Mouse Virulence of K(L) Antigen-Containing Strains of Escherichia coli

G. WOLBERG¹ AND C. W. DEWITT²

Department of Microbiology and Immunology, Tulane University Medical School, New Orleans, Louisiana 70112

Received for publication 30 June 1969

The mouse virulence of two K antigen-containing (L variety) strains of Escherichia coli (serotype O2:K1) isolated from human septicemia, and of their variants which lacked K antigen, was studied. The strains containing envelope antigen (K+) were highly virulent when injected intracerebrally or when suspended in mucin and injected intraperitoneally. After intraperitoneal injection of E-107 K+ (but not K−), there was a marked initial growth in the peritoneal cavity followed by bacteremia and infection of all the organs examined. In the mucin-enhanced lethal infection, this growth continued until death of the animal; in the nonlethal infection, growth ceased and the count dropped quickly after approximately 5 hr. Host defenses were depressed greatly by intraperitoneally, but not intravenously, administered mucin. Bacteria were most virulent when injected intraperitoneally. In vitro phagocytosis of the K+ bacteria required opsonins not needed for phagocytosis of the smooth K− variants. Opsonins were found in immunized rabbit and normal mouse sera. Immune rabbit sera contained antibodies with anti-K specificity which were opsonic in vitro and highly protective in vivo when administered passively. There appears to be a lesser anti-O opsonic and protective activity involving one of the strains (E-107 K+), and colonial morphology, agglutination, and absorption tests indicated a low amount of K antigen on this organism. No anti-O opsonic or protective activity could be shown involving the other strain (E-102 K+). When standard serological typing procedures were used, these two strains appeared to be identical serologically, but they differed greatly in sensitivity to immune rabbit serum in phagocytosis experiments in vitro.

Escherichia coli has been associated with human acute inflammatory systemic infections (32). Although human virulence does not always correlate with mouse virulence, mice have been widely used to study these pathogenic strains (4, 6, 8, 17, 20, 22).

The mechanisms of virulence of E. coli for mice or humans are not known. Attempts to correlate virulence with a specific cell fraction, such as cell wall lipopolysaccharide (20, 22) or compounds containing sialic acid (5), have not been successful.

Mouse virulence is correlated with the presence of certain K antigens, with those strains containing the L or heat-labile varieties being the most toxic (8). The mouse virulence of organisms containing K antigen is greatly increased (about one million-fold) when they are suspended in hog gastric mucin, although the increase in virulence due to mucin of spontaneously occurring smooth mutants, which lack K antigen but retain the O antigen, is minimal (C. W. DeWitt and P. M. Allen, Bacteriol. Proc., p. 119. 1961).

Neither the specificity nor the mode of action of protective antibody in E. coli infections is known. Antiserum directed against those bacteria with envelope (K+) is more active in passive protection of mice than antiserum prepared against bacteria lacking envelopes (K−) (8) or against heat-killed bacteria (4) when K+ organisms are used for challenge. Active immunization with the K+ form will protect against challenge with this form to a much greater extent than will immunization with the K− variant (1).

Unlike the sera of other species, mouse serum appears to lack an in vitro bactericidal mechanism (12, 16) and mouse peritoneal fluid is nonbactericidal in vitro or in vivo (31). This has stimulated interest in the opsonic activities of

¹ Present address: Department of Immunology, The Public Health Research Institute of the City of New York, Inc., New York, N.Y. 10016.
² Present address: Department of Pathology, University of Utah School of Medicine, Salt Lake City, Utah 84112.
mouse serum. The removal by the host of gram-negative bacteria from the mouse peritoneal cavity is dependent on opsonic factors (7, 33) the specificities of which are ill-defined, and horse serum opsonins increase phagocytosis of E. coli by mouse peritoneal macrophages in vitro (21).

The experiments reported here were designed to determine the effect of K antigen and hog gastric mucin in the virulence of E. coli. The strains used in these experiments were mouse-virulent strains containing K antigen and their spontaneously occurring, mutant strains which lack K antigen and are essentially avirulent.

**MATERIALS AND METHODS**

**Bacteria.** Two L variety E. coli strains (E-107 K+, E-102 K+), both serotype 02:K1, isolated from human systemic infections (received from The Upjohn Co., Kalamazoo, Mich.) were used. Neither contained flagellar (H) antigen. Both were found to have a mouse median lethal dose (LD50) of < 10 organisms in the presence of 2% hog gastric mucin (type 1701-W, lot 120401, Wilson & Co., Chicago, Ill.) and 10⁴ to 10⁶ organisms in the absence of mucin. Spontaneously occurring, smooth, translucent variants of each, which retain the somatic antigen but lack the envelope antigen, were isolated. These are referred to as K— variants. Both K— strains have a median lethal dose of 10⁷ to 10⁸ in the absence or presence of hog gastric mucin. In all experiments, 12- to 16-hr Brain Heart Infusion (BHI, Difco) cultures were used, and viable counts were made by standard pour plate procedures with BHI. Total bacterial counts were made with a Petroff-Hauser chamber.

**Sera.** Rabbit anti-O2, anti-K1, and anti-02:K1 sera were prepared as previously described (1), inactivated for 30 min at 56 C, and stored at −70 C. Heterologous anti-0 cross-reacting and non-cross-reacting sera were obtained from the National Communicable Disease Center (Atlanta, Ga.).

Blood obtained from the infrarioral sinuses of a number of normal mice was pooled and allowed to clot; the serum was harvested and stored at −70 C.

**Mice.** Albino CF-1 male mice (Carworth Farms), 4 to 7 weeks old, were used throughout.

**Determination of bacterial virulence.** Serial 10-fold dilutions (0.5-ml volumes) of bacterial cultures were made in BHI, and the last dilution step was made in either 0.85% saline or 2% aqueous hog gastric mucin. Five to ten mice were used in each experimental group, and the median lethal dose was determined from four logarithmic dilutions by the method of Reed and Muench (18). Deaths always occurred within 48 hr after intraperitoneal or intravenous (lateral tail vein) injection, but animals were observed for at least 96 hr.

For intracranial injections, mice received 0.03 ml of saline suspensions of E. coli, 107 K+ or K—, and were observed for 2 weeks.

**In vitro phagocytosis.** Normal mice were killed by cervical fracture and their peritoneal cavities were washed out with 3 ml of medium 199 containing sodium heparin, 2.5 USP units/ml (Lederle Laboratories, Pearl River, N.Y.). These washings were pooled in a complete medium consisting of medium 199, 10% fetal calf serum, penicillin (100 units/ml), and streptomycin (µg/ml; Hyland Laboratories, Los Angeles, Calif.). An average of 4 × 10⁴ to 5 × 10⁴ leukocytes per mouse was obtained. The cells were added to plastic tissue culture plates, each containing a glass cover slip (22 × 22 mm), to a concentration of 4.5 × 10⁴ cells per plate. After 60 min at 37 C in 5% CO₂-air, the cells which did not adhere were decanted, the plates were washed twice with medium 199, and fresh complete medium was added to the plates. Approximately 50% of the peritoneal exudate cells was removed in the washings, and the cells which adhered were 90 to 95% macrophages plus a few monocytes, neutrophils, and lymphocytes.

After an additional 12 to 16 hr of incubation, the medium was replaced with 5 ml of fresh medium, and the test serum and bacteria were added. Unless otherwise stated, 10⁸ bacteria (14- to 16-hr BHI culture) in 1 ml of complete medium, and 0.2 ml of serum or serum dilution, were added per plate. After 75 min, the cover slips were washed twice with medium 199 and once with complete medium, air-dried, and stained with Wright stain. The number of bacteria in 100 consecutive macrophages was counted.

**Passive protection test.** Groups of 5 to 10 mice were injected intraperitoneally with approximately 70 K+ bacteria suspended in 2% mucin (a dose which was fatal to 100% of the controls), followed by intraperitoneal injection of 0.5 ml of serum or serum dilution in medium 199.

**Agglutination tests.** Agglutination tests were performed according to the method of Edwards and Ewing (3). The antigen concentrations were standardized so that the optical density at 660 nm was 0.18 to 0.20.

**RESULTS**

**Determination of bacterial growth and dissemination.** To gain some insight into the relationship among the host, parasite, and hog gastric mucin, the progression of infection after intraperitoneal and intravenous injection was investigated.

Mice were injected as described for the determination of virulence with 650 to 750 viable bacteria. After 0.5, 2, 5, and 10 hr, groups of mice were sacrificed, and counts were made of viable bacteria in blood, peritoneal cavity washings, and saline suspensions of homogenized whole organs (liver, spleen, kidney, and brain). Each experiment was repeated twice, and, although total counts per mouse varied greatly, the relationship of counts among the sites was uniform. Those sites which had the highest counts were the same in repeated experiments, as was the general pattern of bacterial growth or removal. The results shown in Fig. 1-3 are of single

---

*Vol. 100, 1969* MOUSE VIRULENCE OF E. COLI
experiments. Each point is a mean value of two or three mice.

Figure 1 shows the bacterial counts in the peritoneal cavity, blood, spleen, liver, kidney, and brain after intraperitoneal injection of a nonlethal dose of 750 E-107 K+ bacteria in saline. Although it is not possible to compare directly counts in the various tissues, examination of the rates of increase are interesting. A rise in count occurred in the peritoneal cavity, followed by bacteremia and infection of all organs examined at 5 hr. At 10 hr after injection, only very low numbers of bacteria were present in the organs, but counts in the peritoneal cavity remained high.

Figure 2 shows the progress of the mucin-enhanced infection. Increase in bacteria in the peritoneal cavity again preceded bacteremia which preceded appearance of bacteria in the organs. The bacteremia presumably represents spill-out from the peritoneal cavity. At 2 hr after injection, only the peritoneal cavity and blood showed increased bacterial counts, but by 5 hr there were high counts in all organs except the brain. At 10 hr, there was an overwhelming infection, and the mice had signs compatible with endotoxic shock, including inactivity, ruffled hair, diarrhea, and a mucus exudate over the eyelids.

When similar experiments were carried out with the K– variant, no growth occurred in either the peritoneal cavity or the organs.

Next, the progression of disease after intravenous injection of bacteria was investigated to
determine the importance of growth in the peritoneal cavity in development of infection. No growth occurred after intravenous injection of the K+ organism in saline, but when mucin was injected intraperitoneally at the same time all organs examined were infected, and growth in these organs was demonstrated at 5 hr (Fig. 3). By 10 hr after injection, counts in all the organs were greatly decreased. Similar experiments with the K− variant did not show any growth of bacteria.

Thus intraperitoneal injection of mucin appeared to have a major effect on local host defenses and a lesser effect on systemic defenses. These effects were investigated further by reversing the routes of injection of mucin and bacteria. The LD50 of bacteria injected intraperitoneally in saline and in mucin was 10⁶ and <10, respectively, showing the marked mucin depression of host defenses. When mucin was given intravenously, there was little or no effect on the virulence of bacteria injected intraperitoneally or intravenously. However, when mucin was introduced intraperitoneally, the LD50 of intravenously injected bacteria was decreased (Table 1).

![Graph](image)

**Fig. 3.** Multiplication and dissemination of E-107 K+ in the mouse after intravenous injection of 750 organisms in saline concurrent with intraperitoneal injection of 0.5 ml of 2% hog gastric mucin.

### Table 1. Effect of the routes of injection of bacteria and mucin on virulence of E. coli 107 K+

<table>
<thead>
<tr>
<th>Route of injection</th>
<th>LD50 (organisms/mouse)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>Mucin</td>
</tr>
<tr>
<td>ip</td>
<td>ip</td>
</tr>
<tr>
<td>ip</td>
<td>iv</td>
</tr>
<tr>
<td>iv</td>
<td>ip</td>
</tr>
<tr>
<td>iv</td>
<td>—</td>
</tr>
<tr>
<td>iv</td>
<td>iv</td>
</tr>
</tbody>
</table>

* Intraperitoneal (ip) or intravenous (iv).

**In vitro phagocytosis.** The foregoing results indicated that there was a series of events occurring in the peritoneal cavity which determined death or survival of the host. Since phagocytosis by peritoneal macrophages is a prominent defense mechanism, we examined this system in vitro.

The difference in sensitivity of E-107 K+ and K− to in vitro phagocytosis was marked (see Table 2). Phagocytosis of the K+ bacteria was very low even when 10¹⁰ organisms were added to 2 × 10⁶ to 2.5 × 10⁶ macrophages (5,000 bacteria per macrophage). Many of the macrophages appeared to be coated with bacteria. Phagocytosis of K− cells occurred even when the bacteria-to-macrophage ratio was as low as 5:1. The addition of rabbit anti-OK sera led to phagocytosis of the K+ bacteria.

Since the LD50 of E-107 K+ was high (10⁸) in the absence of hog gastric mucin, we postulated that some phagocytosis might occur in vivo as the result of normal mouse opsonins. Indeed, macrophages harvested from mice 15 min after intraperitoneal injection with 10⁴ E-107 K+ organisms showed that 97% contained bacteria, with a mean of 6.4 bacteria per cell.

This was substantiated by in vitro experiments (Table 2). The K+ cells were strongly phagocytized in the presence of 0.5 ml of normal mouse serum, and as little as 0.01 ml was also active.

We then began an examination of the specificity and role of opsonin in host defense mechanisms.

**Passive protection against a lethal dose of E. coli 107 K+ in 2% mucin.** Rabbit anti-107 K+ serum (0.5 ml of a 1:100 dilution) injected intraperitoneally into groups of 10 mice as late as 8 hr after infection completely protected mice against a lethal dose of 60 E-107 K+ plating units in mucin given intraperitoneally, even though a marked bacteremia could be demonstrated after...
5 hr. By 12 hr, the infection following injection of K+ in mucin had progressed to an extent that made it impossible to protect the animals with this concentration of antiserum.

**Further examination of 107 K+.** Since this anti E-107 K+ serum had both an anti-O (1:20, 480) and an anti-K (1:640) agglutination titer, the specificity of the antibody involved in passive protection was not clear. Table 3 shows the protective activities of various rabbit sera. The anti-OK serum was protective, and removal of anti-O antibodies by in vitro absorption did not decrease the protective capacity. Thus anti-K would appear to be the protective factor. However, anti-O antiserum prepared by injection of heated (2 hr at 100 C) E-107 K+ and K−, as well as one heterologous (anti-O 50) cross-reacting serum, all showed some protection. All of these sera contained low levels of anti-K agglutinins and were less protective than the sera with higher anti-K agglutinin titers. It was tempting therefore to explain the protective effect as being due solely to the anti-K activity. But other evidence indicated that the anti-O activity may also be important. First, colonial morphology, as examined by the incident light method (9), showed that E-107 K+ was more translucent and had lighter edges than some of our other K+ strains. This suggested a lower concentration of K antigen. Second, attempts to absorb the anti-K activity from our anti-O antiserum with 16-hr-old E-107 K+ bacteria, by use of the procedure of Edwards and Ewing (3), also led to a decrease in anti-O titer, which indicated incomplete blockade of O antigen by K antigen in post-logarithmic-phase bacteria. A low (1:10 to 1:20) anti-K+ titer remained even after there was a decrease in anti-O titer from 1:10,240 to as low as 1:640 to 1:1,280.

---

**TABLE 2. In vitro phagocytosis of E. coli 107 K+ and K−**

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Bacteria added/ phagocyte</th>
<th>Serum added</th>
<th>Phagocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Phagocytes containing bacteria (%)</td>
</tr>
<tr>
<td>K+</td>
<td>5 × 10⁹</td>
<td>—</td>
<td>9</td>
</tr>
<tr>
<td>K−</td>
<td>5 × 10⁹</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>K−</td>
<td>5 × 10⁹</td>
<td>—</td>
<td>99</td>
</tr>
<tr>
<td>K−</td>
<td>5 × 10⁹</td>
<td>—</td>
<td>75</td>
</tr>
<tr>
<td>K−</td>
<td>5 × 10⁹</td>
<td>—</td>
<td>36</td>
</tr>
<tr>
<td>K+</td>
<td>0.5</td>
<td>Anti-OK, 0.1 ml, of 1:200 dilution</td>
<td>4</td>
</tr>
<tr>
<td>K−</td>
<td>5 × 10⁹</td>
<td>Normal mouse, 0.5 ml</td>
<td>94</td>
</tr>
<tr>
<td>K+</td>
<td>5 × 10⁹</td>
<td>Normal mouse, 0.25 ml</td>
<td>88</td>
</tr>
<tr>
<td>K+</td>
<td>5 × 10⁹</td>
<td>Normal mouse, 0.1 ml</td>
<td>55</td>
</tr>
<tr>
<td>K+</td>
<td>5 × 10⁹</td>
<td>Normal mouse, 0.01 ml</td>
<td>14</td>
</tr>
</tbody>
</table>

---

**TABLE 3. Anti E-107 activity**

<table>
<thead>
<tr>
<th>Serum</th>
<th>Bacterial agglutination*</th>
<th>Passive protection*</th>
<th>In vitro phagocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K+</td>
<td>K−</td>
<td></td>
</tr>
<tr>
<td>Anti-OK</td>
<td>640</td>
<td>20,480</td>
<td>5,000</td>
</tr>
<tr>
<td>Anti-K</td>
<td>640</td>
<td>20,480</td>
<td>5,000</td>
</tr>
<tr>
<td>Anti-ΔK+</td>
<td>640</td>
<td>20,480</td>
<td>5,000</td>
</tr>
<tr>
<td>Anti-ΔK−</td>
<td>640</td>
<td>20,480</td>
<td>5,000</td>
</tr>
<tr>
<td>Anti-O 50</td>
<td>640</td>
<td>20,480</td>
<td>5,000</td>
</tr>
<tr>
<td>Anti-O 3</td>
<td>640</td>
<td>20,480</td>
<td>5,000</td>
</tr>
<tr>
<td>Anti-O 4</td>
<td>640</td>
<td>20,480</td>
<td>5,000</td>
</tr>
<tr>
<td>Anti-O 7</td>
<td>640</td>
<td>20,480</td>
<td>5,000</td>
</tr>
<tr>
<td>Normal rabbit</td>
<td>640</td>
<td>20,480</td>
<td>5,000</td>
</tr>
</tbody>
</table>

* All values given are reciprocals of the highest dilution yielding positive test.

**Comparison of two O, K, isolates (E-107 and E-102).** In an attempt to explain some of these results, we reexamined the relative importance of anti-K and anti-O antibodies in protection, using another strain of the same E. coli serotype O2K (E-102 K+). Morphologically, E-102 K+ appeared to have a higher K antigen content than E-107 K+, as the colonies were opaque without translucent edges.

In vitro, the K− variant of E-102 was strongly...
phagocytized, whereas the K+ variant was not (Table 4). As with E-107, the addition of 0.1 ml of anti-102 OK serum or normal mouse serum led to phagocytosis of the K+ cells. However, whereas E-102 K+ was phagocytized in the presence of an anti-K serum, unlike E-107 K+ it was not phagocytized in an anti-O serum.

Passive protection tests in mice against challenge with E-102 K+ (Table 5) showed good protection by rabbit anti-E-102 OK+ and anti-K sera. Anti-O antiserum (prepared against heated K− cells) had no protective effect.

The in vitro agglutination and opsonic activity of anti-O sera (prepared against heated E-107 K− and E-102 K− cells) when titrated against the homologous and heterologous strains are shown in Table 5. Although the agglutination titers are virtually the same regardless of the target cell, a large difference can be seen in opsonic activity. The E-107 K+ cells were opsonized by both the anti-E-102 (1:200) and the anti-E-107 (1:1,000) sera, whereas the E-102 K+ strain was not opsonized by either. Passive protection against challenge with E-107 K+ was possible with anti-E-102 (1:100) and E-107 (1:1,000). Neither serum protected against challenge with E-102 K+. In both cases, the agglutination titers against the E-107 K+ strain were slightly higher than against the E-102 K+ strain. The results of all three sets of experiments suggest that E-107 K+ is deficient in K antigen and that O antigen is available for reaction with anti-O antibody.

### DISCUSSION

The severe systemic infections of man caused by *E. coli* are associated in a great many cases with other diseases which decrease normal host resistance (11). Likewise, experimental infection of mice requires either reduction of host resistance, as by mucin injection, or injection of the organism into areas, such as the brain, which are relatively isolated from the immediate protective action of blood (2). We found E-107 K+ in the absence of mucin to be relatively avirulent (LD50 = 10⁶ organisms/mouse) by the intraperitoneal route but virulent (LD50 ∼ 55 organisms/mouse) by the intracerebral route. The K− variant was avirulent by both routes. The importance of circumventing or blocking host defenses is further emphasized by our finding that the K+ variant was least virulent when introduced intravenously.

The large number of organisms required for death suggests endotoxic activity rather than virulence and supports the concept of a lower concentration of serum antibody in the peritoneal fluid as compared with serum (31).

We found the peritoneal cavity to be the site of initial multiplication of the K+ bacteria in both the lethal and nonlethal infection. Further, mucin was able to lower the LD₅₀ of the K+ strain by any route only if the mucin was injected intraperitoneally. These results are in agreement with

**Table 4. In vitro phagocytosis of *E. coli* 102 K+ and K−**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Serum</th>
<th>Phagocytes containing bacteria (%)</th>
<th>Bacteria/ phagocyte (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K−</td>
<td>−</td>
<td>100</td>
<td>15</td>
</tr>
<tr>
<td>K+</td>
<td>−</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>K+</td>
<td>Anti-OK (0.1 ml)</td>
<td>92</td>
<td>4.8</td>
</tr>
<tr>
<td>K+</td>
<td>Anti-Δ K− (0.1 ml)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>K+</td>
<td>Anti K (0.1 ml)</td>
<td>90</td>
<td>4.1</td>
</tr>
<tr>
<td>K+</td>
<td>Normal mouse (0.2 ml)</td>
<td>85</td>
<td>3.1</td>
</tr>
</tbody>
</table>

* Addition of 10⁸ bacteria/2 X 10⁴ phagocytes.
* Of a 1:200 dilution.
* Δ = immunizing bacterial suspensions heated at 100 C for 2 hr to inactivate K antigenic activity.

**Table 5. Homologous and heterologous in vitro and in vivo activity of rabbit antisera**

<table>
<thead>
<tr>
<th>Serum</th>
<th>Bacterial agglutination</th>
<th>Passive Protectionb</th>
<th>Phagocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Δ 107 K−</td>
<td>160</td>
<td>10,240</td>
<td>80</td>
</tr>
<tr>
<td>Anti-Δ 102 K−</td>
<td>40</td>
<td>20,480</td>
<td>20</td>
</tr>
<tr>
<td>Anti K</td>
<td>320</td>
<td>320</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Anti-OK</td>
<td>−</td>
<td>320</td>
<td>20,480</td>
</tr>
</tbody>
</table>

* Values given are reciprocals of highest dilution yielding positive test.
* Administration of 0.5 ml of serum dilution intraperitoneally within 10 min of infection with 70 K+ bacteria in 2% mucin.
* Δ = immunizing bacterial suspensions heated at 100 C for 2 hr to inactivate the antigenicity of the K antigen.
the suggestion of Rowley (20) that the virulence of some *E. coli* strains is correlated with ability to grow in the peritoneal cavity.

Our finding that growth continues for approximately 5 hr in the nonlethal infection before the organisms are eliminated indicates that time is required to marshall effective host defense.

The role of hog gastric mucin in enhancement of virulence is far from clear. In a series of papers, Smith and his associates (23–30) reported that the active fractions of mucin included heparin, chondroitin sulfate, blood group A mucoid, and an unidentified water-soluble substance. The many theories that have been suggested for the action of mucin include (i) effects directly on the organism, such as growth stimulation, or acquisition of a protective coating to inhibit phagocytosis or intracellular killing, (ii) direct action on the phagocytic cells, such as inhibition of leukocyteosis or cell toxicity, (iii) inactivation of serum factors such as complement, properdin, or natural antibody, and (iv) increased permeability of the “barrier” between the abdominal cavity and the vascular system.

It is possible that a combination of effects occurs, but if the phagocytes are directly involved it would be difficult to explain the difference in virulence of the K+ and K− bacteria after separate injection of hog gastric mucin. An effect on natural antibody most logically explains the results.

The nonspecific release of protective antibodies after injection of microgram amounts of endotoxin is well known (7, 15). The time of host clearance of *E. coli* in our experiments correlates well with the time of highest serum bactericidal levels against *E. coli* O127:B8 (14) and highest opsonin titers against *Vibrio cholerae* (7) in mice after endotoxin injection. As the strains used in this work are insensitive to in vitro bactericidal activity of either normal mouse serum or mouse anti O2:K1 serum, determined by the test of Landy et al. (10), removal of mucin from normal serum opsonins needed for phagocytosis of K+ cells is a more likely explanation. Elimination of this mucin should have no effect upon phagocytosis of the K− cell, and indeed hog gastric mucin does not affect the virulence of this strain.

Since there is a nonspecific release of many different specific antibodies after injection of endotoxin, an antiserum produced by immunization with one strain of *E. coli* may contain antibodies of other specificities. Thus, determination of the specificity of the antibody involved in protection against the K+ organisms is difficult.

There seems little doubt that anti-K antibody is protective. We found a good protective effect with anti-OK serum, and removal of anti-O antibodies did not decrease this protection. The anti-O sera yielded minimal but significant protection and, although it is tempting to consider this effect to be due to the low titer of anti-K antibodies also present, we have not been able to eliminate the possibility that the anti-O specificity plays a minor role. There is some indication, at least with E-107 K+, that the anti-O specificity has opsonic activity in vitro. It is possible that the bacterial envelope does not completely prevent the reaction between anti-O antibody and the somatic antigen in some K+ strains which appear to have a small amount of K antigen. This may well explain the results obtained with E-107 K+. Since a 14 to 16-hr culture was used, it is possible that a defect in the envelope covering of E-107 K+ cells occurs with age. Although sera produced against heat-killed bacteria are usually less protective than those produced against living bacteria, exceptions do occur (4). These exceptions may involve low K-containing strains similar to E-107 K+.

The highest opsonic titers with both K+ strains were obtained when anti-OK sera were used, indicating that an additive (anti-K plus anti-O) effect may occur. A primary reaction of K antibody with K antigen may allow the somatic antigen to react with its homologous antibody through removal of surface charge.

Recently, Medearis et al. (13) showed a correlation between susceptibility to phagocytosis and the presence or absence of specific sugars in the cell wall lipopolysaccaride of a mouse-virulent *E. coli*, serotype O111:B4. It thus appears that both the O and K antigen are involved in virulence, with the important factor in both cases being susceptibility to phagocytosis. Opsonin, complement, mucin, O antigen, and K antigen may all be factors in determining the degree of virulence and host resistance.

ACKNOWLEDGMENTS

This investigation was supported at various times by Public Health Service research grants AI-04624 from the National Institute of Allergy and Infectious Disease, Public Health Service Training Grant GM-79, and Public Health Service Pre-doctoral Fellowship 1-F1-GM-30,261-01AI from the National Institute of General Medical Sciences.

LITERATURE CITED

4. Ewertsen, H. W. 1946. Experimental studies on the pathogenicity of the Coli bacilli and the effect of colisera (an...

---