 ²	10 ⁵ , 10 ⁴ , 10 ⁴ , 10 ³

* Mean of 6×10^5 inhaled cells; two to four animals examined at each time indicated

† Animals moribund or dead.

‡ Contaminant overgrowth.

ously; however, the organism was isolated more consistently and in greater numbers from the tracheobronchial lymph nodes of animals vaccinated aerogenically.

Quantitative bacteriological data on tissues, other than lung, of animals exposed aerogenically to SCHU S4 are shown in Table 2. SCHU S4 was isolated from the blood of one and from the bone marrow of both animals 2 days after exposure. The viable population of SCHU S4 in the spleen ranged between 10^5 and 10^6 organisms, and, in the liver, approximated 10^5 ; concentrations as high as 10^7 organisms per g of cervical lymph node and 10^8 organisms per g of tracheobronchial lymph node were obtained. On the 3rd day, animals were either moribund or had died, and SCHU S4 had invaded all tissues and organs examined. Blood from these animals contained approximately 10^2 organisms per ml, and bone marrow contained 10^3 to 10^5 organisms per g; 10^8 to 10^9 organisms were present per g of tracheobronchial lymph node.

Gross, histological, and clinical observations. After *intracutaneous* inoculation of monkeys with LVS, a gradually enlarging area of erythema and induration developed at the site, and small central ulcers appeared during the latter part of the 2nd week. These re-epithelized, and inflammation subsided during the 3rd and 4th weeks. The axillary and deep cervical lymph nodes were moderately enlarged by the end of the 2nd week

but did not become necrotic. Histological changes were characteristic of a very mild subacute inflammatory reaction without granuloma formation. Healing with minimal scarring ensued. In the spleen, the changes were very subtle and no miliary granulomas were seen in any organs. ATGG was not identified at the site of dermal vaccination until the 14th day, but was demonstrated in the axillary lymph nodes on the 3rd day and in the spleen on the 5th day. It was also found, though in small quantity, in the liver, in Peyer's patches of the small intestine, and in the coeliac and tracheobronchial lymph nodes. ATGG persisted in the spleen and a few peripheral lymph nodes through the 90th day. With the exception of a 1- to 1.5-F temperature rise of 12- to 24-hr duration in 30% of the animals 3 to 5 days after intracutaneous vaccination, evidence of disease was not observed.

After *aerogenic* administration of the live vaccine, gross changes in the monkey were limited to the lungs and tracheobronchial lymph nodes. Minute areas of erythematous pulmonic parenchyma were seen on the 7th day but had disappeared by the 14th day; they were not detected in roentgenograms made on the 7th day. The tracheobronchial lymph nodes became hyperplastic, reaching a maximal dimension of 1 cm by the 10th day. Petechiae were seen, but no necrosis. Lymphadenitis was not evident on the 28th day. Fluorescent antibody studies showed

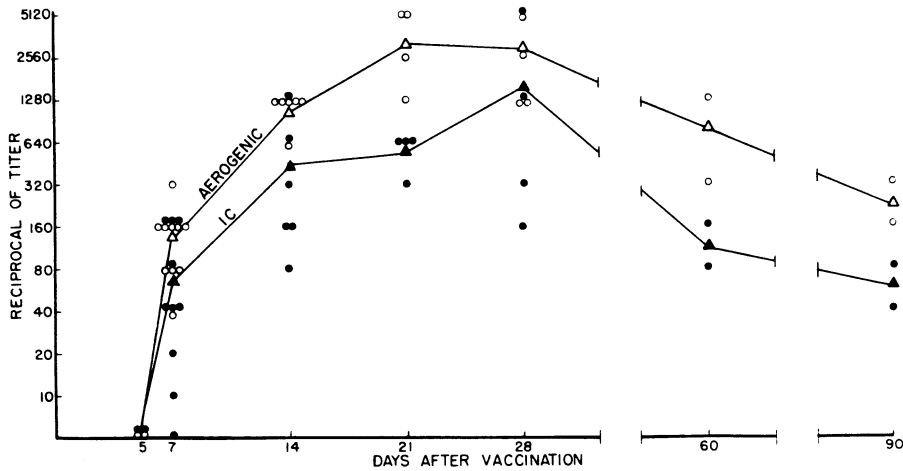


FIG. 2. Agglutinin response of monkeys administered live tularemia vaccine: intracutaneous vaccination, ●; aerogenic vaccination, ○; mean titer, ▲ or △.

that *P. tularensis* organisms had impinged along the walls of the respiratory bronchioles and had initiated small foci of subacute, non-necrotizing inflammation during the 1st week. These were absent by the 14th day, and, on the 28th day, no residua were seen except for a moderate increase in the plasmacyte population of the pulmonic lymphoid tissue. Changes in the spleen, liver, and remaining lymph nodes were comparable with those observed in animals administered LVS intracutaneously. ATGG was present in the tracheobronchial lymph nodes and spleen as early as 5 days after aerogenic vaccination of the animals; on the 7th day, it was demonstrated in the peribronchiolar and perivascular lymphoid tissues of the lung. The number, position, and type of cells involved in production of ATGG in the tracheobronchial lymph nodes, spleen, and liver were comparable in both groups of vaccinated animals. In animals vaccinated aerogenically, ATGG persisted in the lymphoid tissues of the lung, the tracheobronchial lymph nodes, and spleen through the 90th day. After aerogenic vaccination (3 to 5 days), 50% of the animals showed a mild transient temperature response comparable with that seen in animals vaccinated dermally. No other signs of illness were observed.

Strain SCHU S4 administered aerogenically produced a fulminant, acute, necrotizing reaction that was in striking contrast to the subacute, non-necrotizing, and limited inflammatory reaction evoked by the live vaccine. Animals became febrile within 48 hr after exposure to strain

SCHU S4, and exhibited a temperature rise of 2 to 3 F before death or sacrifice. The major sites of tissue reaction to the virulent strain were the bronchioles and adjacent pulmonic parenchyma, the intrapulmonic lymphoid tissues, and the tracheobronchial lymph nodes; the spleen and liver were also involved, but to a lesser extent. Animals were moribund and were sacrificed, or death occurred, within 3 days after exposure, before the more granulomatous lesions of classical tularemia could become manifest.

Serology. The agglutinin response of monkeys after dermal or aerogenic vaccination is shown in Fig. 2. Agglutinins were first detected in the sera of both groups of animals 7 days after vaccination; maximal mean agglutinin titers of 1:1,720 for animals vaccinated dermally and 1:3,520 for animals vaccinated aerogenically were reached within 21 to 28 days, and gradually declined thereafter. The mean agglutinin titer was consistently higher for animals vaccinated aerogenically.

DISCUSSION

Attenuated strains of several microorganisms used for the preparation of live vaccines against various bacterial and viral diseases in man and animals rapidly invade and proliferate in many tissues. In comparison with highly virulent strains, multiplication usually is not as rapid or is reduced appreciably after an inflammatory response, and clearance ensues. Optimally, a

symptomless infection is produced that elicits high-grade, lasting immunity.

Conceivably, the host-parasite relationship and resultant immunity can be influenced by the dose and route of administration of live vaccine. Previous studies in this laboratory indicated that the monkey could be immunized by intracutaneous or aerogenic vaccination with live tularemia vaccine (Eigelsbach et al., 1961). Furthermore, a vaccine dose of approximately 10^5 organisms administered to man by acupuncture, or 3×10^4 cells given aerogenically, elicited a good serological response and produced no undesirable clinical reaction. The present study was designed primarily to provide detailed information on the host-parasite relationship in a primate vaccinated intracutaneously or aerogenically with a relatively large dose (10^5 viable organisms of tularemia vaccine).

The experimental data demonstrate that LVS multiplied at the site of dermal vaccination and in the lungs of monkeys vaccinated aerogenically, and rapidly invaded the regional lymph nodes. Subsequently, LVS was cultured from the spleen and liver. Though dissemination to the spleen and liver was necessarily hematogenous, LVS was not recovered from the blood, indicating that the bacteremia was probably transient and was comprised of a small number of organisms. With few exceptions, the pattern of dissemination, multiplication, and clearance of LVS was comparable in both groups of vaccinated animals. In sharp contrast, the infection produced by inhalation of SCHU S4 organisms proceeded much more rapidly, evoked an acute progressive inflammatory response associated with necrosis, and terminated in a fatal septicemia.

Tissue changes resulting from either intracutaneous or aerogenic vaccination with LVS were mild, and consisted primarily of proliferation of histiocytes without the formation of granuloma; inflammation was readily resolved. However, earlier and more intensive involvement of the regional lymph nodes was observed in animals vaccinated aerogenically. Gefen and Gordon (1961) noted epithelioid and lymphoid granulomas in the lymph nodes, spleen, and liver of guinea pigs that received a Soviet dry, live tularemia vaccine in doses ranging from 10^6 to 10^8 organisms. The absence of granuloma formation in the present study is attributable at least in part to the greater resistance of the monkey to tularemia, in comparison with the guinea pig.

It is postulated that aerogenic vaccination provided opportunity for wide distribution of the live vaccine in the large network of the respiratory bronchioles, with subsequent proliferation at the multiple sites of impingement. The production and persistence of ATGG in the respiratory bronchioles and associated lymphoid aggregates and in other pulmonic lymphoid tissues, as well as the production of higher agglutinin titers in animals vaccinated aerogenically, might be associated with the trend toward increased immunity to respiratory challenge observed by Eigelsbach et al. (1961) in comparison with animals vaccinated dermally. The mild tissue changes without granuloma formation, rapid resolution of the inflammatory response, and complete clearance of LVS within 90 days after aerogenic vaccination (with 10^5 viable organisms) of the monkey depict the benign nature of this route of vaccination. The monkey, though more comparable to man in resistance to tularemia than most laboratory animals, is nevertheless somewhat less resistant than man. It is predicted that aerogenic vaccination of man, with LVS in doses up to 10^5 organisms, would produce no more and probably less tissue reaction than observed in the monkey, and that individuals so vaccinated will be protected against tularemia.

ACKNOWLEDGMENTS

Appreciation is expressed to Joseph V. Jemski for exposure of the monkeys to aerosols and to George G. Wright and Lloyd H. Graf for helpful suggestions concerning the manuscript.

LITERATURE CITED

- ALEKSANDROV, N. I., N. E. GEFEN, N. W. GARIN, K. G. GAPOCHKO, I. I. DAAL-BERG, AND V. M. SERGEYEV. 1958. Reactogenicity and effectiveness of aerogenic vaccination against certain zoonoses. *Voenno-Med. Zhur.*, No. 12, p. 34-38.
- BRIGHAM, G. C. 1950. *In* Diagnostic procedures and reagents, p. 262, 3rd ed. American Public Health Association, Inc., New York.
- CORIELL, L. L., E. O. KING, AND M. G. SMITH. 1948. Studies on tularemia. IV. Observations on tularemia in normal and vaccinated monkeys. *J. Immunol.* **58**:183-202.
- EIGELSBACH, H. T., W. BRAUN, AND R. D. HERRING. 1951. Studies on the variation of *Bacterium tularemense*. *J. Bacteriol.* **61**:557-569.
- EIGELSBACH, H. T., AND C. M. DOWNS. 1961. Prophylactic effectiveness of live and killed

- tularemia vaccines. I. Production of vaccine and evaluation in the white mouse and guinea pig. *J. Immunol.* **87**:415-425.
- EIGELSBACH, H. T., C. M. DOWNS, AND R. D. HERRING. 1958. Comparative immunogenicity of live and killed tularemia vaccines for the mouse and guinea pig. *Bacteriol. Proc.*, p. 74.
- EIGELSBACH, H. T., W. D. TIGERTT, S. SASLAW, AND F. R. McCRUMB, JR. 1962. Live and killed tularemia vaccine: evaluation in animals and man. *Proc. 1962 Army Science Conf., U.S. Army Military Acad., West Point, N.Y.*, vol. 1, p. 235-246.
- EIGELSBACH, H. T., J. J. TULIS, E. L. OVERHOLT, AND W. S. GOCHENOUR, JR. 1959. Immunogenicity of live tularemia vaccine for the monkey. *Bacteriol. Proc.*, p. 87-88.
- EIGELSBACH, H. T., J. J. TULIS, E. L. OVERHOLT, AND W. R. GRIFFITH. 1961. Aerogenic immunization of the monkey and guinea pig with live tularemia vaccine. *Proc. Soc. Exptl. Biol. Med.* **108**:732-734.
- GEFEN, N. E., AND G. Y. GORDON. 1961. Aerosol immunization with dry live vaccines and toxoids. Report 5. Study of the morphological changes in aerosol immunization with some combined vaccines and toxoids. *Zhur. Mikrobiol. Epidemiol. Immunobiol.* **32**:40-46.
- McCRUMB, F. R., JR. 1961. Aerosol infection of man with *Pasteurella tularensis*. *Bacteriol. Rev.* **25**:262-267.
- McGAVRAN, M. H., J. D. WHITE, H. T. EIGELSBACH, AND R. W. KERPSACK. 1962. Morphological and immunohistochemical studies of the pathogenesis of infection and antibody formation subsequent to vaccination of *Macaca irus* with an attenuated strain of *Pasteurella tularensis* I. Intracutaneous vaccination. *Am. J. Pathol.* **41**:259-271.
- MILLS, R. C., H. BERTHELSEN, E. DONALDSON, AND P. L. WILHELM. 1949. Nutritional requirements of *Bacterium tularense*. *Bacteriol. Proc.*, p. 37-38.
- RIGGS, J. L., R. J. SEIWALD, J. H. BURCKHALTER, C. M. DOWNS, AND T. G. METCALF. 1958. Isothiocyanate compounds as fluorescent labeling agents for immune serum. *Am. J. Pathol.* **34**:1081-1097.
- SASLAW, S., H. T. EIGELSBACH, J. A. PRIOR, H. E. WILSON, AND S. CARHART. 1961a. Tularemia vaccine study. I. Intracutaneous challenge. *A.M.A. Arch. Internal Med.* **107**:689-701.
- SASLAW, S., H. T. EIGELSBACH, J. A. PRIOR, H. E. WILSON, AND S. CARHART. 1961b. Tularemia vaccine study. II. Respiratory challenge. *A.M.A. Arch. Internal Med.* **107**:702-714.
- WOLFE, E. K., JR. 1961. Quantitative characterization of aerosols. *Bacteriol. Rev.* **25**:194-202.