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Experimental *Yersinia pestis* Infection in Rodents After Intragastric Inoculation and Ingestion of Bacteria

THOMAS BUTLER,[1] YAO-SHI FU,[1]* LYDIA FURMAN,[1] CELIO ALMEIDA,[2] AND ALZIRA ALMEIDA[2]

Departments of Medicine and Pathology, University Hospitals of Cleveland and Case Western Reserve University, Cleveland, Ohio 44106,[1] and the Central Laboratory of Plague, Garanhuns, Pernambuco State, Brazil[2]

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To clarify the pathogenesis of oral plague infection, we studied the susceptibility of three species of rodents to intragastric inoculation of *Yersinia pestis*, described the pathology and progression of infection, and measured antibody responses to fraction IA antigen of *Y. pestis*. The 50% lethal doses of bacteria by intragastric inoculation for *Mus musculus*, *Zygodontomys pixuna*, and *Rattus rattus* were $\log_{10}$ = 6.32, 5.46, and 9.62, respectively, which were at least 1,000-fold higher than the values obtained by subcutaneous inoculation. *M. musculus* was shown to be susceptible to lethal infection also when bacteria were ingested in drinking water. Microscopic pathology was consistent with heavy systemic infection. Quantitative cultures of tissues at different times after intragastric inoculation revealed that infections of blood, liver, and spleen preceded infections of Peyer's patches and mesenteric lymph nodes. Stool cultures were negative. The strain of *Y. pestis* used for inoculation was killed when exposed to a buffered solution at pH ≤3. Antibody responses were observed in some of the surviving rodents after intragastric challenge. These results showed that *Y. pestis* was an effective oral pathogen that produced fatal systemic infections and self-limited infections with immunity but did not produce enteric pathology or lead to fecal excretion of bacteria.

Plague caused by *Yersinia pestis* is a natural infection of rodents. Transmission among rodent reservoirs occurs predominately by flea bites but also may occur by ingestion. In 1894, Yersin first showed that rats and mice could be fatally infected by feeding them fragments of spleen or liver from animals that had died of plague (21). Although rodents are not naturally carnivorous, they eat dead littermates in captivity and may do so occasionally in their natural burrows. Natural carnivores such as bobcats, coyotes, and cats can develop fatal systemic infections with plague after ingesting infected rodents, and these carnivores have transmitted plague to people, such as hunters, who have handled the animals (14, 18). An outbreak of plague in Libya in 1976 was associated with skinning and eating the meat of a sick camel (10).

Y. pestis has been shown to be extraordinarily lethal to rodents by parenteral injection (3), but little work has been done since the time of Yersin to clarify the role of *Y. pestis* as an oral pathogen in rodents. Rust and colleagues showed that dogs and cats which were fed *Y. pestis* all developed antibody rises and that three cats died with overwhelming purulent infections (16). Chen et al. showed that vervet monkeys fed 10 organisms of the avirulent strain EV76 developed fever, diarrhea, and passage of blood and mucus in stools (9). Most of these animals developed antibody responses after nonfatal illnesses. Chen and co-workers found that instilling organisms into the oral cavity was more effective than placing bacteria into the food of monkeys and concluded that *Y. pestis* crossed the oral mucosa more efficiently than the mucosa of the lower gastrointestinal tract (9).

The classification of the plague bacillus in the family *Enterobacteriaceae* suggests that *Y. pestis* could have a larger role in the intestinal niche than has been previously appreciated. In this study, we attempted to determine the susceptibility of laboratory and field rodents to *Y. pestis* by the oral route. The pathology of orally acquired plague infection suggested that it is not an enteric pathogen but kills by producing a systemic infection. Furthermore, the failure of bicarbonate or streptomycin pretreatment of mice to render them more susceptible to infection demonstrated basic differences between *Y. pes-*

tis and the other enteric pathogenic bacteria *Vibrio cholerae* and *Salmonella typhimurium* (2, 12).

MATERIALS AND METHODS

Bacterial strains. A strain of *Y. pestis* obtained from a patient with plague in Vietnam in 1974 was used to inoculate mice. It had been passaged through mice to maintain virulence and was stored at room temperature on nutrient agar slants. To prepare bacteria for experimental infections, growth was obtained by inoculation of Mueller-Hinton broth and incubation at 35°C for 6 h or overnight. In some experiments, to obtain inocula containing $>10^8$ bacteria per ml, chocolate agar plates were streaked and, after incubation for 48 h at 35°C, colonies were suspended in 0.9% NaCl. Viable colony counts were performed by serial dilutions of turbid suspensions and inoculation of 0.1 ml onto nutrient agar plates. In Brazil, a local strain of *Y. pestis* isolated from human plague in 1977 was used to inoculate *Rattus rattus* and *Zygodontomys pixuna*. This strain had been stored on nutrient agar without animal passage and was cultivated for 48 h on nutrient agar at 35°C and suspended in 0.9% NaCl. The suspension was diluted serially 10-fold in 0.9% NaCl for animal inoculation and bacterial enumeration.

Experimental infections. Laboratory mice used were *Mus musculus* CF-1 females that were 6 to 8 weeks old and weighed approximately 20 g (Charles River, Wilmington, Mass.). They had free access to tap water and Formulab chow 5008 (Ralston Purina Co., St. Louis, Mo.) and were fasted overnight before inoculations and intubated for intragastric (i.g.) inoculation with a no. 16 metal feeding cannula attached to a syringe. To minimize the possibility of introducing bacteria by abrading the oral mucosa and esophagus, the tip of the feeding cannula was filed smooth and, before each inoculation, the suspension of bacteria was withdrawn from the cannula, which was then lightly flamed and wiped with cotton containing 70% ethanol. Bacteria were delivered in 0.2 ml of 0.9% NaCl in 10-fold dilutions of dosages ranging from about 10^2 to 10^8 bacteria. For subcutaneous inoculations, bacteria were delivered with a no. 26 needle in 0.2 ml of 0.9% NaCl beneath the skin of the lower abdomen. Bacteria were given in 10-fold dilutions of dosages ranging from approximately one viable organism to about 10^6 organisms. Each group inoculated by the same dosage and route comprised 10 mice. Animals were observed for death during 21 days. Dead animals were autopsied for cultures of blood and spleen. Surviving mice were killed 21 days after inoculation. Cultures of blood and spleen were obtained, and serum was taken for serological testing.

In Brazil, the two most frequently infected species of wild rodents in Pernambuco, *R. rattus* and *Z. pixuna* (C. R. Almeida, A. R. Almeida, J. B. Vieira, U. Guida, and T. Butler, Bull. W.H.O., in press) were used for experimental infections. Pixunas are brown, burrowing, rural rodents which are smaller than rats and live near cultivated fields, where they eat corn and other grains. The rats in rural Brazil, on the other hand, live in or near people's houses. Animals that had been trapped in Pernambuco and quarantined for about 30 days were selected for inoculation. Rats ranged in weight from 37 to 147 g, and pixunas ranged in weight from 27 to 58 g. Both sexes were used. All animals were bled from the retroorbital plexus for serological testing before inoculation. Five animals composed a group for each dosage and route of infection. Rats received doses of bacteria of from 4.2×10^2 to 4.2×10^6 organisms subcutaneously and 4.2×10^5 to 4.2×10^9 organisms i.g.; pixunas received 1.7 to 1.7×10^4 organisms subcutaneously and 1.7×10^5 to 1.7×10^9 organisms i.g. Inoculations given i.g. were delivered with a no. 16 metal feeding cannula, and subcutaneous inoculations were delivered with a no. 26 needle. Dead animals were autopsied for cultures of blood and spleen. After 4 weeks, surviving animals were bled for serological testing, and these animals were sacrificed for cultures of blood and spleen. Cultures were confirmed as *Y. pestis* by specific bacteriophage lysis.

The 50% lethal dose (LD_{50}) and 50% infective dose (ID_{50}) were calculated by the method of Reed and Muench (15). For lethal doses, only dead animals with positive postmortem cultures for *Y. pestis* were considered. For infective doses, all animals were included that died with positive cultures, had positive cultures at the time of sacrifice, or showed an elevated passive hemagglutinin (PHA) titer ($\geq$1:16) at 3 or 4 weeks after infection.

Serological testing. The PHA test for antibodies against fraction IA of *Y. pestis*, which is sensitive and specific for the detection of both human and rodent plague in endemic areas (8), was performed by the method of the Plague Branch of the Centers for Disease Control (CDC), Fort Collins, Colo. (4, 20). Briefly, sheep erythrocytes collected in Alsever solution were washed and fixed in glutaraldehyde. The cells were exposed to tannic acid and sensitized with fraction IA antigen of *Y. pestis* (obtained through the courtesy of the Plague Branch, CDC) in a final concentration of 100 μg of antigen per ml. The PHA test was performed by a microtiter technique and controlled by concomitant passive hemagglutination inhibition (PHI) testing. Into each well of the PHA microtiter plate was pipetted 0.025 ml of 1:100 normal rabbit serum in 0.9% NaCl, and into each well of the PHI control was placed 0.025 ml of 1:100 normal rabbit serum in 0.9% NaCl containing 100 μg of antigen per ml. Using a microdiluter with a volume of 0.025 ml, test and control sera were delivered to both PHA and PHI wells, and 11 twofold dilutions were made of each serum in a row. Finally, 0.025 ml of sensitized erythrocytes, 0.5% by volume, in 1:250 normal rabbit serum in 0.9% NaCl was delivered to all wells. The plates were swirled, covered, and allowed to stand overnight at 4°C. The highest dilutions of sera showing hemagglutination were recorded and compared with the PHI titers. Sera with PHA titers of $\geq$1:16 were readsorbed with sheep erythrocytes and retested. Sera were considered positive only when repeated testing showed a PHA titer of $\geq$1:16 that was eightfold or more above the PHI titer.

Pathology. Dead mice were autopsied for cultures and smears of spleens, livers, and blood. Tissues were fixed in 95% ethanol before being placed into Formalin for preparation of paraffin blocks. Sections of stomach, small bowel, lymph nodes, spleen, liver, lungs, and kidney were cut for staining with hematoxylin and eosin and Brown-Brenn bacterial stain.

Treatment. In some experiments, mice received by feeding cannula 0.1 ml of a 20% aqueous solution of

TABLE 1. Susceptibility of rodents to *Y. pestis* infection by the i.g. and subcutaneous routes

Rodent species	Log_{10} viable bacteria by following route[a]:			
	I.g.		Subcutaneous	
	LD_{50}	ID_{50}	LD_{50}	ID_{50}
M. musculus[b]	6.32	5.04	−0.15	−0.15
Z. pixuna[c]	5.46	5.46	1.73	1.46
R. rattus[c]	9.62	7.26	6.62	3.30

[a] Numbers are the log_{10} viable bacteria that were the LD_{50} and ID_{50} calculated by the method of Reed and Muench.

[b] CF-1 laboratory mice were inoculated with a strain of *Y. pestis* isolated in Vietnam.

[c] *Z. pixuna* and *R. rattus* trapped in rural Brazil were inoculated with a strain of *Y. pestis* isolated in Brazil.

sodium bicarbonate (Fisher Scientific Co., Pittsburgh, Pa.) 5 min before bacterial inoculation. Other mice received by feeding cannula 50 mg of streptomycin sulfate (Eli Lilly & Co., Indianapolis, Ind.) in 0.25 ml 24 h before bacterial inoculation.

Effects of acidity on viability of bacteria. Log phase turbid cultures of *Y. pestis* were obtained in Mueller-Hinton broth at 37°C. Other bacteria examined were *Salmonella typhimurium* LT-2, *Vibrio cholerae* V-17881 isolated from a patient in Bangladesh, *Shigella flexneri* V-17768 isolated from a patient in Bangladesh, and *Yersinia enterocolitica* A9466 obtained from the Centers for Disease Control, Atlanta, Ga. A 0.1-ml amount of broth was placed into 0.9 ml of buffered solutions with pH values of 2, 3, 4, and 5, which were prepared by mixing varying proportions of 0.2 M sodium diphosphate and 0.1 M citric acid. The pH of the Mueller-Hinton broth was 7.4, and addition of 0.1 ml of the Mueller-Hinton broth to 0.9 ml of the buffers resulted in pH changes of no more than 0.1 pH unit for each of the buffered solutions. After the bacterial suspensions were mixed with the buffered solutions and were incubated at 37°C for 1 h, quantitative cultures were performed.

RESULTS

Susceptibility of rodents to infection by i.g. inoculation. To compare the infectivity of *Y. pestis* by the i.g. and subcutaneous routes, different groups of rodents received graded doses of *Y. pestis* by both routes. All dead rodents were autopsied and had positive organ cultures. Surviving rodents were sacrificed, and those with positive organ cultures or titers of PHA antibody against fraction IA of ≥16 were added to those that died with positive organ cultures to obtain infective doses. All three species were susceptible to fatal infection with *Y. pestis* by the i.g. route (Table 1). Of the two Brazilian rodent species inoculated with the same strain of *Y. pestis*, *R. rattus* was more resistant to infection than was *Z. pixuna*. The ID_{50} values for *M. musculus* and *R. rattus* were lower than the LD_{50} values because some of these rodents showed serological responses to infection. For all species, inoculation by the subcutaneous route produced LD_{50} and ID_{50} values that were at least 1,000-fold lower than the corresponding values obtained by i.g. inoculation. The values of 0.7 (log_{10} = −0.15) bacterium for the LD_{50} and ID_{50} for mice by the subcutaneous route demonstrated both the high susceptibility of these rodents and the virulence of *Y. pestis* for this species that permitted one bacterium to kill some of the mice. Most deaths occurred between 3 and 8 days after inoculation. Early deaths usually occurred in those animals that received the largest inoculum.

Susceptibility of rodents by ingestion of bacteria in drinking water. To determine whether *Y. pestis* was also virulent when naturally ingested, bacterial suspensions were prepared from colonies on a chocolate agar plate and diluted with distilled water to a final concentration of about 10^8 organisms per ml. Five mice were allowed to drink 20 ml of infected water. All died 3 days later, and smears and cultures of tissues revealed heavy *Y. pestis* infection.

Pathology of orally infected mice. Gross and microscopic pathological examinations were made of 10 mice infected by either i.g. inoculation or allowing them to ingest water containing bacteria. Animals were selected that were moribund or freshly dead, and infection with *Y. pestis* was confirmed by positive spleen, liver, and blood cultures. Gross and microscopic examinations of lungs revealed no evidence of pneumonia. Thus, aspiration of bacteria either from the stomach or during drinking was excluded as a mechanism for the introduction of bacteria across mucosal surfaces. The spleens were moderately enlarged, and the small intestines did not show changes of enteritis or abscess formation. Microscopically, most animals had minimal changes of increased mitosis of intestinal epithelial cells. Peyer's patches showed lymphoid hyperplasia with an increase of mononuclear cells. The livers were grossly normal, but microscopically they showed abscesses, congestion, and focal necrosis. Kupffer cells contained bacteria. The spleens were also congested with lymphoid hyperplasia of the white pulp and necrosis in the red pulp. High numbers of bacteria were visible in liver sinusoids and in the red pulp of the spleens. The mesenteric lymph nodes demonstrated lymphoid hyperplasia without necrosis or abscess formation. In most animals, there were increased numbers of mononuclear cells with phagocytic activity (Fig. 1).

To observe the sequential appearance of pathological changes, another group of 15 mice were sacrificed at intervals of 6, 12, 24, 48, and

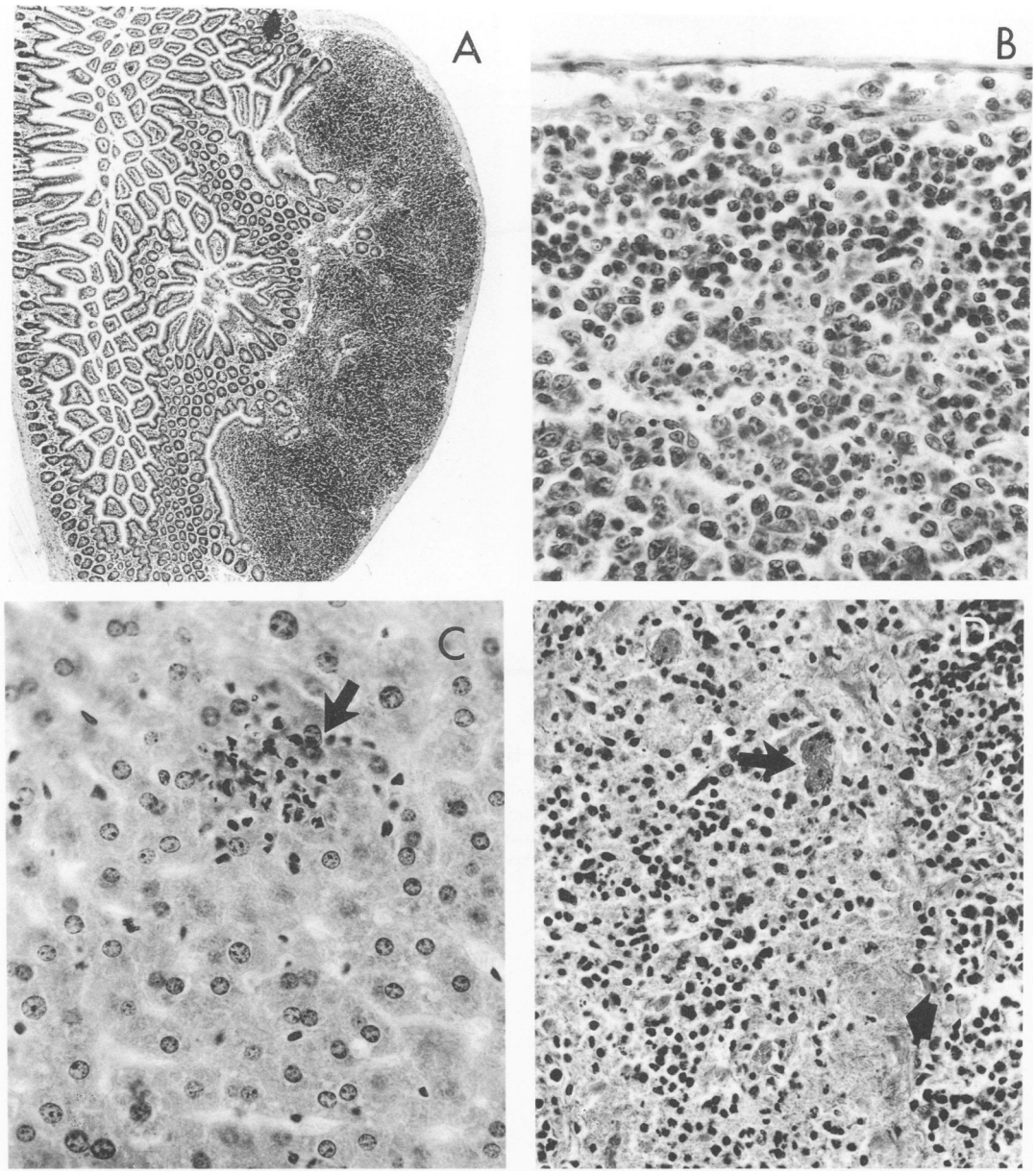

FIG. 1. Characteristic pathological changes in CF-1 mice with fatal *Y. pestis* infection acquired by i.g. inoculation. (A) In the terminal ileum, a Peyer's patch shows lymphoid hyperplasia. The mucosa overlying the Peyer's patch and villi is intact (magnification, ×31). (B) A mesenteric lymph node shows increased numbers of mononuclear cells (magnification, ×320). (C) A section of liver shows microabscesses with collection of neutrophils (arrow) (magnification, ×400). (D) The red pulp of a spleen shows extensive necrosis and neutrophilic infiltration. Macrophages (arrow) and massive numbers of extracellular bacteria are present (arrowhead) (magnification, ×320).

72 h after i.g. inoculation of 8×10^5 organisms. No pathological changes were noted in the first 12 h, but changes became apparent 24 h after the infection. In the gastrointestinal tract, the overlying intestinal mucosa was intact throughout. Peyer's patches demonstrated mononuclear cells with phagocytosed nuclear debris, and the number of mononuclear cells increased with time. In the liver and spleen, organisms were present in the sinusoidal spaces and were engulfed by the cells of the reticuloendothelial lining at 24 h. Microabscesses containing bacte-

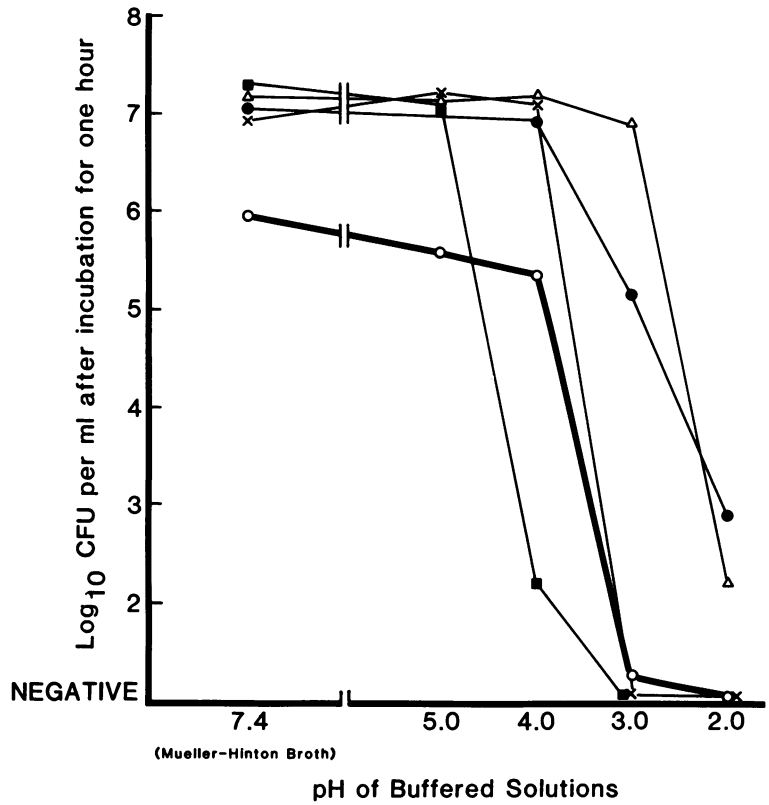

FIG. 2. Antibacterial effect of acidity on suspensions of *Y. pestis* (Vietnam) (○). Each point is the mean of triplicate plate counts determining the colony-forming units (CFU) of bacteria after 1 h of incubation at 37°C in citrate-phosphate buffers of a given pH. Results of cultures of *V. cholerae* (■), *Salmonella typhimurium* (X), *Y. enterocolitica* (△), and *Shigella flexneri* (●) are also shown.

ria were evident at 48 h, and extensive necrosis was noted at 72 h. The mesenteric lymph nodes were hyperplastic, with increased numbers of mononuclear cells in the sinuses. Some of the mononuclear cells contained bacterial organisms at 72 h. No pathology was found in the lungs, kidney, or pancreas. Bacteria were, however, found in the blood vessels of all organs.

To determine whether the observed changes in intestinal epithelium, Peyer's patches, and mesenteric lymph nodes were attributable to the introduction of organisms into the gastrointestinal tract, these tissues were compared between groups of mice inoculated by the i.g. and subcutaneous routes. Both groups showed comparable, similar changes. This identical pathology in infections introduced by both routes indicated that the lesions of orally induced plague infections were probably the result of systemic infection rather than of local enteric infection.

Susceptibility of *Y. pestis* to lethal effects of acidity. To assess the ability of *Y. pestis* to survive the acid pH of the stomach after ingestion or i.g. inoculation, we placed one part of log phase cultures in Mueller-Hinton broth into nine parts of sodium diphosphate-citric acid buffers at different pH values. After 1 h of incubation at 37°C, viable bacteria were counted. This strain of *Y. pestis* survived pH values of 4 and higher but was sharply killed at pH values of 3 and lower (Fig. 2). This pattern was similar to that seen with the LT-2 strain of *S. typhimurium*. When compared with other strains of bacteria, *Y. pestis* was more resistant to acid pH than was *V. cholerae* but was more sensitive than were tested strains of *Yersinia enterocolitica* and *Shigella flexneri*.

Effects of bicarbonate and streptomycin pretreatment on susceptibility of mice to infection. Reducing gastric acidity with bicarbonate and reducing the normal intestinal flora with streptomycin have been shown to increase the susceptibility of animals to infection by other enteric bacteria (2, 11). Groups of mice were given i.g. 0.1 ml of a 20% solution of sodium bicarbonate or 50 mg of streptomycin at 5 min or 24 h, respectively, before i.g. challenges with *Y. pestis* in serial 10-fold-diluted doses ranging from

TABLE 2. Progression of infection through tissues of CF-1 mice inoculated i.g. with a suspension of 3×10^8 viable *Y. pestis* organisms in 0.2 ml of 0.9% NaCl

H after inoculation	Mean ± SEM $\log_{10}$ CFU of *Y. pestis* in following type of tissue[a]:				
	Peyer's patches	Mesenteric lymph nodes	Spleen	Liver	Blood
6	None	None	0.89 ± 0.19	None	None
12	0.77 ± 0.08	0.80 ± 0.19	4.09 ± 0.39	4.91 ± 0.44	2.49 ± 0.77
24	1.21 ± 0.30	2.21 ± 0.51	3.86 ± 1.12	4.93 ± 1.42	3.60 ± 0.99
48	0.89 ± 0.19	4.94 ± 0.47	5.83 ± 0.49	7.02 ± 0.42	4.95 ± 0.43

[a] For Peyer's patches, mesenteric lymph nodes, and spleen, each number is the mean ± standard error of the mean of the $\log_{10}$ colony-forming units (CFU) of *Y. pestis* per total tissue obtained in each of four mice. For liver and blood, results are the mean ± standard error of the mean of the $\log_{10}$ colony-forming units of *Y. pestis* per gram of tissue obtained in each of four mice. The limit of detectability was 10 bacteria per tissue. Negative cultures were counted as five bacteria, or $\log_{10} = 0.70$. None, All specimens showed negative cultures.

10^2 to 10^7 viable organisms. There were no significant differences in the LD_{50} and ID_{50} values between the pretreated groups of mice and the untreated controls.

Progression of *Y. pestis* infection through tissues after i.g. inoculation. To elucidate the sequence of bacterial proliferation in different tissues, quantitative cultures of homogenized organs and tissues were obtained at 6, 12, 24, and 48 h after i.g. challenge with 3×10^8 viable organisms. Bacteria accumulated to levels greater than $\log_{10}$ 2 per tissue at 12 h in the spleen, liver, and blood (Table 2). In these tissues, the numbers of bacteria continued to accumulate until 48 h. In the intestinal lymphoid tissue of Peyer's patches and mesenteric lymph nodes, bacteria appeared in smaller quantities and more slowly than in the spleen, liver, and blood. Only at 48 h had the mesenteric lymph nodes acquired more than $\log_{10}$ 4 bacteria per node. This level of infection had been reached in the spleen and liver as early as 12 h after inoculation. These findings indicated that *Y. pestis* infection by the oral route bypasses intestinal tissues by proceeding directly to become a systemic infection of the blood, spleen, and liver.

Cultures of stool and intestinal contents. After i.g. challenges with doses as high as 10^9 *Y. pestis* organisms stools were collected daily for 3 days from 25 mice and from 10 rats. Stools were emulsified in saline, serially diluted, and plated onto tryptic soy agar and deoxycholate agar. All stool cultures were negative for *Y. pestis*. To determine how well inoculated *Y. pestis* survives in the intestine, five mice were killed 1 h after i.g. challenge with 3×10^8 organisms. The stomach and small bowel were flushed with saline. These effluents gave negative cultures for *Y. pestis*, indicating that bacteria did not survive in the lumen of the stomach and bowel.

TABLE 3. Immune response in rodents surviving i.g. inoculation with virulent *Y. pestis*

Rodent species	Dose range of inoculated viable bacteria	No. of rodents with elevated PHA titers/ total no. of survivors
M. musculus	1.1×10^2 to 1.1×10^7	6/57
Z. pixuna	1.7×10^4 to 1.7×10^9	0/8
R. rattus	4.2×10^5 to 4.2×10^9	8/15

Immune response after i.g. inoculation with *Y. pestis*. In experiments done to determine the LD_{50} and ID_{50} values of *Y. pestis* by i.g. challenge, rodents that survived for 21 days or longer were killed, and serum was obtained for measurement of antibodies against fraction IA of *Y. pestis*. Most animals that received the larger doses died before the time of serological testing. Of 57 surviving mice, 6 (11%) showed increased titers of antibody (Table 3). All 57 mice had negative spleen cultures at this time, indicating that the six with elevated antibody titers had developed self-limited infections. None of eight tested *Z. pixuna* animals had antibody elevations; the low number available for testing resulted from the high mortality rate in this group, indicating that they were susceptible to lethal infection but not to self-limited infection. *R. rattus* showed a higher rate of serological responses (8 of 15 [53%]) that was associated with higher LD_{50} and ID_{50} values, indicating that the rats were relatively resistant to developing infection but that some did develop self-limited infections.

DISCUSSION

Both laboratory mice and wild rodents were susceptible to lethal *Y. pestis* infection when they were inoculated with approximately 10^5 or more viable organisms by orogastric intubation. Furthermore, mice were susceptible to infection

when they drank water containing high numbers of bacteria. Although the anatomical site at which the bacteria crossed epithelial surfaces to produce fatal systemic infection was not determined, the absence of lung pathology rules out inhalation or aspiration as the model of infection. Thus, it appears likely that *Y. pestis* crosses the gastrointestinal epithelium at some site after bacteria enter the stomach.

The failure to reculture organisms from intestinal contents or stool after i.g. inoculation indicates that *Y. pestis* does not multiply in the lumen of the gut and is probably susceptible to the lethal action of stomach acid and other antibacterial factors in the bowel. The mechanism by which the bacteria cross the gastrointestinal epithelium likely resembles the persorption of *Candida* organisms from the gut directly into the thoracic duct that has been described in infant mice inoculated i.g. (11) and the translocation of *Salmonella* from the gut to mesenteric lymph nodes that has been described in germfree mice (1). In the case of *Salmonella*, Takeuchi observed organisms by electron microscopy within and between intestinal epithelial cells (17). In our studies of oral plague infection, however, bacteria did not appear initially and build up in Peyer's patches and mesenteric lymph nodes, as Carter and co-workers showed in experimental *Salmonella* and *Yersinia enterocolitica* infections (6, 7). After oral challenge, *Y. pestis* appeared in the liver, spleen, and blood in high numbers by 24 h after inoculation. The presence of lower numbers of bacteria at this time in the mesenteric lymph nodes and Peyer's patches indicated that *Y. pestis* had bypassed the intestinal lymphoid tissues to become a systemic infection.

In contrast to the pathology of *Y. enterocolitica* infection in mice by the oral route, which produces acute abscesses of Peyer's patches and mesenteric lymph nodes (5), *Y. pestis* produced minimal evidence of any local enteric pathology. Mice showed no signs of acute enteritis or abscess formation. The lymphoid tissue of Peyer's patches and mesenteric lymph nodes revealed only moderate increases of mononuclear cells with phagocytosis. The pathology of these orally acquired infections was typical of overwhelming systemic infection, consisting of heavy infection of the blood, acute inflammation and necrosis of the splenic red pulp, and microabscesses in the liver. The lesions in these tissues were identical to those seen in mice infected by the subcutaneous route. This consistent systemic pathology establishes that *Y. pestis* is not an enteric pathogen but is an effective systemic pathogen whether introduced by the subcutaneous or the oral route.

Further differences between oral *Y. pestis* infections and the classical enteric pathogens *Salmonella* and *Vibrio cholerae* were demonstrated by the failure of bicarbonate or streptomycin pretreatment to enhance susceptibility to infection (2, 12). Although *Y. pestis* was killed in buffers at pH ≤ 3, the failure to enhance infection by neutralizing stomach acid may have resulted from the rapid absorption of a few organisms out of a large inoculum. Likewise, the inability of streptomycin to enhance infection could have resulted from the failure of *Y. pestis* to survive and multiply in regions of the bowel that are changed by reducing *Bacteroides* species through streptomycin treatment (13).

Our studies of antibody responses in rodents surviving oral challenge of *Y. pestis* showed that mice and *Z. pixuna* rarely survived with an immune response but that wild rats more commonly resisted fatal infections and developed antibody rises. Rats are naturally more resistant to fatal plague infection than are either *M. musculus* or *Z. pixuna*. Accordingly, rats survived larger inocula of *Y. pestis* in our experiments than did the more susceptible species of rodents. The larger doses given to rats may explain the higher rate of seroconversions in the rats than in the other species, which succumbed to the larger doses of bacteria. This dosage relationship to seroconversion has been documented also by Williams et al. (19) in parenteral inoculations of ground squirrels.

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