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ANTIBODY RESPONSE OF RABBITS TO PASTEURELLA TULARENSIS: I. EFFECT OF VACCINE, ROUTE, AND SCHEDULE

John E. Nutter

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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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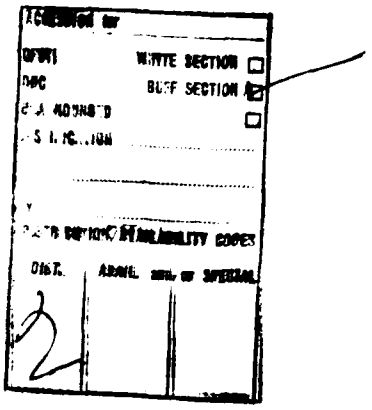

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Medical Bacteriology Division
BIOLOGICAL SCIENCES LABORATORIES

Project 1B662706A071

December 1968

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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ABSTRACT

The response of the rabbit to viable or killed whole-cell *Pasteurella tularensis* vaccines was studied. The most practical preparation for the production of anti-*P. tularensis* antibodies was viable organisms of the live vaccine strain (LVS). The intravenous route of administration proved superior to either the subcutaneous or intradermal routes, and incorporation of LVS into Freund's adjuvants did not result in increased levels of antibody. Short-term hyperimmunization, three injections at weekly intervals, constituted the most efficient method for increasing levels of the antibodies.

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I. INTRODUCTION*

The rabbit is commonly used for the production of antisera against a wide variety of antigens.[3] There is, however, little information concerning the antibody response of this animal to Pasteurella tularensis.[3,4,7] The objectives of this study were to determine optimal procedures for antibody production and to study the response of the rabbit to viable or killed whole-cell P. tularensis vaccines.

II. MATERIALS AND METHODS

A. RABBITS

New Zealand white rabbits, weighing between 1.8 and 2.5 kg, were used throughout the investigation. Except where noted, all experimental groups contained five animals.

B. VACCINES AND VACCINATION TECHNIQUES

P. tularensis was grown in a modified casein hydrolyzate medium (MCPH) similar to that described by Mills et al.[6] Numbers of viable cells were estimated by cultivation of appropriate dilutions on glucose cysteine blood agar.[7]

The live vaccine strain (LVS) described by Eigelsbach and Downs[5] was used to produce viable vaccine. The usual preparation was a saline dilution of an MCPH culture containing 10^8 or 10^9 viable organisms per ml. In one experiment, equal portions of LVS in saline were mixed with either Freund's complete or incomplete adjuvant (Baltimore Biological Laboratory) to give a final concentration of 10^9 viable organisms per ml.

Strain SCHU S4, a fully virulent North American strain, was employed as a killed (phenol-merthiolate) or viable vaccine; the killed preparation contained 10^9 organisms per ml. One thousand viable cells were used to infect rabbits intravenously; after clinical symptoms appeared (3 to 4 days) the animals were treated with streptomycin.[7]

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C. SEROLOGICAL TECHNIQUES

Trial bleedings were made from the marginal ear vein and animals were exsanguinated by cardiac puncture. Three serological tests were performed on sera: (i) Bacterial agglutination tests using formalinized SCHU S4 cells as antigen;[1] (ii) passive hemagglutination tests with polysaccharide-treated sheep erythrocytes;[11] and (iii) precipitin assays by double diffusion in agar, using the technique and strain SCHU S4 antigen (sonic lysate) described by Tulis and Eigelsbach.[10] Precipitates were allowed to develop for 21 days at 37 C in a moist atmosphere.

III. RESULTS

A. PRIMARY AND SECONDARY ANTIBODY RESPONSES TO THREE VACCINES

The agglutinin responses of three groups of 10 rabbits each inoculated intravenously with 10^9 killed strain SCHU S4, 10^9 viable strain LVS, or 10^3 viable strain SCHU S4, respectively, are presented in Table 1. During the primary response, highest peak titer was obtained with viable strain SCHU S4; killed strain SCHU S4 induced the poorest response. The peak titers were compared by Student's t test, and the difference between titers produced by viable SCHU S4 and killed strain SCHU S4 was the only significant one ($P = 0.05$).

TABLE 1. BACTERIAL AGGLUTININ RESPONSE OF RABBITS FOLLOWING THE INTRAVENOUS ADMINISTRATION OF *P. TULARENSIS* LIVE OR KILLED VACCINE

	Mean Reciprocal Agglutinin Titer		
Day	Killed SCHU S4	Viable LVS	Viable SCHU S4
7	352	576	56
14	120	640	1,280
21	76	256	640
28	40[a]	224[a]	512
35	208	704	480
42	152	480	512
49	112	320	320
56	80	352	416

a. Animals revaccinated.

Rabbits receiving viable strain SCHU S4 maintained significantly higher titers on the 21st and 28th days than those given LVS; at those times animals inoculated with killed SCHU S4 exhibited the lowest titers. Twenty-eight days after vaccination, the killed SCHU S4 and LVS groups were revaccinated with 10^9 cells of the appropriate vaccine by the intravenous route. The animals in the viable SCHU S4 group were not revaccinated because only three animals survived the infection despite streptomycin therapy. Both revaccinated groups of rabbits had elevated agglutinin titers 7 days after revaccination and maximum titers were significantly higher ($P = 0.05$) than the declining titers observed during the late primary response (28 days). However, when the maximum secondary and primary response titers were compared, there were no significant differences in the values. At the 42nd, 49th, and 56th days, the titers of the rabbits revaccinated with viable LVS and the surviving animals undergoing a prolonged primary response to viable SCHU S4 were similar.

The passive hemagglutinin titers of two groups of rabbits for each of the three vaccines are presented in Table 2. Highest primary response titers (1:10,240) were obtained from animals vaccinated with viable SCHU S4. Second highest titers (1:5,120) were exhibited by animals that had received the LVS vaccine. Killed SCHU S4 vaccinated rabbits had the lowest titers. Revaccination of the animals previously given killed strain SCHU S4 or viable strain LVS induced maximal passive hemagglutinin titers 7 days after administration of the vaccines. When LVS was employed, the maximal secondary titers were fourfold to eightfold higher than during the primary response. The hemagglutinin response of rabbits surviving the viable SCHU S4 infection showed that these animals maintained relatively high primary titers (1:5,120).

TABLE 2. PASSIVE HEMAGGLUTININ RESPONSE OF RABBITS FOLLOWING THE INTRAVENOUS ADMINISTRATION OF P. TULARENSIS LIVE OR KILLED VACCINE

	Reciprocal Passive Hemagglutinin Titer[a]					
Day	Killed SCHU S4		Viable LVS		Viable SCHU S4	
7	640	1,280	2,560	2,560	320	160
14	640	640	5,120	2,560	5,120	5,120
21	320	640	2,560	5,120	10,240	10,240
28	160[b]	320[b]	2,560[b]	1,280[b]	10,240	-[c]
35	5,120	5,120	40,960	20,480	5,120	-
42	1,280	1,280	5,120	2,560	5,120	-
49	640	1,280	2,560	2,560	5,120	-
56	160	640	5,120	2,560	5,120	-

a. Two pooled serum samples per vaccine group.
b. Animals revaccinated.
c. No survivors in this pool.

The agar gel precipitin response of the three groups of rabbits is presented in Table 3. During the primary response the viable LVS and viable SCHU S4 vaccines induced similar precipitin patterns with four to five bands; the killed SCHU S4 vaccine induced a poorer response. Revaccination caused an increased response with one of the two killed SCHU S4 pooled samples; the number of precipitin bands in animals given live strain LVS was not increased over that observed during the primary response.

TABLE 3. PRECIPITIN RESPONSE OF RABBITS FOLLOWING THE INTRAVENOUS ADMINISTRATION OF P. TULARENSIS LIVE OR KILLED VACCINE

	Number of Precipitin Bands[a]					
Day	Killed SCHU S4		Viable LVS		Viable SCHU S4	
7	2	2	4	4	2	2
14	2	2	4	5	4	4
21	1	0	4	4	5	5
28	0[b]	0[b]	4[b]	3[b]	4	-[c]
35	2	4	4	5	5	-
42	2	4	4	4	5	-
49	0	2	4	4	4	-
56	0	1	4	3	4	-

a. Two pooled serum samples per vaccine group.
b. Animals revaccinated.
c. No survivors.

B. VACCINATION BY VARIOUS ROUTES

The bacterial agglutinin responses of rabbits administered 10^9 viable LVS cells via three routes are presented in Table 4. The routes employed were: intravenous (IV), subcutaneous (SC), or intradermal (ID). For the latter, one group of animals was inoculated at one site (ID x 1) and another at five sites (ID x 5; each extremity and interscapular region). During the primary response maximal titers of all groups occurred on the 14th day; the highest mean titer (1:768) was obtained by IV vaccination. During the secondary response the group revaccinated by the IV route had significantly higher titers than those vaccinated by other routes. In no case was the maximum titer during the secondary response significantly different from that following primary vaccination. These findings are supported by passive hemagglutination data obtained on the sera.

TABLE 4. EFFECT OF THE ROUTE OF VACCINATION WITH VIABLE P. TULARENSIS LVS ON THE BACTERIAL AGGLUTININ RESPONSE OF RABBITS

Day	Mean Reciprocal Agglutinin Titer			
	Intravenous	Subcutaneous	Intradermal	
			One site	Five sites
7	384	136	160	190
14	768	136	416	208
21	416	104	192	136
28[a/]	208	68	120	104
35	960	192	384	416
42	384	96	192	240
49	272	96	136	160
56	176	38	104	136

a. Animals revaccinated.

C. ANTIBODY RESPONSE OF RABBITS TO VIABLE LVS CONTAINED IN FREUND'S ADJUVANTS

The primary administration of 10^9 LVS organisms in complete or incomplete adjuvant by the SC route did not result in greater antibody production than was observed in animals that received 10^9 LVS without adjuvant by the IV route (Table 5). Revaccination on the 28th day did not result in an appreciable increase in agglutinin titers over maximum levels observed following primary vaccination. Similar conclusions resulted from the passive hemagglutination and agar gel precipitation studies.

D. HYPERIMMUNIZATION WITH KILLED AND VIABLE VACCINES

The antibody response of 10 rabbits to 8 weekly IV injections of 10^9 killed SCHU S4 cells is presented in Table 6. Regardless of the assay technique employed, hyperimmunization did not result in a higher antibody response than previously observed following a secondary response to the killed SCHU S4 vaccine. Maximum values during the hyperimmunization were comparable with those obtained following primary immunization with viable LVS.

TABLE 5. BACTERIAL AGGLUTININ RESPONSE OF RABBITS VACCINATED WITH VIABLE P. TULARENSIS LVS CONTAINED IN FREUND'S COMPLETE OR INCOMPLETE ADJUVANT

Day	Mean Reciprocal Agglutinin Titer		
	Complete adjuvant	Incomplete adjuvant	No adjuvant
7	104	160	640
14	160	160	544
21	160	136	192
28[a]	176	160	224
35	136	240	544
42	192	208	640
49	160	272	384
56	112	160	192

a. Animals revaccinated.

The mean agglutinin titers and passive hemagglutinin titers of pooled sera from rabbits vaccinated IV with 10^8 or 10^9 viable LVS at weekly intervals for 8 successive weeks are presented in Table 6. Maximum titers by both techniques were obtained on the 21st day (three prior doses of vaccine) with sera from animals vaccinated with 10^9 cells; the agglutinin titer was 1:1,920 and the passive hemagglutinin titer was 1:81,920. Antibody levels then decreased even though vaccination was continued. The agar gel precipitation technique revealed that the animals responded with an early and continued production of four to five precipitin bands (Fig. 1). Some groups of rabbits were extremely sensitive to this hyperimmunization technique and mortality rates as high as 80% were observed.

To alleviate the high mortality, rabbits were hyperimmunized with 10^8 viable LVS cells at weekly intervals for 8 weeks. The antibody response to this vaccination schedule was somewhat lower than with hyperimmunization by 10^9 cells per dose. Maximal bacterial agglutinin and hemagglutinin titers had been reached by the 21st day with titers of 1:1,024 and 1:20,480, respectively. Subsequently the titers, as assayed by both techniques, declined. The precipitin response was slower with 10^8 than with 10^9 cells per dose and comparable patterns were not observed until the 35th day.

The bacterial agglutinin response of rabbits hyperimmunized with three doses of 10^8 viable LVS cells administered IV at weekly intervals was studied. Termination of the hyperimmunization after 3 weeks did not result in data appreciably different from those for hyperimmunization continued for 8 weeks.

TABLE 6. ANTIBODY RESPONSE OF RABBITS FOLLOWING INTRAVENOUS HYPERIMMUNIZATION WITH VIABLE OR KILLED P. TULARENSIS[a]

Vaccine Dose	Serological Technique	Reciprocal Titer at Indicated Day							
		7	14	21	28	35	42	49	56
10^8 viable LVS	Bacterial agglutination[b]	272	1,024	1,024	512	448	448	320	448
	Passive hemagglutination[c]	2,560	10,240	20,480	10,240	5,120	5,120	5,120	5,120
10^9 viable LVS	Bacterial agglutination[b]	240	1,160	1,920	770	830	270	770	640
	Passive hemagglutination[c]	2,560	40,960	81,920	40,960	10,240	10,240	5,120	5,120
10^9 killed SCHU S4	Bacterial agglutination[b]	224	384	176	240	224	176	176	176
	Passive hemagglutination[c]	2,560	5,120	5,120	5,120	5,120	1,280	1,280	1,280

a. Injections at 8 weekly intervals.
b. Mean values.
c. Pooled samples.

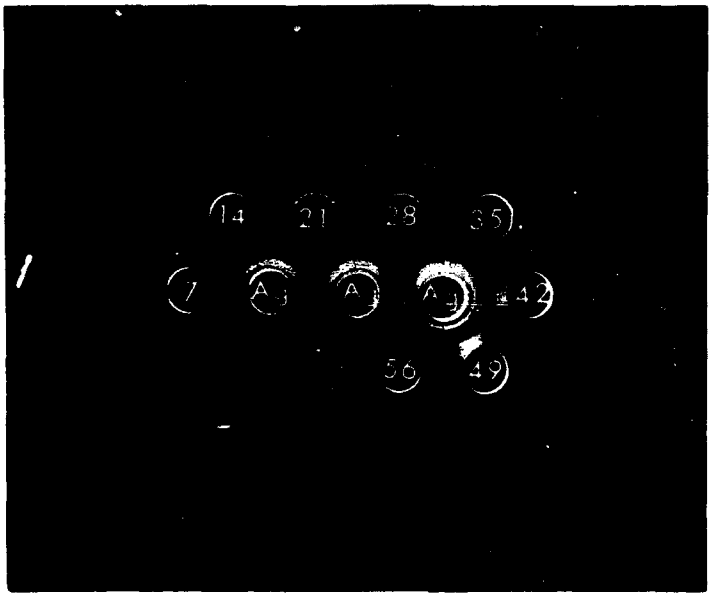

FIGURE 1. Precipitin Response of Rabbits Hyperimmunized with Viable P. tularensis LVS. Ag = antigen wells. Numbered wells are the antiserum wells; the numbers indicate the days after initiation of immunization.

IV. DISCUSSION

These experiments demonstrated that the most practical whole-cell preparation for the production of anti-P. tularensis antibodies in rabbits was viable strain LVS. The initiation of an overt infection with virulent SCHU S4 organisms followed by eradicative streptomycin therapy induced a slightly better response but this technique is certainly more cumbersome and hazardous. The use of a killed vaccine consistently resulted in the poorest antibody responses.

The antibody response of rabbits following vaccination with viable LVS administered by one of three routes showed that the IV route resulted in the best response but the reason for the clear superiority of this route is not known. This finding is similar to that of Snyder et al.[9] who studied the antibody responses of rabbits following injection of viable Mycobacterium tuberculosis. Undoubtedly there is widespread dissemination of the viable organisms and their antigenic products to most, if not all, of the lymphoid tissues following IV administration, and this could account for the better response. The similarity in responses between the animals vaccinated at either one ID or five ID sites indicates that a subdivision of the initial antigenic mass into five equal portions and the involvement of five rather than one draining lymphoid network is insufficient to induce a better response than that following IV administration. The pathogenesis of the LVS "vaccination infection" for the rabbit has not been studied; possibly, when the organisms are administered by either the SC or ID route, the organisms and their antigenic components of importance remain localized.

The failure of 8 weekly IV injections of killed SCHU S4 vaccine to induce better antibody responses than one injection of viable LVS or secondary vaccination with killed SCHU S4 is interesting because hyper-immunization is commonly used for the production of high-titered antisera against a variety of antigens.[3] In contrast, short-term hyperimmunization of rabbits with viable LVS constituted the most practical method for increasing the levels of anti-P. tularensis antibodies. The viable LVS schedule could be terminated after three injections at weekly intervals because the titers subsequently declined in spite of a continuing antigenic challenge. The decline in antibody levels despite repeated vaccination has also been observed in chickens administered killed or viable SCHU S4 organisms.*

In general, the results obtained with the killed strain SCHU S4 antigen compare favorably with those reported by Carlisle et al.[2] Maximum levels of antibody in the rabbits were achieved 1 week after both primary and secondary vaccination; however, those investigators obtained somewhat

* Unpublished observations.

higher antibody titers; this discrepancy most likely resulted from the use of an estimated 700-fold more bacteria for the primary and 100-fold more for the secondary response.[8]

These studies have shown that the rabbit can be used for the production of high-titered anti-P. tularensis antiserum and that the optimum method of vaccination is the IV administration of viable LVS cells at three weekly intervals.

LITERATURE CITED

1. Brigham, G.D. 1950. Page 262. In Diagnostic procedures and reagents. 3rd ed. American Public Health Association, Inc., New York.

2. Carlisle, H.N.; Hinchliffe, V.; Saslaw, S. 1962. Immunodiffusion studies with Pasteurella tularensis antigen-rabbit antibody systems. J. Immmol. 89:638-644.

3. Chase, M.W. 1967. Page 213. In Methods in immunology and immunochemistry. Vol. I. Academic Press, New York.

4. Downs, C.M. 1932. Immunologic studies on tularemia in rabbits. J. Infect. Dis. 51:315-323.

5. Eigelsbach, H.T.; Downs, C.M. 1961. Prophylactic effectiveness of live and killed tularemia vaccines: I. Production of vaccine and evaluation in the white mouse and guinea pig. J. Immmol. 87:415-425.

6. Mills, R.C.; Berthelsen, H.; Donaldson, D.; Wilhelm, P.L. 1949. Nutritional requirements of Bacterium tularense. Bacteriol. Proc. p. 37-38.

7. Nutter, J.E.; Myrvik, Q.N. 1966. In vitro interactions between rabbit alveolar macrophages and Pasteurella tularensis. J. Bacteriol. 92:645-651.

8. Roessler, W.G.; Brewer, C.R. 1967. Permanent turbidity standards. Appl. Microbiol. 15:1114-1121.

9. Snyder, I.S.; O'Connor, J.E.; Koch, N.A. 1967. Antibody response following injection with M. tuberculosis. Proc. Soc. Exp. Biol. Med. 126:740-745.

10. Tulis, J.J.; Eigelsbach, H.T. 1961. Use of double diffusion in agar for the study of Pasteurella tularensis antigens and precipitins. Bacteriol. Proc. p. 138.

11. Wright, G.G.; Feinberg, R.J. 1952. Hemagglutination by tularemia antisera: Further observations on agglutination of polysaccharide-treated erythrocytes and its inhibition by polysaccharide. J. Immmol. 68:65-71.

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13. ABSTRACT

The response of the rabbit to viable or killed whole-cell Pasteurella tularensis vaccines was studied. The most practical preparation for the production of anti-P. tularensis antibodies was viable organisms of the live vaccine strain (LVS). The intravenous route of administration proved superior to either the subcutaneous or intradermal routes, and incorporation of LVS into Freund's adjuvants did not result in increased levels of antibody. Short-term hyperimmunization, three injections at weekly intervals, constituted the most efficient method for increasing levels of the antibodies. ( )

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