Lethal Gram-Negative Bacterial Superinfection in Guinea Pigs Given Bacitracin

W. EDMUND FARRAR, JR., THOMAS H. KENT, AND VAN B. ELLIOTT

Departments of Applied Immunology and Experimental Pathology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C.

Received for publication 19 April 1966

ABSTRACT

FARRAR, W. EDMUND, JR. (Walter Reed Army Institute of Research, Washington, D.C.), THOMAS H. KENT, AND VAN B. ELLIOTT. Lethal gram-negative bacterial superinfection in guinea pigs given bacitracin. J. Bacteriol. 92:496-501. 1966.—Oral administration of a single dose of bacitracin (either 2,000 or 10,000 units) was lethal to more than 80% of guinea pigs. Within the first 12 hr, there was a 2,000-fold fall in the number of gram-positive organisms in the cecum. An increase in the number of coliform bacteria in the cecum was demonstrable within 6 hr, and, by 48 hr, these organisms had increased from the normal level of less than 100 per gram to approximately 1 billion per gram. The changes in intestinal bacterial flora were associated with development of a severe cecitis, mild ileitis, and acute regional lymphadenitis. Bacteremia, primarily due to coliform bacteria, was demonstrated in approximately 40% of the animals killed between 72 and 96 hr after administration of bacitracin. Development of this disease syndrome was suppressed by the administration of neomycin and polymyxin B, nonabsorbable antibiotics effective against coliform bacteria. The lethal disease produced by bacitracin in the guinea pig is similar to that produced by penicillin.

Penicillin was shown to be lethal for the guinea pig more than 20 years ago by Hamre and associates (8). DeSommer et al. (4) and Seeliger and Werner (13) demonstrated that, in this animal, penicillin administration results in replacement of the normal gram-positive intestinal flora by enormous numbers of gram-negative coliform organisms. Farrar and Kent (7) quantitated the changes in the microbial flora of the guinea pig cecum after penicillin administration and found that the number of coliform organisms increased approximately 10 million-fold within the first 48 hr. They also demonstrated that these changes in the bacterial flora were accompanied by severe inflammation of the cecum, regional lymphadenitis, and a high incidence of bacteremia due to coliform organisms, thus confirming a direct causal relationship between the gram-negative superinfection and the development of the fatal disease.

Although the administration of bacitracin also results in the appearance of large numbers of coliform bacteria in the gut and in the development of a disease syndrome in the guinea pig similar to that produced by penicillin (4, 5, 6), strictly quantitative studies of its effect on the bacterial flora of the cecum have not been done, nor have the histological changes which it produces been described. This antibiotic resembles penicillin in its spectrum of antibacterial activity in being much more effective against gram-positive than against gram-negative organisms. It is poorly absorbed and presumably has an antibacterial effect only within the lumen of the gastrointestinal tract after oral administration. This study was undertaken to investigate the effects of intragastric administration of bacitracin on the microbial flora and morphology of the gut in an effort to make clear the mechanism by which this antibiotic produces disease and death in the guinea pig.

MATERIALS AND METHODS

Female Hartley strain guinea pigs weighing 300 to 400 g each were used throughout. They were housed six to eight per cage in a room which contained no other species of experimental animals. Diet consisted of Purina rabbit chow supplemented with lettuce and other greens and water ad libitum.

Sterile bacitracin powder was dissolved in distilled
water so that the dose was contained in a volume of 10 ml. In all experiments, bacitracin was administered as a single dose by intragastric tube.

The techniques used in quantitative isolation and identification of intestinal microorganisms have been described previously (7). Gram-negative bacilli were counted on MacConkey Agar (Difco) plates incubated aerobically for 24 hr. Streptococci and bacteroides were enumerated on sheep blood-agar plates incubated anaerobically (H2 + 10% CO2) for 4 days.

Tissues for histologic study (jejunum, ileum, cecum, and mesenteric lymph nodes) were fixed in 10% neutral formaldehyde, and sections were stained with hematoxylin and cosin.

**RESULTS**

*Clinical effects of bacitracin.* The clinical features of the syndrome produced in guinea pigs by oral bacitracin administration were very similar to those which followed the parenteral injection of penicillin (7). This was characterized initially by diminished activity and loss of appetite, with severe obstipation. Terminally, the animals manifested progressive prostration, cyanosis, and respiratory difficulty.

Figure 1 shows the number of animals dying each day after bacitracin administration in three separate experiments. Fifty animals were used in each experiment; in two experiments, the dose of bacitracin was 2,000 units, and, in the third experiment, the dose was 10,000 units. The overall mortality in the two experiments in which 2,000 units were used was 82 and 88%, and in the experiment in which 10,000 units were given, it was 88%. Deaths began to occur between 24 and 48 hr after administration of bacitracin, in contrast with the experiments with penicillin, in which no animal died before 48 hr. A majority of the animals died between 48 and 72 hr when 2,000 units were given, whereas after administration of 10,000 units, more died in the 72- to 96-hr period. Thus, after the smaller dose of bacitracin, the peak mortality occurred about 24 hr sooner than in the penicillin experiments, whereas the time of death after administration of 10,000 units of bacitracin was closer to that seen after administration of penicillin. A possible explanation for this apparently paradoxical finding will be presented.

*Microbiological effects of bacitracin.* The microbiological findings in the cecal contents of six normal guinea pigs did not differ greatly from those reported earlier in experiments with penicillin. The most abundant organisms were microaerophilic or anaerobic streptococci, which were present at a level of from 10^4 to 10^4.4 organisms per gram of cecal contents (mean, 10^4.3 per gram). Most of these organisms grew on primary isolation only under anaerobic conditions, but frequently showed good growth on subculture in an aerobic environment. On the basis of biochemical criteria given in *Bergey's Manual* (7th ed.), three species were identified: *Streptococcus anaerobius*, *S. micros*, and *S. foetidus*. As in previous experiments, coliform bacteria were either absent or present in very small numbers (rarely above 10^3; 1 animal of 20 had 10^5 *Escherichia coli*).

Changes in the bacterial flora of the cecum after bacitracin administration are shown in Fig. 2. Within 12 hr after a dose of 10,000 units, the number of streptococci had fallen from more than 10^9 organisms to less than 10^7 per gram (i.e., approximately 2,000-fold). An explosive increase in the number of gram-negative coliform organisms was seen, apparently beginning very early after administration of bacitracin. A significant rise in the number of coliforms was detectable within 6 hr, and by 24 hr it had reached 10^8 organisms per gram. The peak number of approximately 10^9 coliforms was reached at 48 hr, making the total increase at least 10 million-fold. The magnitude of the increase in numbers of coliforms was similar to that seen after administration of penicillin, but the increase occurred much more rapidly after administration of bacitracin. (The coliform count did not change significantly for at least 24 hr after administration of penicillin.)
Abnormal numbers of coliforms (greater than $10^3$) were present in four of six surviving animals studied at the end of 2 weeks. As in the penicillin experiments, no one type of coliform organism appeared to be predominantly responsible for the superinfection. *E. coli*, *Klebsiella-Aerobacter*, and paracolon organisms were all found with approximately equal frequency. Two or more different colony types of coliforms were usually found in a given animal. Significant numbers of bacteroides were found in a few animals.

It was not possible to obtain accurate counts of streptococci in the cecal contents after the massive proliferation of coliform bacteria had occurred. Attempts were made to suppress the growth of coliform bacteria on the plates by incorporating sodium azide into the medium, but effective concentrations of this substance also greatly lowered the count of streptococci.

Blood cultures positive for coliform organisms were obtained as early as 12 hr after administration of bacitracin (Fig. 3). At 72 to 96 hr, the incidence of bacteremia was 40 to 50%. Blood cultures taken from five animals 1 week after administration of bacitracin were negative. Of the 19 positive blood cultures, 13 contained coliform organisms; the remaining 6 contained bacteroides.

**Morphological changes after administration of bacitracin.** Guinea pigs were killed 1, 2, 3, and 4 days after oral administration of either 2,000 or 10,000 units of bacitracin. Within 24 hr, the cecal contents had become liquid. At 72 and 96 hr, the cecum was greatly distended with liquid feces, and the cecal wall was congested. By 72 hr, mesenteric lymph nodes appeared enlarged and congested.

All animals given 10,000 units of bacitracin exhibited a severe acute inflammation of the cecum at 72 and 96 hr (Fig. 4 and 5). This was characterized by diffuse infiltration of the mucosa by polymorphonuclear leukocytes, prominent sloughing of surface epithelial cells, and severe vascular congestion. A similar reaction was seen...
in most of the animals given 2,000 units of bacitracin, but, in a few animals, this lesion was not demonstrable. Variable but generally mild acute inflammatory changes were noted in the distal ileum in approximately half the animals killed 12 to 96 hr after bacitracin administration. Acute mesenteric lymphadenitis was usually present at 72 and 96 hr. In general, the lesions seen in guinea pigs after bacitracin administration were quite similar in extent and time of appearance to the lesions seen after penicillin injection.

Protection against the lethal effects of bacitracin. A group of 25 animals was given neomycin sulfate (10 mg) and polymyxin B sulfate (3.3 mg) twice daily by gastric tube for 5 days after bacitracin administration. As shown in Fig. 6, these non-absorbable antibiotics completely protected the animals from the lethal effect of bacitracin for the period during which they were administered. Some deaths occurred in this group after the non-absorbable antibiotics were discontinued, but the overall mortality was still significantly less than that in the group receiving bacitracin alone. The number of gram-negative bacteria in the gut was also significantly less in the group which received neomycin and polymyxin B, and, at 48 and 96 hr, only Proteus organisms could be demonstrated (Table 1). A mixture of Proteus and coliform organisms was found in two of three surviving animals in this group cultured 1 week after administration of bacitracin. These findings are very similar to those obtained when neomycin and polymyxin B were given to animals treated with penicillin (7).

DISCUSSION

Intragastric administration of a single dose of bacitracin in the guinea pig produces a rapid fall in the total number of viable organisms in the cecum. This is accompanied by a prodigious increase in the numbers of coliform bacteria in the gut. In association with this superinfection, severe acute inflammatory lesions appear in the cecum. At this time, bacteremia is present in nearly half the animals. The demonstration of this sequence of events makes clear that the disease produced in the guinea pig by bacitracin administration is due to the actual invasion of the host tissues by gram-negative bacteria proliferating within the intestine. These bacteria, which are normally present only in very small numbers in the guinea pig, are presumably able to gain predominance because the normal gram-positive bacterial flora is virtually eliminated by the bacitracin.

The exact mechanisms by which antibiotic suppression of the normal intestinal bacterial flora may lead to bacterial superinfection in man or in experimental animals are not well understood. Meynell (9) and Bohnhoff et al. (2) presented evidence that streptomycin administration in the mouse causes a rise in intestinal pH and a fall in the concentration of volatile fatty acids produced by the normal bacteroides flora. These substances
are capable of inhibiting the proliferation of *Salmonella typhimurium* in the mouse intestine, and the streptomycin-treated mouse is rendered exquisitely sensitive to oral challenge with these organisms. Meynell (9) also suggested that there may be mechanisms in the normal mouse gut which inhibit the indigenous enterobacteria and maintain their numbers within certain limits. Studies of this type have not been carried out in the guinea pig, but, if the paucity of coliform organisms in this animal is due to the presence of inhibitory agents in the intestine, these must be substances which are very rapidly dissipated or inactivated after antibiotic administration, since an appreciable increase in the numbers of coliform organisms in the cecum is consistently demonstrable within 6 hr after bacitracin administration.

Bacitracin is a polypeptide antibiotic which, like penicillin, possesses a five-membered, sulfur-containing ring (1). Like penicillin, it inhibits the synthesis of bacterial cell walls, but it differs from penicillin in that it also interferes with the synthesis of certain bacterial enzymes (15) and inhibits the growth of lysozyme-induced streptococcal protoplasts at the same concentration required for inhibition of the growth of complete cells (14). Bacitracin is much more active against gram-positive than against gram-negative bacteria (10). Since it is minimally absorbed from the gastrointestinal tract (3, 12), any effects observed after its oral administration may be presumed to be due to its action within the intestinal lumen rather than on distant organs.

The clinical, microbiological, and morphological effects of intragastrically administered bacitracin in the guinea pig are quite similar to those produced by parenterally injected penicillin. The fact that this essentially nonabsorbable antibiotic is capable of producing such a similar disease in the guinea pig strongly supports the hypothesis that both syndromes are initiated by suppression of the normal gram-positive flora within the intestinal lumen, with subsequent superinfection by gram-negative coliform organisms. This suggestion is further substantiated by the finding that oral administration of antibiotics highly effective against gram-negative bacteria protects against the lethal effects of both penicillin and bacitracin.

Deaths occurred earlier after a small dose of bacitracin (2,000 units) than after either penicillin (50,000 units) or a large dose of bacitracin (10,000 units). This finding may be related to the fact that, whereas penicillin and bacitracin are much more active against gram-positive organisms, very high concentrations will inhibit many strains of gram-negative bacteria. In the experiments with penicillin, where a very high dose was used, the increase in coliform bacteria did not begin until some time after 24 hr. In the bacitracin experiments, the number of coliform bacteria in the cecum at 24 hr was approximately 10-fold lower after 10,000 units were administered than in the two experiments in which only 2,000 units were given (Fig. 2). Thus, it seems possible that high concentrations of either antibiotic may actually delay the development of the gram-negative superinfection. In the guinea pig, unlike the mouse, penicillin is detectable in the large bowel mucosa

### Table 1. Effect of neomycin and polymyxin B on cecal flora of guinea pigs given bacitracin

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Time after bacitracin (hr)</th>
<th>Animal no.</th>
<th>Log_{10} organisms per g^2</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacitracin</td>
<td>48</td>
<td>B1</td>
<td>9.5</td>
<td>Coliforms</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>B2</td>
<td>9.3</td>
<td>Coliforms</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>B3</td>
<td>9.0</td>
<td>Coliforms</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>B4</td>
<td>9.6</td>
<td>Coliforms</td>
</tr>
<tr>
<td>Bacitracin plus neomycin and polymyxin B</td>
<td>48</td>
<td>N1</td>
<td>7.0</td>
<td>Proteus</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>N2</td>
<td>&lt;2.0</td>
<td>Proteus</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>N3</td>
<td>5.2</td>
<td>Proteus</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>N4</td>
<td>8.7</td>
<td>Proteus</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>N5</td>
<td>8.5</td>
<td>Proteus</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>N6</td>
<td>6.0</td>
<td>Proteus</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>N7</td>
<td>3.5</td>
<td>Proteus</td>
</tr>
<tr>
<td></td>
<td>168</td>
<td>N8</td>
<td>8.3</td>
<td>Proteus, coliforms</td>
</tr>
<tr>
<td></td>
<td>168</td>
<td>N9</td>
<td>8.6</td>
<td>Proteus (1% coliforms)</td>
</tr>
<tr>
<td></td>
<td>168</td>
<td>N10</td>
<td>8.0</td>
<td>Proteus</td>
</tr>
</tbody>
</table>

* Cultures made on MacConkey Agar.
* Administration: 10,000 units by gastric tube.
* Neomycin sulfate (10 mg) and polymyxin B sulfate (3.3 mg) were given twice daily by gastric tube.
for at least 24 hr after parenteral injection (11). Comparable data for bacitracin are not available.

In our earlier paper on penicillin, an analogy was drawn between the disease produced by that agent in the guinea pig and the antibiotic-induced intestinal superinfections sometimes encountered in human patients. The possibility was suggested that study of the experimental model might lead to a better understanding of the analogous human disease. The findings of the present study indicate that bacitracin should be equally useful in this regard.

**Literature Cited**

13. Seeliger, H. P. R., and H. Werner. 1962. Die toxische Wirkung des Methicillins (2,6-Dimethoxy - benzamidopenicillin - Natrium) auf Meerschweinchen im Vergleich zu seiner toxi-