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Serum Resistance Associated with Virulence in *Yersinia enterocolitica*†

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Yersinia enterocolitica strains that exhibited a calcium requirement for growth and autoagglutination at 37°C were invariably virulent in rabbits, causing diarrhea and a high degree of lethality, and were capable of colonizing the intestinal lumen and establishing foci of infection on the Peyer's patches of mice. Strains that had lost the properties of calcium dependency and autoagglutinability were totally avirulent in rabbits and were quickly eliminated from the intestinal lumen and tissues of mice. Virulent and avirulent strains were shown to be equally invasive to HeLa cells. However, the virulent strains were resistant to the bactericidal action of normal serum, and this serum resistance was lost with the loss of virulence. Furthermore, the serum resistance of virulent strains was expressed, as were other properties, when strains were grown at 37°C, but not at 25°C. These results suggest that a virulence factor associated with serum resistance plays an essential role in the pathogenicity of *Y. enterocolitica*.

Yersinia enterocolitica is an important cause of bacterial gastroenteritis in children (15, 19). Acute mesenteric lymphadenitis, terminal ileitis, and other suppurative infections have also been reported (4). Histopathological findings from human cases (5) and experimental infections (9, 25, 31) have indicated that the invasion of the intestinal mucosa is of prime importance to the pathogenesis of enteritis caused by *Y. enterocolitica*, but specific virulence factors involved in pathogenicity have yet to be defined.

The large majority of clinical isolates and many environmental isolates of *Y. enterocolitica* produce heat-stable enterotoxin (ST) (11, 24, 26), but there is no evidence to suggest that the enterotoxin is produced in vivo (25; C. H. Pai, V. Mors, and T. A. Seemayer, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, B12, p. 19). The abilities of *Y. enterocolitica* to penetrate HeLa or HEp-2 cells in tissue cultures and to evoke keratoconjunctivitis in guinea pigs have been considered important pathogenic properties of the organism (13, 18, 21, 32, 33). However, a HeLa-positive strain of *Y. enterocolitica* which was highly virulent in rabbits when it was freshly isolated from a patient with diarrhea was found to have lost virulence during laboratory cultivation even though the HeLa cell invasiveness of the strain remained unchanged (25). Furthermore, the virulence of the laboratory stock strains that are HeLa cell invasive was not predictable (C. H. Pai and V. Mors, unpublished data). These observations suggested that *Y. enterocolitica* must possess an additional virulence factor, apart from tissue invasiveness as determined in vitro, to be fully virulent. The difficulty in defining the virulence determinants of *Y. enterocolitica* was due in part to the lack of isogenic strains with altered virulence. Recently, however, some virulent strains of *Y. enterocolitica* have been shown to possess a species of plasmid that is associated with the ability of organisms to evoke a positive Sereny test (33) or with virulence for mice (13) or gerbils (28). Furthermore, evidence also exists suggesting that a number of temperature-dependent properties are determined by the plasmid. These include a calcium requirement for growth (10, 13), a phenomenon of autoagglutination when cells are grown in tissue culture media (16), production of V and W antigens (10), and alteration in outer membrane proteins (28). The V and W antigens have long been recognized as a major determinant of virulence in *Y. pestis* and *Y. pseudotuberculosis* (8, 17), although the precise role of the proteins in pathogenesis is not known. In *Y. pestis* and *Y. pseudotuberculosis*, the production of the V and W antigens is correlated to calcium requirement (6, 7). Recent reports by Gemski et al. (14) and Ferber and Brubaker (12) suggested that these pathogens

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also possess virulence-associated plasmids. A common mechanism of pathogenicity may exist among all three species of *Yersinia*.

In this study, we characterize the virulence of the isogenic strains that differed in calcium dependency and autoagglutinability and describe a possible determinant of virulence in *Y. enterocolitica*.

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MATERIALS AND METHODS

Bacteria. The clinical isolates, and their derivatives, of *Y. enterocolitica* used in this study are listed in Table 1. Strains MCH700, MCH291, MCH674, and MCH159 were originally isolated from patients with diarrhea at the Montreal Children's Hospital and were stored at −70°C in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) with 20% glycerol.

Autoagglutination. The ability of *Y. enterocolitica* strains to autoagglutinate was tested by a modification of the method described by Laird and Cavanaugh (16). A single colony was inoculated into a pair of tubes (13 by 100 mm) containing 2 ml of Earle minimum essential medium (Flow Laboratories, Rockville, Md.) One tube was incubated at 37°C and the other was incubated at 25°C for 24 h. Autoagglutination-positive (Agg^+) colonies grew as large clumps at the bottom of the tubes with clear supernatant fractions at 37°C, but they grew as homogeneous turbid suspensions at 25°C. The growth of autoagglutination-negative (Agg^-) colonies remained as a suspension without agglutination at both incubation temperatures. The tissue culture medium was not essential for the autoagglutination test. Methyl red-Voges-Proskauer medium (Difco) was found to be equally satisfactory.

Nalidixic acid-resistant mutants. A suspension of bacteria was spread onto brain heart infusion agar (Difco) containing nalidixic acid (20 μg/ml). After 48 h of incubation at 25°C, a few colonies that grew on the plates were picked and purified. Isolates were then tested for biochemical reactions and autoagglutination to ensure that the relevant characteristics of the parent strains were unchanged. Strains were stored at −70°C.

Virulence test in rabbits. The young rabbit model described by Pai et al. (25) was used to test the virulence of *Y. enterocolitica* strains. New Zealand white rabbits (Canadian Breeding Farm Laboratories Ltd., St. Constant, Quebec) weighing 0.5 to 0.8 kg were inoculated intragastrically by means of a feeding tube with 10^{10} bacterial cells grown overnight on sheep blood agar at 25°C and suspended in 10 ml of 10% (wt/vol) $NaHCO_3$ solution. Animals were weighed daily and observed for diarrhea for a period of 2 weeks. Rabbits were considered to have diarrhea when feces were semisolid and their perineum or hind legs were wet and soiled.

Infection of mice. Swiss albino mice (Canadian Breeding Farm Laboratories Ltd.) weighing about 20 g were housed in groups of six per cage and were infected with Nal^r mutants of *Y. enterocolitica* strains by the method described previously (13). Animals were deprived of water for 18 h, and then each group was allowed to drink ad libitum for 24 h from a 100-ml water suspension of a bacterial strain containing about 10^9 organisms per ml. After the 24-h period, about one-half of the bacterial suspension was usually left in the bottle, and the viable counts of each suspension remained unchanged. Mice were then allowed access to sterile water for the duration of the experiments.

Recovery and enumeration of bacteria from infected mice. At specified times after infection, groups of three mice were killed by cervical dislocation. A 2- to 5-cm portion of the distal ileum was removed and cut open longitudinally. Ileal contents were removed gently onto a weighing dish, the Peyer's patches were excised from the intestine, and the Peyer's patches and the remainder of the small intestine section were washed in 10 ml of ice-cold saline by blending in a Vortex mixer for 15 s. The Peyer's patches (four per mouse) and the section of ileum without Peyer's patches from each mouse were homogenized in 2 ml of ice-cold saline, using sterile mortars and pestles. Ileal contents were similarly homogenized. Each homogenate was diluted in saline, and 0.1-ml aliquots of each 10-fold

TABLE 1. *Y. enterocolitica* strains and their source

Strain	Origin	Serotype/biotype[a]	Source
MCH700	Diarrhea	O:3/4	Montreal Children's Hospital (25)
MCH700-S	Agg^+ derivative of MCH700	O:3/4	This study
MCH700-L	Agg^- derivative of MCH700-S	O:3/4	This study
MCH700-S1	Nal^r mutant of MCH700-S	O:3/4	This study
MCH700-L1	Nal^r mutant of MCH700-L	O:3/4	This study
MCH291	Diarrhea	O:5,27/2	Montreal Children's Hospital
MCH291-S	Agg^+ derivative of MCH291	O:5,27/2	This study
MCH291-L	Agg^- derivative of MCH291-S	O:5,27/2	This study
MCH674	Diarrhea	O:1,2,3/3	Montreal Children's Hospital
MCH674-S	Agg^+ derivative of MCH674	O:1,2,3/3	This study
MCH159	Diarrhea	O:6,30/1	Montreal Children's Hospital
MCH159-1	Nal^r mutant of MCH159	O:6,30/1	This study

[a] Nilehn's biotyping scheme (22).

dilution were plated in triplicate onto salmonella-shigella agar (Difco) containing nalidixic acid (20 μg/ml) and incubated for 2 days at 37°C. The number of organisms recovered was expressed as colony-forming units (CFU) per Peyer's patch, per 1-cm portion of ileal tissue without Peyer's patches, or per gram of ileal contents.

Histological examination. Sections of ileum and Peyer's patches were fixed with 10% buffered Formalin, dehydrated in graded alcohol, embedded in paraffin, sectioned, and stained with hematoxylin-phloxine-saffron or Giemsa solution.

Enterotoxin assay. ST was assayed in infant mice, using sterile culture filtrate as described previously (24).

HeLa cell invasiveness. The ability of *Y. enterocolitica* strains to penetrate HeLa cells was examined by modification of the methods described by Lee et al. (18) and Mehlman et al. (20). HeLa cells were grown to about 50% confluence on cover slips (22 mm) placed in petri dishes (35-mm diameter) containing 2 ml of Earle minimum essential medium with 10% fetal calf serum at 37°C in an atmosphere of 5% CO_2. The cell cultures were washed three times with Hanks balanced salt solution (HBSS; GIBCO Laboratories, Grand Island, N.Y.) and then were infected with 2×10^8 bacterial cells which had been grown overnight on tryptic soy agar (Difco). After 90 min of incubation at 37°C in an atmosphere of 5% CO_2, the cover slips were rinsed twice with HBSS and once with minimum essential medium containing penicillin (200 μg/ml), gentamicin (40 μg/ml), and lysozyme (100 μg/ml; Sigma Chemical Co., St. Louis, Mo.). Cell cultures were further incubated for 3 h in minimum essential medium with 10% fetal calf serum, antibiotics, and lysozyme and were washed, fixed with methanol for 10 min, and stained with Giemsa stain for 30 min. Stained preparations were examined in a microscope under oil immersion.

Serum sensitivity test. Normal human serum was obtained from healthy volunteers, pooled, and stored in small portions at −70°C until required. Normal rabbit serum was also used in some experiments. Once thawed, serum was never refrozen and was discarded after 18 h at 4°C. Bacteria were grown overnight on tryptone soya agar, supplemented with glucose (0.01 M), $MgCl_2$ (0.02 M), and sodium oxalate (0.02 M), at 37 or 25°C, and colonies were harvested into saline to a concentration of 10^8 CFU/ml. The saline suspension was diluted 10-fold in HBSS containing 0.1% (wt/vol) gelatin (Difco). (The inclusion of 0.1% gelatin was found necessary to neutralize the bactericidal action of HBSS itself.) At time zero, 0.3 ml of the bacterial suspension (10^7 CFU/ml) was added to a tube containing 2.7 ml of HBSS with 0.1% gelatin and 10% (vol/vol) normal serum. Control tubes contained the same reaction mixture, but without normal serum. Tubes were incubated at 37°C; at various intervals, samples were taken and serially diluted in saline, and 0.1-ml aliquots of each 10-fold dilution were plated in triplicate onto tryptic soy agar for viable counts.

RESULTS

Isolation of Agg^+ and Agg^- derivatives. Previous reports have shown that virulent strains of *Y. enterocolitica* have a calcium requirement for growth at 37°C (10, 13) or autoagglutinate when grown at 37°C in tissue culture media (16). The calcium requirement was shown by the inhibition of growth at 37°C on magnesium oxalate agar. We compared the difference in colony sizes of strain MCH700 attainable at 37 and 25°C on a number of commercially available nutrient agar media supplemented with glucose (0.01 M), sodium oxalate (0.02 M), and $MgCl_2$ (0.02 M). The virulence of strain MCH700 in rabbits has been clearly documented in our previous studies (19). Of the four media tested, nutrient agar (Difco), tryptic soy agar (Difco), tryptone soya agar (Oxoid), and Columbia blood agar base (Difco), the largest difference in colony size attainable at the two incubation temperatures was observed with supplemented tryptone soya agar. A pinpoint colony (MCH700-S) of MCH700 that developed after overnight incubation at 37°C was found to autoagglutinate when grown at 37°C in Earle minimum essential medium. When an Agg^+ colony was subcultured on supplemented tryptone soya agar at 37°C, more than 99% of colonies were pinpoint in size, but a few (<1%) large colonies were also observed. These large colonies (MCH700-L) were found to have lost the property of auto-agglutination. Similarly, Agg^+ and Agg^- strain pairs of MCH291 and MCH674 were isolated. No small colonies were observed with strain MCH159. Randomly picked colonies of strain MCH159 were invariably Agg^-. Thus, calcium dependency and autoagglutination appeared to be expressed by the same determinant.

Virulence in rabbits. Using the rabbit model developed in our laboratory (25), the virulence of Agg^+ and Agg^- variants were compared (Table 2). All Agg^+ variants of the various strains produced diarrhea in 100% of rabbits inoculated orally, with a significant weight loss and mortality. Histological examination of sections of intestine and the Peyer's patches of rabbits challenged with MCH700-S revealed crypt abscesses with infiltration of neutrophils and eosinophils and necrosis in the Peyer's patches. In contrast, Agg^- variants of strains MCH700 and MCH291 were totally avirulent. No pathological changes were noted in the sections of the intestine obtained from rabbits challenged with MCH700-L. These findings indicated that a determinant(s) associated with the phenomenon of autoagglutination played an essential role in the pathogenicity of *Y. enterocolitica*.

Virulence in mice. The virulence of *Y. enterocolitica* serotype O:8 strains may be tested in mice by 50% lethal dose determination, but other serotypes are not lethal to mice (1, 6, 21). Although mice were reported to have diarrhea after oral challenge with some nonlethal strains

TABLE 2. Virulence of *Y. enterocolitica* in rabbits[a]

Strain	No. of animals with diarrhea/no. inoculated	Wt change (%)[b]	Mortality (no. that died/no. observed)
MCH700-S	10/10	−13	5/7
MCH700-L	1/10	+40	0/8
MCH291-S	12/12	−14	11/12
MCH291-L	0/8	+35	0/8
MCH674-S	6/6	− 6	3/6
MCH159	0/4	+43	0/4

[a] Rabbits (600 to 800 g) were inoculated intragastrically with organisms (10^9 CFU/ml) suspended in 10 ml of $NaHCO_3$ solution (10%).
[b] At day 9 post-inoculation.

(16), diarrhea in mice was difficult to define. Each mouse would have to be isolated in a special cage for up to 2 h to collect stool specimens on filter paper (D. A. Schiemann, personal communication). We decided, therefore, to compare the ability of our *Y. enterocolitica* strains to colonize the intestine and to multiply in the Peyer's patches of mice after oral challenge. The Peyer's patches of the distal ileum have been shown to be a primary site of infection in mice challenged orally with a virulent strain of *Y. enterocolitica* (2, 9).

The number of bacteria recovered from the ileal contents of mice after oral challenge is shown in Fig. 1. At day 1 post-inoculation, both Agg^+ (MCH700-Sl) and Agg^- (MCH700-L1 and MCH159-1) strains of *Y. enterocolitica* were present in the ileal contents in a large number, but by day 3, the Agg^- strains were no longer detectable ($<10^2$ CFU/g of ileal contents). In contrast, the Agg^+ strain was capable of maintaining the colonization without an appreciable decline in number up to day 10.

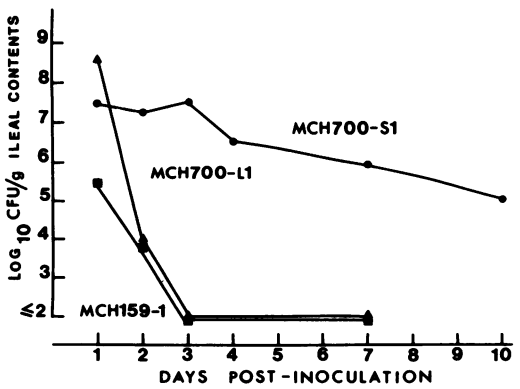

FIG. 1. Number of *Y. enterocolitica* recovered from the ileal contents of mice at specific times after oral challenge. Mice were allowed to drink ad libitum from a suspension of cells (10^9/ml) for 24 h. Each point represents the mean of numbers recovered from three mice.

The number of bacteria recovered from the Peyer's patches and intestinal tissue of the distal ileum is shown in Fig. 2. The Agg^- strains appeared in the Peyer's patches and ileal tissue in limited numbers at day 1, but by day 2 to 3, the organisms were almost completely eliminated. The Agg^+ strain, however, was recovered from the Peyer's patches and ileal tissue in a much greater number at day 1, and the number increased approximately 100-fold in the following 2 days, after which time a steady decline was seen, but the organism was still recovered in significant numbers from the tissue even at day 10 post-inoculation. Sections of the Peyer's patches and the intestinal tissue of the distal ileum were obtained at day 3 post-inoculation and examined for pathological changes. Crypt abscesses and minute areas of mucosal ulceration were present in mice inoculated with MCH700-S1. The Peyer's patches were necrotic. No pathological findings were observed in mice inoculated with MCH700-L1 or MCH159-1.

ST production. The original clinical isolates of *Y. enterocolitica* used in this study are all ST positive. ST production remained unchanged in both Agg^+ and Agg^- variants.

HeLa cell invasiveness. Since the ability of *Y. enterocolitica* to invade epithelial cells in tissue culture is considered an important property of virulent strains, we compared the Agg^+ and Agg^- strains for their HeLa cell invasiveness. All Agg^+ strains were HeLa invasive, and Agg^- strains, MCH700-L and MCH291-L, were found to be as invasive as their Agg^+ counterparts. Since the phenomenon of autoagglutination was temperature dependent, we examined the possible effect of growth temperature on HeLa cell infectivity; MCH700-S and MCH700-L grown at 37 or 25°C were equally invasive. These results

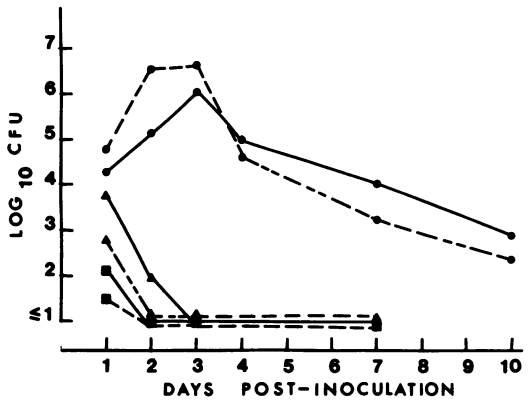

FIG. 2. Number of *Y. enterocolitica* strains [MCH700-S1 (●), MCH700-L1 (▲), and MCH159-1 (■)] recovered from the Peyer's patches (---) and intestinal wall (—) of the ileum of mice at specific times after oral challenge. Mice were allowed to drink ad libitum from a suspension of cells (10^9/ml) for 24 h. Each point represents the mean of numbers ($\log_{10}$ CFU per Peyer's patch or per 1-cm portion of ileal tissue) recovered from 3 mice.

indicated that the loss of virulence in Agg^- strains was not due to the loss of tissue invasiveness.

Sensitivity to bactericidal action of normal serum. In the course of studies to further define the role of the autoagglutination-associated virulence determinant, we found that 10% normal human serum was bactericidal to the Agg^- variants, but not to Agg^+ strains. Strains MCH700-S and MCH700-L were grown overnight on magnesium oxalate agar at 37°C, and colonies were suspended in HBSS with 0.1% gelatin. Care was taken not to include a few large colonies of MCH700-S that always developed at 37°C on magnesium oxalate agar. The bacteria were incubated at 37°C in a reaction mixture containing fresh normal human serum (10%). The number of CFU of MCH700-L decreased by more than 5 logs during 120 min, whereas that of MCH700-S remained unchanged during the same incubation period (Fig. 3). The bactericidal action of normal serum was completely abolished when the serum was heated at 56°C for 30 min (data not shown), suggesting that complement was involved in the bactericidal action of the serum.

We also observed that the serum resistance of Agg^+ strains was expressed only under certain conditions. When strain MCH700-S was first grown at 25°C and tested for its serum sensitivity, it was equally susceptible to the bactericidal action of normal serum, as was MCH700-L (Fig. 3), indicating that serum resistance of MCH700-S was expressed only after growth at the elevated temperature. Strain MCH700-L was serum sensitive regardless of the growth temperature. The serum sensitivity was also tested with other strains (Fig. 4). All virulent strains were resistant to normal serum when grown at 37°C, whereas avirulent strains were sensitive regardless of the growth temperature.

The same results were obtained with normal rabbit serum, although twice the concentration of rabbit serum was required to exert the same degree of bactericidal activity as human serum (data not shown).

DISCUSSION

The results of this study demonstrate that *Y. enterocolitica* strains that grow poorly at 37°C on magnesium oxalate agar and autoagglutinate when grown at 37°C in tissue culture media are virulent and strains that have lost the property for calcium dependency and autoagglutination are avirulent. Agg^+ strains produced severe diarrhea in rabbits with a high degree of lethality. In mice, Agg^+ strains were able to colonize the intestine and multiply in the Peyer's patches and the intestinal tissue of the distal ileum. In contrast, Agg^- strains derived from Agg^+ parents were shown to be totally devoid of virulence in rabbits and were rapidly eliminated from the mouse intestine within a few days of oral challenge. These results confirm the previous findings that calcium dependency or autoagglutination is correlated to the virulence of *Y. enterocolitica* (10, 13, 16).

Examination of the in vitro properties of Agg^+ and Agg^- strains revealed that the autoagglutination-associated virulence factor was not relat-

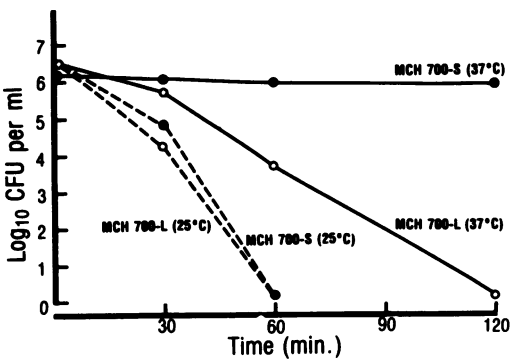

FIG. 3. Survival of *Y. enterocolitica* in normal human serum (10%). Bacteria were grown overnight at 37°C (solid lines) or 25°C (broken lines) on agar media and suspended in saline. The bacterial suspension was diluted in reaction mixtures consisting of HBSS, gelatin (0.1%), and fresh normal human serum (10%) and incubated at 37°C. Viable counts were made at intervals by spreading 0.1-ml aliquots of appropriate dilutions onto tryptic soy agar plates. Tubes containing no serum or serum heated at 56°C for 30 min were included as controls. Viable counts did not change in the control reaction mixtures.

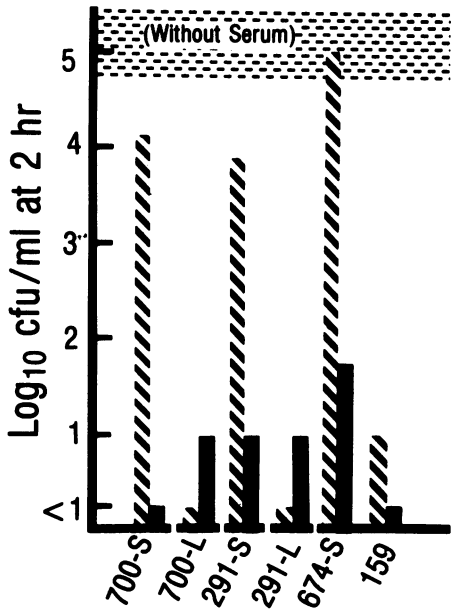

FIG. 4. Survival of *Y. enterocolitica* in normal human serum (10%). Bacteria were grown overnight at 37°C (shaded bars) or 25°C (solid bars) and incubated in fresh normal human serum (10%), as described in the legend to Fig. 3. Data represent the number of CFU recovered after 2 h of incubation. Horizontal, dotted bar represents the range of viable bacteria recovered in reaction mixtures without serum.

ed to ST production and HeLa cell invasiveness. There is growing evidence suggesting that the enterotoxin of *Y. entercolitica* may not be involved in the pathogenesis of diarrheal disease; ST was produced in vitro at 25°C, but not at 37°C (24), and no ST activity was detectable in the diarrheal stool or in the intestinal contents of rabbits infected with enterotoxigenic clinical isolates (25). Tissue invasiveness, however, is considered an important property of the virulent strains of *Y. enterocolitica* (18, 21, 32). Zink et al. (33) and Gemski et al. (13) showed that tissue invasiveness and virulence for mice are lost simultaneously in *Y. enterocolitica* O:8 strains cured of a virulence plasmid. Our present results indicate that Agg^- derivatives which lack one or more virulence determinants for mice or rabbits remain invasive for HeLa cells. It should be noted that we tested tissue invasiveness by HeLa cell assay, whereas Zink et al. (33) and Gemski et al. (13) examined invasiveness by the Sereny test (29). The virulent strains used in our study are Sereny negative. While this manuscript was in preparation, Portnoy et al. (28) reported that strains of *Y. enterocolitica* (not serotype O:8) that have lost virulence for gerbils due to the loss of a plasmid are still able to penetrate epithelial cells in tissue cultures.

The results of this study show that the susceptibility of organisms to the bactericidal action of normal serum is related to virulence in *Y. enterocolitica*. Virulent strains were able to survive in fresh normal sera of humans or rabbits whereas their avirulent variants were readily killed. Furthermore, we found that serum resistance was expressed at 37°C but not at 25°C. Thus, serum resistance may be added to a list of the temperature-dependent properties previously shown to be associated with virulence in *Y. enterocolitica*, i.e., calcium dependency (10, 13), autoagglutination (16), altered outer membrane proteins (28), and production of V and W antigens (10). It remains to be determined, however, whether these properties are indicators of the same virulence determinant. Furthermore, many of these properties have previously been shown to be associated with virulence in *Y. pestis* and *Y. pseudotuberculosis*, suggesting a common mechanism of pathogenicity among all three species of *Yersinia* (6–8, 17). Virulence-associated plasmids have been demonstrated in *Y. enterocolitica* (13, 28, 33), and, recently, similar-sized plasmids have been found in *Y. pestis* (12) and *Y. pseudotuberculosis* (14).

The relationship between serum resistance and virulence of certain strains of *Escherichia coli* has been well defined by Binns et al. (3); a restriction endonuclease fragment of plasmid ColV, I-K94, increased the virulence of *E. coli* for 1-day-old chicks, lowering the 50% lethal dose 100-fold, and a genetic determinant for serum resistance was mapped within the fragment. Furthermore, plasmid derivatives which greatly increased the survival of the host bacterium in serum also enhanced the virulence of the strain for chicks. There is evidence to suggest that serum resistance may contribute toward the pathogenicity of other enterobacterial strains (23).

The biochemical basis for the increased serum resistance of Agg^+ strains of *Y. enterocolitica* is not known. Recently, Portnoy et al. (28) reported that a species of plasmids are associated with virulence in *Y. enterocolitica* and that strains bearing the plasmid are drastically altered in their outer membrane proteins when grown at 37°C. Although we did not examine the presence of a plasmid in Agg^+ strains, a similar colony morphology at 37°C of plasmid-bearing and Agg^+ strains and the frequent segregation of both Agg^- and plasmidless derivatives at 37°C suggested strongly that the autoagglutination-associated property was also determined by similar plasmids. Therefore, the serum resistance may be associated with the altered outer membrane proteins of virulent strains reported by Portnoy et al. (28), particularly since both properties are expressed when grown at 37°C,

but not at 25°C. Also, in some *E. coli* strains the presence of an additional protein surface antigen has been shown to be partly responsible for serum resistance (30).

Serum resistance is not, of course, the only factor required to convert an otherwise benign strain of *Y. enterocolitica* into a virulent strain. Virulent strains presumably must be capable of invading the intestinal mucosa and resisting the natural defense mechanisms of the host. The ability of bacteria to resist the bactericidal action of serum could be an important factor in this host-parasite relationship. The role of serum resistance in the pathogenicity of *Y. enterocolitica* could be investigated by examining infection with HeLa cell-invasive but serum-sensitive strain such as MCH700-L in an animal deficient in its serum bactericidal activity.

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