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Virulence of Non-Type 1-Fimbriated and Nonfimbriated Nonflagellated *Salmonella typhimurium* Mutants in Murine Typhoid Fever

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The virulence of *Salmonella typhimurium* mutants that were unable to synthesize type 1 fimbriae was tested in a murine typhoid fever model. Nonfimbriated mutants (*fim*) exhibited a lower 50% lethal dose than a wild-type (*fim*$^+$) strain and produced significantly higher mortality (*fim*, 55%; *fim*$^+$, 37% [$P < 0.002$]) in mice that were challenged orally. There was no difference in virulence when the wild-type and mutant strains were injected intraperitoneally into mice. The progress of a short-term lethal infection was monitored after oral inoculation of mice with a mixture containing equivalent numbers of *fim*$^+$ wild-type and *fim* mutant bacteria. The results indicated that while both strains colonized the intestinal tract equally well and invaded internal organs, the *S. typhimurium fim* mutant proliferated in the blood of the mice faster than the *fim*$^+$ strain. The results of the mixed oral challenge suggested that bacteremia caused by *fim*$^+$ *S. typhimurium* was reduced or delayed by the sequestration of the fimbriated bacteria in the spleen, liver, and kidneys. Thus, type 1 fimbriae were not virulence factors for *S. typhimurium* in this model, and the fimbriae may be an impediment to the pathogen in this setting. An *S. typhimurium* double mutant lacking type 1 fimbriae and flagella (*fla*) also was tested in mice. The virulence of the *fim fla* mutant was greatly reduced compared with that of the wild-type strain (mortality from *fim fla* challenge, 11% [$P < 0.0005$]). The significance of this latter result is discussed in relation to host adaptation by pathogenic salmonellae.

Type 1 fimbriae (pili) on enteric bacteria mediate attachment of the bacteria to mannosyl receptors on mammalian cells (8, 10) and represent a potential virulence trait for enteric pathogens. A variety of alternate fimbriae have been implicated as virulence factors for *Escherichia coli* (25), and the pathogenic significance of type 1 fimbriae has generally been dismissed owing to the ubiquity of type 1 (common) fimbriae among gram-negative bacteria (4). Nevertheless, the expression of type 1 fimbriae enhanced the colonization of the mouse urinary tract by *E. coli* in an ascending infection (18, 23), and the frequency of prodromal colonization of the oropharynx was greater in mice that were fed fimbriated strains of *E. coli* serotype K1 than in mice that were fed nonfimbriated strains (3, 15). Type 1 fimbriae did not appear to be beneficial to bacteria in the bloodstream, however, where fimbriated bacteria were removed by the liver more efficiently than nonfimbriated bacteria (28, 36, 41). The incidence of bacteremia was similar in mice that were infected orally with either fimbriated or nonfimbriated strains of *E. coli* K1 (3, 15), but bacteremia in mice that were challenged orally with a fimbriated *E. coli* K1 strain developed with a concomitant selection for nonfimbriated bacteria in the blood (15). A change in the fimbrial phenotype of an *E. coli* strain was also observed in peritoneal infections of mice and rats. Non-type 1-fimbriated *E. coli* was isolated from animals that had been injected intraperitoneally with fimbriated bacteria (1, 42).

The results of experimentally induced bacteremia and peritonitis in animals suggest that fimbrial phase variation occurs in vivo. Bacteria that exhibit type 1 fimbrial phase variation are genotypically *fim*$^+$ but alternate rapidly between phenotypically fimbriated (Fim$^+$) and nonfimbriated (Fim$^-$) states. The fimbrial phenotype of *E. coli* is controlled by the reversible inversion of a 314-nucleotide portion of the chromosome that is adjacent to the gene encoding the major subunit of the fimbrial filament (*fimA*) (10). The position of the *fimA* promoter on the invertible segment results in the promoter being oriented in the same direction as ("on") or opposite from ("off") the *fimA* gene. Phase variation of type 1 fimbriae has been studied in *E. coli* and *Salmonella typhimurium*, but the rate of change between Fim$^+$ and Fim$^-$ states has been measured accurately only in *E. coli*. Phase variation in *E. coli* occurs at a frequency of $\approx 10^{-3}$ per cell per generation (10, 35), and it is influenced in *E. coli* and *S. typhimurium* by the ambient environment such that bacterial growth conditions can be used to enrich for Fim$^+$ cells or to reduce the number of Fim$^+$ cells present in a culture (8, 10, 35).

The differences that have been observed between Fim$^+$ and Fim$^-$ bacteria in some animal infections (1, 6, 9, 18, 36, 41, 42, 47) may be difficult to evaluate given the variable nature of the phenotype of genotypically *fim*$^+$ cells and the lack of knowledge of the factors that affect phase variation in vivo. Stable *E. coli fim* mutants were used in some of the experimental urinary tract and bacteremic infections cited above (3, 23), but analogous *S. typhimurium fim* mutants have not been used in studies of virulence in animals. Many studies of the role of type 1 fimbriae in *S. typhimurium* pathogenesis were done with cultures of *fim*$^+$ strains that were enriched for Fim$^+$ or Fim$^-$ cells (11, 29) or strains of *S. typhimurium* that were not stably nonfimbriated (6, 9, 47). Several authors concluded that type 1 fimbriae provided *S. typhimurium* with an advantage in colonizing the intestinal

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tracts of infected mice and increased the incidence of fatal infections (6, 9, 47).

The following report describes the results of experimental infections in mice with nonreverting *S. typhimurium fim* mutants and the attenuation of virulence that resulted from combining *fim* and *fla* mutations in the same strain.

MATERIALS AND METHODS

Bacterial strains and growth conditions. All of the strains used in this study were isogenic derivatives of *S. typhimurium* SR-11 (32, 44) and were isolated by selecting for the loss of tetracycline resistance, which was encoded by Tn*10* inserted adjacent to the genes for type 1 fimbriae (*fim*). A Tn*10* insertion in *ahp* was transduced from strain TA4190 (46) to χ3181 (SR-11 wild type) to produce χ3893. χ3893 (*ahp*::Tn*10*) was plated on fusaric acid agar (33), and fusaric acid-resistant, tetracycline-sensitive isolates were screened for the absence of mannose-sensitive agglutination of guinea pig erythrocytes or yeast cells. Agglutination-negative isolates were also examined by electron microscopy and for reactivity with antiserum specific for *S. typhimurium* type 1 fimbriae. χ4252 (*ahp-251*) was a *fim*$^+$ control that was saved from the mutant screening procedure, and χ4253 [(*fim-ahp*)-*391*] and χ4254 [(*fim-ahp*)-*401*] were independent *fim* mutants. χ4253 was transduced with P22HT *int* (43), which had been propagated on χ3376 (*fli-8007*::Tn*10*) (30), and a tetracycline-resistant, nonmotile transductant was saved as strain χ4308 [(*fim-ahp*)-*391 fli-8007*::Tn*10*]. χ4333 was a spontaneous nalidixic acid-resistant derivative of χ4252 (Fim$^+$), and χ4334 was a spontaneous streptomycin-resistant derivative of χ4253 (Fim$^-$). Bacteria were cultivated at 37°C in L broth (27) as described previously (30) or in Mueller-Hinton broth under static conditions for the production of type 1 fimbriae. A mixture of χ4333 and χ4334 was prepared by growing the two strains separately and adjusting the cell densities of the cultures to equivalence. Equal volumes of the two cultures were mixed, and the bacteria were concentrated by centrifugation and suspended in buffered saline containing 0.1% gelatin.

Southern blot analysis. Bacteria from 1.5 ml of saturated overnight culture broth were pelleted in a Microfuge tube, washed once with 1 ml of ice-cold TE (10 mM Tris, 1 mM EDTA [pH 8.0]), and repelleted. The cells were suspended in 0.5 ml of an ice-cold solution containing 2 mg of lysozyme per ml, 25 mM Tris (pH 8.0), 10 mM EDTA (pH 8.0), and 5 mM glucose and incubated on ice for 30 min. Sodium dodecyl sulfate (0.5%, final wt/vol) and proteinase K (1 mg/ml, final wt/vol) were added, and the mixture was incubated at 65°C for 1 h. The lysate was extracted with TE-saturated phenol and then with $CHCl_3$. CH_3COONa was added to a final concentration of 0.3 M, and the nucleic acids were precipitated with cold 2-propanol and collected by centrifugation. The nucleic acids were dried, dissolved in TE, and digested with 50 μg of RNase A per ml at 65°C for 1 h. The solution was extracted successively with TE-saturated phenol, $CHCl_3$, and H_2O-saturated ethyl ether, and the DNA was precipitated as described above. Chromosomal DNAs were stored in TE at 4°C.

Chromosomal DNAs were digested with *Sph*I (International Biotechnologies, Inc., New Haven, Conn., and Promega Corp., Madison, Wis.), separated on 1% agarose gels, and blotted onto Immobilon-P membranes (Millipore Corp., Bedford, Mass.) under alkaline conditions (38). A *fim*-specific DNA fragment from pISF101 (5) was obtained by digesting the plasmid with *Sph*I, separating the digestion fragments by electrophoresis on an agarose gel, and electroeluting the fragment containing the *fim* operon from a slice of the gel. The probe was labelled with [^{32}P]dCTP by use of a random priming kit (Boehringer Mannheim, Indianapolis, Ind.) and separated from unincorporated radionucleotides by Sephadex G-50 column chromatography. Hybridizations were performed with 50% formamide–0.75 M NaCl at 37°C, and the filters were washed in 0.75 M NaCl at 65°C as previously described (21).

Animal infections. Six- to eight-week-old female BALB/c mice were used in all experiments. All of the bacterial strains were passaged in mice and stored at −70°C prior to virulence assays. The method of passaging bacterial strains, the preparation of the animals, and the challenge regimen for the determination of 50% lethal doses (LD_{50}s) have been described previously (30). Mice that received the mixed inoculum were prepared in the same manner as for the LD_{50} experiments.

Sampling regimen. Five mice per day were dissected at daily intervals after they were infected orally with the mixture of χ4333 (Fim$^+$) and χ4334 (Fim$^-$). All specimens that were taken from the animals were immediately placed in 3 ml of ice-cold buffered saline–0.1% gelatin and kept on ice until they could be processed. Each mouse was anesthetized with a mixture of xylazine (2.6 mg/ml) and ketamine (17.4 mg/ml) delivered by intraperitoneal injection (0.1 ml/20 g of mouse), and blood (0.1 to 0.3 ml) was obtained by retro-orbital puncture. The mice were killed by CO_2 asphyxiation, and the spleen, kidneys, and portions of the liver were removed aseptically. The small intestine was resected, and the Peyer's patches (8 to 10 aggregated lymphoid follicles per animal) were isolated, rinsed free of loosely associated material, and pooled. The remaining small intestinal segment was cut longitudinally and rinsed free of loosely associated material. The specimens were not normalized with respect to volume or mass. Tissue specimens were homogenized with a motor-driven homogenizer (Brinkman Instruments, Inc., Westbury, N.Y.), and samples were plated on L agar containing nalidixic acid (100 μg/ml) or streptomycin (100 μg/ml). These plates were incubated overnight at 37°C. The numbers of *S. typhimurium* cells in the samples were recorded as the CFU per milliliter of homogenate.

Statistical analyses. The LD_{50}s of χ4252 (Fim$^+$) and χ4253 (Fim$^-$) were calculated by linear regression analysis of the cumulative dose-response data obtained 30 days after challenge. The LD_{50} of χ4254 (Fim$^-$) was determined by the method of Reed and Muench (39) for five mice per dose. The significance of differences in mortality was calculated with a chi-square test, with a correction for continuity (24).

The ratios of CFU in infected mice were determined for individual animals, and the mean ± standard deviation of the ratios was calculated from the four or five samples that were taken on the same day (because of death from infection, only four mice were available on day 5). The differences between the ratios of CFU in mixed infections were analyzed in a *t* test of unpaired samples (24).

RESULTS

Isolation of *fim* mutants. Derivatives of χ3893 (*ahp*::Tn*10*) that were isolated from fusaric acid agar were sensitive to tetracycline and remained hypersensitive to organic peroxides (46) because of imprecise excision of Tn*10* from the gene for alkyl hydroperoxide reductase (*ahp*) (46). χ4253 and χ4254 did not agglutinate guinea pig erythrocytes or yeast cells, and antiserum specific for *S. typhimurium* type 1

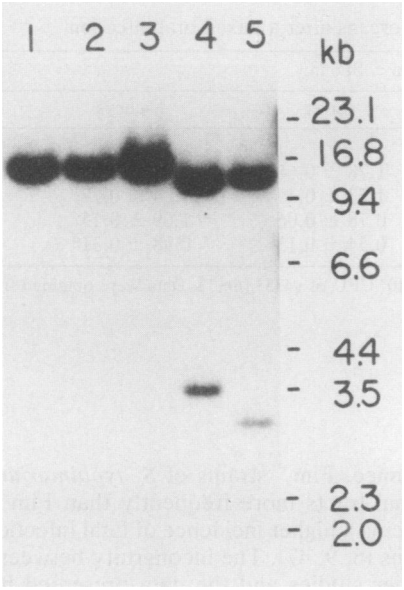

FIG. 1. Southern blot analysis of *S. typhimurium fim* mutants. The blot was hybridized with a probe consisting of the entire *S. typhimurium fim* operon. Lanes: 1, χ3181 (*fim*$^+$ *ahp*$^+$); 2, χ3893 (*fim*$^+$ *ahp*::Tn*10*); 3, χ4252 (*fim*$^+$ *ahp-251*); 4, χ4253 [(*fim-ahp*)-*391*]; 5, χ4254 [(*fim-ahp*)-*401*].

fimbriae did not react with these *fim* mutants. The *fim* mutations were stable, and reversion to agglutination proficiency or reactivity with anti-type 1 fimbria antiserum was not detected. The *S. typhimurium fim* mutations have been characterized (31).

Southern blot analysis of chromosomal DNAs obtained from isogenic *fim*$^+$ and *fim* mutant strains revealed that the mutations which eliminated the expression of type 1 fimbriae were associated with the introduction of a novel restriction site in the *fim* operon. The chromosomes of *fim*$^+$ strains contained 13.7-kb *Sph*I fragments that were recognized by a probe consisting of the *fim* operon (Fig. 1, lanes 1 to 3). The *fim*-specific chromosomal DNAs from the *fim* mutants were separated into two fragments, however. The *fim* genes in χ4253 (*fim-391*) were located on 10.2- and 3.5-kb chromosomal DNA fragments (Fig. 1, lane 4), and the mutation in χ4254 (*fim-401*) produced 10.6- and 3.1-kb *fim*-specific *Sph*I chromosomal fragments (Fig. 1, lane 5). These results were confirmed with probes that were specific for internal regions of the *fim* operon (31).

Pathogenesis of *fim* mutants. The results of virulence tests of isogenic *fim*$^+$ and *fim* mutant strains of *S. typhimurium* are summarized in Table 1. The presence of an *ahp* mutation did not affect the virulence of *S. typhimurium*, as the LD_{50} of strain χ4252 (Ahp$^-$ Fim$^+$) was comparable to that of wild-type *S. typhimurium* SR-11 (30). Initial observations indicated that there was little, if any, distinction between the virulence of the independent *fim* mutants, χ4253 and χ4254, so most of the comparisons were made between χ4252 (Fim$^+$) and χ4253 (Fim$^-$). Infections with χ4253 produced significantly more deaths among mice challenged orally than did infections with χ4252. The mortality data in Table 1 were obtained for similar cohorts. Nine groups of mice were challenged orally with doses of χ4252 ranging from 7.7×10^2 to 7.2×10^6 CFU, and 10 groups of mice received χ4253 in doses of 6.9×10^2 to 7.2×10^6 CFU (n = 3 to 12 mice per group). The dose-response curves for the animals challenged

TABLE 1. Virulence of type 1-fimbriated and nonfimbriated strains of *S. typhimurium* SR-11[a]

Strain	No. of mice dead/no. tested (% mortality)[b]	LD_{50} (CFU)	No. of days to death (mean ± SD)[c]
χ4252 *fim*$^+$ *fla*$^+$	56/150 (37)	4.2×10^4	11.6 ± 6.2
χ4253 *fim-391*	83/151 (55) ($P < 0.002$)[d]	2.3×10^4	12.6 ± 5.0
χ4254 *fim-401*	11/26 (42)	3.3×10^4	11.0 ± 2.8
χ4308 *fim-391* *fli-8007*	10/90 (11) ($P < 0.0005$)[d]	$>>10^7$	8.3 ± 4.0

[a] Data were obtained 30 days postchallenge for orally infected mice.
[b] Aggregated results for mice given equivalent doses.
[c] Calculated for animals that received approximately 1 LD_{50}.
[d] Significance versus χ4252.

with χ4252 and χ4253 were linear over the range of doses that were administered (after linear regression analysis of $\log_{10}$ CFU versus percent mortality, $r = 0.94$ for χ4253 and $r = 0.90$ for χ4253). The difference in the incidence of mortality from infections by the two strains of *S. typhimurium* was paralleled by a lower LD_{50} for χ4253 than for χ4252 (Table 1). The difference between the mean number of days to death for mice infected with either χ4252 or χ4253 was insignificant (Table 1). There was a weak correlation between the numbers of bacteria that were given to mice and the mean number of days to death (after linear regression analysis of $\log_{10}$ CFU versus mean number of days to death, $r = -0.74$ for χ4252 and $r = -0.76$ for χ4253). The virulence of χ4254 (*fim-401*) was similar to that of χ4253 (*fim-391*) (Table 1). There were no differences in the virulence of the strains when they were given to mice parenterally. The LD_{100} for χ4252, χ4253, and χ4254 was <20 CFU when the bacteria were injected intraperitoneally.

Virulence of the *fim fla* double mutant. Table 1 shows the results of assays of the virulence of an *S. typhimurium fim fla* double mutant. The mortality caused by oral infections with χ4308 (Fim$^-$ Fla$^-$) was greatly reduced compared with that caused by oral infections with χ4252 (Fim$^+$ Fla$^+$), and the LD_{50} of χ4308 was more than 250 times the LD_{50} of χ4252. When mice were infected orally with 10^7 CFU of χ4308, only 20% mortality ensued.

Mixed oral infections with *fim*$^+$ wild-type and *fim* mutant strains. Mice were challenged orally with a mixture of differentially marked *fim*$^+$ wild-type and *fim* mutant strains of *S. typhimurium* to discern differences between the two strains during the course of a lethal infection. The numbers of CFU recovered from these mice increased with increasing time after inoculation irrespective of the *fim* genotype of the strain (data not shown). Table 2 shows the ratios of CFU per milliliter in homogenates of specimens taken from mice infected with the mixture. There were no meaningful changes in the ratios of CFU in the intestinal tract over 5 days postchallenge (Table 2). In contrast, there was a two- to fourfold inversion of the CFU ratios in the spleen, liver, and kidneys during the course of the experimental infection. The increases in the proportions of CFU of Fim$^+$ *S. typhimurium* in the spleen, liver, and kidneys were significant when the ratios from day 2 were compared with those from day 5 (Table 2). The CFU of χ4334 and χ4333 in the blood also changed significantly during the 5-day infection, but in this case, the proportion of the Fim$^-$ strain increased threefold over that of the Fim$^+$ strain (Table 2).

TABLE 2. Ratios of *fim-391* to *fim*$^+$ *S. typhimurium* in various organs after a mixed oral infection[a]

Time after challenge (days)	Ratio of CFU (mean ± SD) in:					
	Peyer's patches	Intestinal wall	Spleen	Liver	Kidneys	Blood
1	2.10 ± 0.36	1.75 ± 0.41				
2	1.87 ± 0.59	1.98 ± 0.88	1.85 ± 0.43	1.78 ± 0.66	1.93 ± 0.86	2.01 ± 0.11
3	2.30 ± 0.42	2.00 ± 0.46	1.89 ± 0.24	1.73 ± 0.49	1.24 ± 0.77	2.34 ± 0.23
4	2.10 ± 0.61	1.93 ± 0.17	0.94 ± 0.24	0.78 ± 0.06	1.09 ± 0.15	3.45 ± 0.09
5	1.80 ± 0.29	2.02 ± 0.21	0.56 ± 0.12[b]	0.54 ± 0.13[c]	0.98 ± 0.31[d]	6.01 ± 0.12[e]

[a] Mice were infected with a mixture containing 2.0×10^9 CFU of χ4334 (*fim-391*) and 1.0×10^9 CFU of χ4333 (*fim*$^+$). Data were obtained from four or five mice.
[b] Significantly different from spleen ratio at day 2 ($P < 0.001$).
[c] Significantly different from liver ratio at day 2 ($P < 0.005$).
[d] Significantly different from kidney ratio at day 2 ($P < 0.05$).
[e] Significantly different from blood ratio at day 2 ($P < 0.00005$).

DISCUSSION

The results of assays of the virulence of isogenic *fim*$^+$ wild-type and *fim* mutant strains of *S. typhimurium* in mice showed that type 1 fimbriae were not essential virulence factors for the bacteria (Table 1). The hypothesis that type 1 fimbriae provide an advantage to *S. typhimurium* during an infection was not supported by the fact that the *S. typhimurium fim* mutant strain was significantly more virulent than the wild-type strain in oral infections of mice. The results obtained from a short-term infection with a mixture of *fim*$^+$ and *fim* strains (Table 2) demonstrated that the expression of type 1 fimbriae in *S. typhimurium* was neither an advantage nor a disadvantage for bacterial colonization of the intestinal tract. Bloch and Orndorff reached a similar conclusion from the results of comparisons of isogenic *fim*$^+$ and *fim* strains of *E. coli* K1 in infections of mice (3).

The changes in the ratios of CFU in the spleen, liver, and kidneys in mice infected with a mixture of strains indicated that *fim*$^+$ bacteria proliferated or were trapped in those organs to a greater extent than *fim* bacteria. The changes in the ratios of CFU in the blood were the converse of those observed in the internal organs (Table 2). At 5 days postchallenge, the ratios of CFU of *fim* and *fim*$^+$ *S. typhimurium* in the blood had increased from 2:1 to 6:1 (Table 2). The results of the mixed challenge were consistent with the pathogenesis of the infection as well as the results of studies of the removal of Fim$^+$ bacteria from the blood. Murine typhoid fever progresses with the translocation of the pathogen from the intestinal lumen and the invasion of internal organs by the bacteria. Untreated animals die from bacteremia caused by the infiltration of *S. typhimurium* into the bloodstream (34). The results of several independent studies demonstrated that when bacteria were introduced parenterally into mice or rats, the numbers of Fim$^+$ bacteria in the animals decreased more quickly than the numbers of Fim$^-$ bacteria (28, 36, 41, 42). An *S. typhimurium fim*$^+$ strain that was injected intravenously into mice was filtered from the blood and retained in the liver more efficiently than a *fim* mutant strain (28). The higher incidence of mortality and the lower LD_{50} seen here in mice that were fed *S. typhimurium fim* mutants (Table 1) can be explained by the proliferation of *fim* bacteria in the blood faster or in larger numbers than *fim*$^+$ bacteria. Fim$^+$ bacteria that entered the blood would be removed more effectively than Fim$^-$ bacteria, and bacteria expressing type 1 fimbriae might not escape from the internal organs to the blood as well as bacteria without fimbriae. Thus, type 1 fimbriae may interfere with the dissemination of *S. typhimurium* in infected mice.

Several earlier reports proposed that following oral inoculation of mice, Fim$^+$ strains of *S. typhimurium* colonized the intestinal tracts more frequently than Fim$^-$ strains, in turn producing a higher incidence of fatal infections from the Fim$^+$ strains (6, 9, 47). The incongruity between the results of the earlier studies and the data presented here may be partially explained by differences in materials or methods. The Fim$^-$ strain of *S. typhimurium* that was used by Duguid et al. was not a stable *fim* mutant, and *fim*$^+$ revertants were isolated from mice that had been infected with the Fim$^-$ strain (9). The phenotype of the *fim* mutants that were used in the experiments described in this report was stable, and reversion was not observed. The stability of the Fim$^-$ phenotype of the strains used by Darekar and Eyer (6) and Tanaka and Katsube (47) was not reported by those authors.

Duguid et al. infected mice orally with *S. typhimurium* that was in the log or stationary phase of growth, and the differences in the mortality caused by Fim$^+$ or Fim$^-$ bacteria were significant when the results of several unrelated challenges were analyzed together (9). When the results of Duguid et al. are analyzed on the basis of the mice that received bacteria grown under the same conditions, the differences between the Fim$^+$ and Fim$^-$ strains of *S. typhimurium* are not consistent. The culture conditions described by Duguid et al. are now known to affect the efficiency with which salmonellae invade tissue culture cells (11, 26), and the same conditions may alter animal infectivity. When the data of Duguid et al. are reexamined in light of this recent finding, the incidence of infection or mortality is significantly higher in mice given log-phase bacteria than in mice given stationary-phase bacteria.

The role of phase variation in pathogenesis cannot be evaluated from the outcome of the experiments described in this report. Selection for Fim$^-$ isolates of *E. coli* in rats and mice that were infected with Fim$^+$ strains suggests that phase variation occurs in vivo (15, 42). It is possible that the differential expression of type 1 fimbriae results from the conditions encountered by a facultative intracellular pathogen such as *S. typhimurium*. Attachment and survival in phagocytes mediated by type 1 fimbriae have been reported for *E. coli* (14, 22), and expression of the fimbriae may benefit *S. typhimurium* as well. The data in Table 2 suggest that type 1 fimbriae may be a disadvantage to *S. typhimurium* when the bacteria are extracellular, i.e., in the blood. The variable expression of type 1 fimbriae may permit *S. typhimurium* to adapt to and take advantage of different niches encountered in an infected host. Nonetheless, the absence of type 1 fimbriae and the attendant phase variation in the *fim* mutants did not impair the virulence of these strains (Table 1).

Although *fim* (this report) and *fla* (30) mutations did not independently reduce the virulence of *S. typhimurium* SR-11, a striking attenuation was produced when *fim* and *fla* mutations were introduced into the same strain (Table 1). The *fli-8007*::Tn*10* mutation was shown previously to have no effect on the virulence of *S. typhimurium* SR-11 (30), but a cumulative effect of the two mutations may explain the loss of virulence in χ4308. The pathogenesis of *S. typhimurium* is probably multifactorial, and *S. typhimurium* possesses adhesins in addition to type 1 fimbriae (16, 20, 40, 48). The attenuation associated with the simultaneous absence of type 1 fimbriae and flagella may indicate the limited ability of *S. typhimurium* to circumvent the host during infection. The flagella of *E. coli*, *S. typhimurium*, *Vibrio cholerae*, and *V. parahaemolyticus* have been reported to mediate attachment (2, 12, 13, 17, 19), and the avirulence of χ4308 may reflect a severely reduced adherence of that strain to host tissues.

A possible alternative is that the avirulence of χ4308 was a result of the assay that was used to measure virulence. Type 1 fimbriae and flagella may be more critical in infections of inbred BALB/c mice than other susceptible hosts. The phenotype of χ4308 (Fim^- Fla^-) has been observed in clinical isolates of salmonellae. Duguid et al. found that 0.9% (18 of 2,030) of the isolates of *S. typhimurium* that were examined lacked both type 1 fimbriae and flagella (7). These Fim^- Fla^- strains of *S. typhimurium* were isolated primarily from cases of avian diseases (7). *S. typhimurium* isolates that were missing only type 1 fimbriae (16% [319 of 2,030]) or flagella (1% [22 of 2,030]) came from a variety of human and other animal sources (7). The association between the Fim^- Fla^- phenotype and virulence in birds is strengthened by the fact that two major avian pathogens, *S. gallinarum* and *S. pullorum*, are nonflagellated and lack type 1 fimbriae (8, 37, 45). Thus, although the results of experiments with mice suggest that flagella and mannose-sensitive adherence play a role in the pathogenesis of *S. typhimurium*, these virulence factors may have a restricted host range.

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