Oral Vaccination against Tularemia in the Monkey[1] (34331)

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It has been established that live tularemia vaccine prepared from *Pasteurella tularensis* LVS in our laboratories and administered dermally or aerogenically to animals and man is innocuous and immunologically superior to killed preparations (1–4). Data obtained in the monkey and in man on the pathogenesis and immunogenicity of live vaccine strain LVS, administered via the dermal or respiratory route, (4–6) were sufficiently encouraging to warrant an evaluation of oral vaccination against tularemia in the monkey and then in man (7). This report considers some of the studies on oral vaccine prophylaxis in the monkey prerequisite to a comparable investigation in volunteers.

The rationale for studies on oral tularemia prophylaxis was based on the ability of *P. tularensis* to survive the acidity of the stomach. This organism can be readily isolated from the gastric washings of patients with the typhoidal-pneumonic form of tularemia (8). Our studies on the development and evaluation of a live tularemia vaccine have involved the exposure of 372 volunteers to aerosols of highly virulent *P. tularensis* SCHU S4 (3). In only one instance did a volunteer develop symptoms suggestive of gastrointestinal involvement although it is known that some of the aerosolized organisms were brought up from the trachea and swallowed or were impinged in the upper airways and flushed into the oropharynx.

Our previous studies on *P. tularensis* infection and on tularemia prophylaxis showed that the monkey was suitable as a model for man. The studies described below include data on the oral infectivity and pathogenicity of highly virulent *P. tularensis* SCHU S4 for the monkey as well as on the comparative immunity provided by oral and dermal administration of live vaccines prepared from *P. tularensis* LVS and from *P. tularensis* 425, a strain of high virulence for the mouse and guinea pig but considerably less virulent for the rabbit and monkey (9).

Materials and Methods. *P. tularensis* strains used for vaccination or for challenge were grown in casein partial hydrolyzate liquid media and viable populations were estimated by colony count on glucose cysteine blood agar (1).

Groups of eight conditioned monkeys (*Macaca mulatta*) were force fed 10^4, 10^6, 10^8, or 10^{10} live SCHU S4 organisms mixed in milk. A group of five animals was compelled to swallow gelatin capsules containing 10^{10} organisms, to prevent contamination of the upper respiratory tract.

For studies on the oral vaccination of monkeys, the following strains of *P. tularensis* were used; live vaccine strain LVS, immunogenic but innocuous for the monkey when administered by the dermal or respiratory route, and strain 425, capable of producing a mild illness that is seldom lethal in the monkey. Groups of 16 monkeys were force fed 10^8–10^{10} live organisms of strain LVS or 425 in milk. Another group of eight monkeys was inoculated with strain LVS dermally by multiple puncture in the manner used for vaccination against smallpox. Eight animals were used as nonvaccinated controls.

Rectal temperatures were recorded twice daily several days prior to vaccination of

[1] In conducting the research described in this report, the investigators 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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animals and for 30 days after administration of vaccine or challenge organisms; weights were recorded weekly. C-reactive protein (CRP) and corrected erythrocyte sedimentation rate (ESR) determinations were made on heparinized blood samples drawn twice before and three times weekly after administration of the test organisms. Gel diffusion and bacterial agglutination studies were conducted on serum from blood samples obtained on a weekly basis. Animals were frequently examined for clinical signs of illness (anorexia, weakness, and diarrhea). All animals that died after challenge with strain SCHU S4, one to two animals per group of nonchallenged vaccinees, and one nonvaccinated nonchallenged control were necropsied and subjected to pathologic and bacteriologic examinations.

Results. As indicated in Table I, the oral ID_{50} of strain SCHU S4 for the monkey was in the range of 10^7 cells. Evidence for infection included development of a febrile response or overt disease but was considered positive on basis of a diagnostic agglutinin titer alone. Monkeys administered 10^4 cells did not become infected. At doses of 10^6 cells or greater some or all animals became infected and died. As indicated, when the dose was increased, time to death was decreased appreciably. Animals given the organism–milk mixture became febrile and anorectic approximately 12–24 hr earlier and died on the average 2 days earlier than those that swallowed the capsule.

Considerable differences in pathologic changes were observed in animals dying from the various oral doses of strain SCHU S4. Ingestion of 10^6–10^8 cells produced lesions in the lung and other organs comparable to those usually seen after an aerogenic exposure (5); only one animal exhibited inflammation of the large intestine. In contrast, all animals administered 10^{10} organisms showed severe inflammation and ulceration of the intestinal mucosa, especially in the ileum. The majority of these animals also showed inflammation and circumscribed ulceration in the cardiac region of the stomach. One to 2 days prior to death, these monkeys passed blood rectally. *P. tularensis* organisms were routinely isolated from the mesenteric lymph nodes as well as from the lung, liver, and spleen.

TABLE I. Oral Infectivity of *Pasteurella tularensis* SCHU S4 for the Monkey.

Oral dose[a]	Infected (%)	Dead (%)	Mean day of death
10^6	100	100	18
10^4	0	0	
10^6	25	25	18
10^8	100	63	11
10^{10}	100	100	5
10^{10b}	100	100	7

[a] Five to eight animals per group.

[b] Bacteria contained in gelatin capsules; other animals force fed organisms in milk.

In contrast to the severe disease associated with oral administration of strain SCHU S4, 10^{10} cells of either strain LVS or 425 resulted in only a benign response. One animal force fed strain LVS showed a transient low grade fever. With this exception, none of the monkeys vaccinated with LVS exhibited overt signs of disease. The majority of the animals force fed strain 425 showed a transient low grade febrile response but no other signs of disease; CRP and ESR data were not indicative of an active disease process.

A diagnostic serological response was detected earlier in monkeys vaccinated dermally with LVS or orally with strain 425 than in those administered LVS orally; all vaccinees developed agglutinins (Table II). Highest titers were recorded 21–28 days after vaccination but may have risen to even greater levels before animals were challenged 10 weeks after vaccination. At that time agglutinin titers in general had dropped to approximately two-thirds to one-half the maximal reported titer. Twenty-one days after vaccination and thereafter agglutinin titers in animals vaccinated orally with strain 425 were appreciably higher than those in animals given LVS dermally or orally. Gel diffusion studies showed comparable precipitin production by animals vaccinated dermally or orally with LVS but usually more (eight versus six) and denser precipitin bands were ob-

TABLE II. Agglutinin Response of Monkeys Vaccinated with *P. tularensis*, Strain LVS or 425.

Strain	Route	No. of vaccinees	Reciprocal mean titer; after vaccination Days: 7	14	21	28	70
LVS	Dermal[a]	8[c]	118	143	220	275	187
	Oral[b]	16[d]	8	78	340	364	188
425	Oral[b]	16[d]	85	248	1200	1780	803

[a] Sixty intracutaneous punctures made with a needle through a drop of live vaccine containing approximately 10^7 organisms.

[b] Between 10^8 and 10^{10} organisms force fed in milk.

[c] One animal sacrificed for pathologic and bacteriologic study 7 weeks after vaccination.

[d] Two animals sacrificed for pathologic and bacteriologic study after vaccination.

served in the sera of animals vaccinated orally with strain 425.

Two of the 16 animals force fed strain LVS, 2 of 16 force fed strain 425, and 1 of 8 vaccinated percutaneously were sacrificed for pathologic and bacteriologic examination approximately 7 weeks after exposure. No pathologic residua were observed grossly or microscopically and *P. tularensis* was not isolated from the tissues. Ten weeks after vaccination, the remaining animals were challenged via the respiratory route with 10^4 organisms of virulent strain SCHU S4. On the basis of our previous experience (4), it was estimated that this formidable aerogenic dose would result in the infection of all dermal vaccinees with a survival of 50–60%. Results are presented in Table III. The disease was characterized as mild, moderate, severe, or fatal. Mild illness consisted of low grade fever for 3–5 days; little or no anorexia, loss of weight, apparent weakness, or diarrhea; no greater than 1+ CRP, and a corrected ESR of less than 15 mm/hr. Illness was classified moderate if the rectal temperature exceeded 104°F for a week or more; anorexia, weakness, and diarrhea were noted for 3 or more days, CRP values of 2+ or greater were obtained; and the corrected ESR exceeded 15 mm/hr. More intense and continuing symptoms and signs were noted in animals that died and in the only control animal that survived. Marked prostration characterized the course of the illness in the latter animal that incurred severe disease. Although all vaccinees and controls became ill following challenge, 64–71% of the vaccinees survived in comparison to 14% of the controls. Vaccinated survivors were febrile for 3–14 days and anorectic for 1–3 days during the acute phase of disease. No appreciable differences were noted in the grade of immunity engendered by the vaccines or the route by which administered. The sole surviving control animal experienced a severe illness and for several days appeared moribund.

Discussion. The data on the oral infectivity of highly virulent *P. tularensis* SCHU S4 for the monkey indicated that 10^4 viable cells were not sufficient to infect but doses of 10^6 and 10^8 infected 25 and 100% of the animals, respectively. In contrast, 10–25 orga-

TABLE III. Immunogenicity of *P. tularensis*, Strains LVS and 425, for the Monkey.

Strain	Route	Vaccinees challenged[a]	Animals showing disease: Mild	Moderate	Severe	Fatal	Survival (%)
LVS	Dermal	7	3	2	0	2	71
	Oral	14	6	3	0	5	64
425	Oral	14	9	1	0	4	71
None		7	0	0	1	6	14

[a] Dosages, 10^4 cells of strain SCHU S4 via the respiratory route.

nisms of this strain have proved sufficient to infect either man or monkey dermally or via the respiratory route (4). The diversity in infectivity of SCHU S4 with regard to portal of entry was subsequently corroborated in studies in man (7); 10^8 live SCHU S4 given orally produced illness and serologic conversion in all of five volunteers but 10^6 organisms caused neither clinical disease nor an agglutinin response in a comparable number of volunteers.

Monkeys infected orally with high doses of SCHU S4 exhibited pathologic manifestations previously not observed by gross and histologic examination of monkeys infected by the dermal or respiratory route. The severity of the gastrointestinal involvement in the monkey as evidenced in intestinal bleeding and severe inflammation and ulceration of the intestinal mucosa and the stomach, indicated how the clinical and pathologic progression of an infectious bacterial disease may vary dependent upon the portal of entry and the dose.

Results on oral vaccination of monkeys with strains LVS or 425 demonstrated that this immunization procedure is not associated with significant untoward effects. High grade immunity was provided animals against a substantial respiratory challenge. No appreciable differences in the grade of immunity was observed between animals vaccinated with strains LVS or 425 nor between animals vaccinated orally or dermally. Subsequent studies on the effectiveness of oral vaccination of man with LVS demonstrated that immunity derived therefrom was comparable to that after vaccination by the dermal or aerogenic route (7). The monkey again was proved an excellent model for man in studies on vaccine prophylaxis against tularemia.

Summary. Studies on the immunogenicity of live tularemia vaccine for animals and man vaccinated dermally or aerogenically were sufficiently encouraging to warrant an evaluation of vaccination of the monkey via the oral route. A preliminary investigation of the oral infectivity and virulence of *P. tularensis* SCHU S4, fatal for the monkey infected dermally or by the respiratory route with 10–25 organisms, revealed that 10^4 viable cells swallowed with milk did not result in infection but that 10^6 organisms infected two of eight animals, both of which died. An oral dose of 10^8 or 10^{10} cells infected all monkeys tested and the majority died; severe inflammation and ulceration of the gastrointestinal tract were found at necropsy. The course and outcome of the disease were comparable when animals were compelled to swallow intact a gelatin capsule containing 10^{10} organisms.

In a study on the reactivity and immunogenicity of live vaccine strains LVS and of strain 425, highly infectious but seldom lethal for the monkey inoculated dermally or inhaling up to 10^4 cells, groups of animals were force fed 10^{10} live organisms in milk. An additional group of monkeys was vaccinated dermally with strain LVS administered by multiple puncture. Both the oral and the dermal inocula of LVS proved innocuous but the majority of the animals given strain 425 showed a transient low grade febrile response. The sera of all vaccinees gave positive agglutinin and precipitin reactions. Ten weeks after vaccination those animals and a group of nonvaccinated controls were administered an aerogenic challenge of 10^4 organisms of strain SCHU S4. All animals became infected but 64–71% of the vaccinees survived in contrast to 14% of the nonvaccinated controls. A potential for oral vaccination with live tularemia vaccine prepared from strain LVS was demonstrated by these studies and led to tests in volunteers which showed that vaccination with strain LVS by the oral route is useful and practical for the immunization of man against tularemia (7).

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