ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1988, p. 337–340 Vol. 32, No. 3
0066-4804/88/030337-04$02.00/0
Copyright © 1988, American Society for Microbiology

Impact of LY146032 on *Streptococcus (Enterococcus) faecalis* Translocation in Mice

STEVEN H. DOUGHERTY,[1]* DAVID J. HENTGES,[2] SHARON W. CASEY,[2] AND WENDY R. THAL[2]

Department of Surgery, Texas Tech University Health Sciences Center, El Paso, Texas 79905,[1] and Department of Microbiology, Texas Tech Health Sciences Center, Lubbock, Texas 79430[2]

Received 27 August 1987/Accepted 8 December 1987

The susceptibility of Swiss White mice to colonization with *Streptococcus (Enterococcus) faecalis* was greatly increased when the animals were given 5 mg of streptomycin sulfate per ml in their drinking water. One week after initiation of streptomycin treatment, the mice were challenged orogastrically with graded doses of streptomycin-resistant *S. faecalis*. The number of *S. faecalis* cells required to implant the intestinal tract of 50% of untreated mice was 2.9×10^9, but was only 4.8×10^3 for streptomycin-treated animals. When both groups of mice were challenged orogastrically with 4.6×10^6 viable *S. faecalis* cells, the cecum and small intestine of 100% of the streptomycin-treated animals, but only 10% of the untreated animals, were colonized with the organism. Similarly, translocation of *S. faecalis* to extraintestinal sites occurred in a majority of streptomycin-treated mice, but in only a small number of untreated mice. Subcutaneous administration of the experimental antibiotic LY146032 (Eli Lilly & Co., Indianapolis, Ind.) to streptomycin-treated mice concomitant with orogastric challenge with 5.5×10^5 viable *S. faecalis* cells resulted in a significant decrease in the incidence of intestinal colonization by the organism, a significant reduction in *S. faecalis* populations, and the absence of the organism in the liver, spleen, and heart. However, once intestinal colonization had occurred and extraintestinal infections were established, LY146032 did not significantly reduce *S. faecalis* populations or ameliorate the infections. We conclude that LY146032 effectively prevents translocation of *S. faecalis* from the intestinal tract of mice but does not resolve established extraintestinal infections.

The significance of enterococci as nosocomial pathogens is being increasingly recognized, especially since clinical use of broad-spectrum cephalosporins and other new beta-lactam antibiotics has become commonplace (3, 4, 11, 19). In a recent study from the Centers for Disease Control, Hughes et al. (J. M. Hughes, D. R. Olson, T. G. Emori, W. R. Jarvis, D. H. Culver, and C. Thornsberry, Program Abstr. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1029, 1985) reported that nosocomial enterococcal infections had increased from 3.4 to 3.8 per 1,000 hospital discharges between 1980 and 1984; during this same period, the incidence of enterococcal bacteremias almost doubled (0.12 to 0.20 per 1,000 hospital discharges). Overall infection rates were highest on surgery services (5.6 per 1,000 discharges), with the urinary tract, surgical wounds, and blood accounting for 59, 23, and 4% of the infections, respectively. This may represent a shift in the traditional epidemiology of enterococcal infections, which have typically involved the biliary tract, genitourinary tract, or heart valves. Perhaps even more ominous is the fact that isolates recovered from surgical infections are increasingly resistant to aminoglycosides (20).

Surgical infections involving enterococci, especially *Streptococcus (Enterococcus) faecalis*, often occur in seriously ill patients with peritonitis who have received broad-spectrum antibiotic therapy effective against most enteric pathogens except enterococci (5). We have observed that in some of these patients, enterococcal bacteremia may arise even when the organism has not been previously recovered from any known septic focus, including the urinary tract, and in the apparent absence of endocarditis, biliary tract disease, or pus (S. Dougherty, unpublished data). Global depression of host immune defenses usually appears to be present as well. We speculate that in seriously ill patients with peritonitis who receive broad-spectrum antibiotic therapy ineffective against enterococci, intestinal overgrowth of these organisms and extraintestinal spread by translocation sometimes occurs.

Surprisingly little experimental data exist, however, on the ability of enterococci to translocate to extraintestinal sites (18) or on the ability of specific antibiotic therapy to prevent translocation. In this report, we describe *S. faecalis* colonization of the intestines of mice and translocation of the organism to extraintestinal sites. We further report on the effectiveness of the experimental antibiotic LY146032 (Eli Lilly & Co., Indianapolis, Ind.) in preventing these extraintestinal infections and in ameliorating infections already established.

MATERIALS AND METHODS

Bacterial strain. The *S. faecalis* strain was kindly provided by Robert C. Moellering, New England Deaconess Hospital and Harvard Medical School. The MIC of streptomycin sulfate for this strain was greater than 10,000 μg/ml.

Mice. Outbred Swiss White mice (Cox variety; Laboratory Supply Co., Indianapolis, Ind.) were used in all experiments. The mice were housed individually in cages with wire-mesh bottoms to minimize coprophagy and were given Purina lab rodent diet and water ad libitum. Seven days before challenge with *S. faecalis* and after challenge for the duration of the experiments, the mice were given drinking water containing 5 mg of streptomycin sulfate per ml. Each mouse consumed 3 to 5 ml of drinking water per day whether or not the water contained streptomycin. Previous studies in this laboratory have shown that oral administration of streptomycin to mice disrupts the indigenous intestinal flora and predisposes the animals to colonization by exogenous bacteria (8, 14).

* Corresponding author.

ID_{50} determination. The challenge dose of *S. faecalis* that resulted in implantation of the intestinal tract of 50% of the mice (ID_{50}) was determined for streptomycin-treated and untreated animals. Seven days after initiation of streptomycin treatment, mice receiving the antibiotic and untreated control animals were challenged orogastrically with graded numbers of *S. faecalis* cells suspended in 0.1 ml of saline. Challenge doses were introduced directly into the stomach with a feeding needle. Three days after challenge, three fecal pellets were collected from each mouse, and the pellets were emulsified in 1 ml of sterile saline. A 0.1-ml sample of the emulsion was plated on bile-esculin-azide agar (Difco Laboratories, Detroit, Mich.) containing 1 mg of streptomycin sulfate per ml (S-BEA). The plates were incubated for 48 h, and the presence or absence of *S. faecalis* was recorded. The ID_{50}, based on the isolation of *S. faecalis* from the feces, was calculated by the method of Reed and Muench (15).

Localization studies. Streptomycin-treated and untreated mice were challenged with 4.6×10^6 viable *S. faecalis* cells as described above. Seven days after challenge, the mice were sacrificed by cervical dislocation and the cecum, small intestine, liver, spleen, and heart were aseptically removed, weighed, and homogenized in 9 volumes (vol/wt) of 0.05% yeast extract. Serial 10-fold dilutions of the homogenates prepared in yeast extract were plated on S-BEA. The numbers of *S. faecalis* cells were recorded as CFU per gram of organ (wet weight).

MIC of LY146032 for *S. faecalis*. The MIC of LY146032 for *S. faecalis* was determined in both the presence and absence of streptomycin. LY146032 was added in concentrations ranging from 0.25 to 64 μg/ml either to Todd-Hewitt broth (Difco) containing 1.65 mg of streptomycin per ml (mean concentration measured in the cecal contents of treated mice) or to Todd-Hewitt broth without added streptomycin. Broth tubes containing various concentrations of LY146032 were inoculated with 0.1 ml of a dilution of an 18-h-old culture of *S. faecalis* that had been adjusted to a McFarland standard of 1. The tubes were then incubated at 37°C for 24 h. The MIC of LY146032 was defined as the lowest concentration of the experimental antibiotic that prevented visible growth.

Administration of LY146032. LY146032 was administered subcutaneously to streptomycin-treated mice twice daily in 0.1-ml doses of 10 mg/kg of body weight. This dosage provided maximum plasma levels in mice without producing toxic effects (Eli Lilly & Co., personal communication). In one set of experiments (prevention studies), the mice received the first dose of LY146032 at the time of orogastric challenge with *S. faecalis* and daily thereafter for 7 days. In another set of experiments (amelioration studies), the initial dose of LY146032 was given 1 week after challenge with *S. faecalis* and administration continued daily for another 7 days.

Statistical analyses. Results were analyzed with the Statistical Package for the Social Sciences (SPSS, Inc., Chicago, Ill.). Since the skewness (8.2) and kurtosis (73.8) of the data were considerably greater than 0, nonparametric tests were used to compare means. Kruskal-Wallis one-way analysis of variance and the Mann-Whitney U test were used to compare the means of interval variables. Chi-square and Fisher's exact tests were used as tests of independence among nominal variables.

RESULTS

ID_{50}. The influence of streptomycin treatment on the susceptibility of mice to implantation with *S. faecalis* was determined first. The ID_{50} for untreated mice was 2.9×10^9 viable organisms. When the mice were given streptomycin for 7 days before challenge with *S. faecalis*, only 4.8×10^3 organisms were needed for colonization of 50% of the animals. Streptomycin administration therefore increased susceptibility to colonization with *S. faecalis* by almost 10^6-fold.

Localization studies. To determine where *S. faecalis* cells localized in colonized animals, we challenged 10 streptomycin-treated and 10 untreated control mice with 4.6×10^6 viable *S. faecalis* cells, an inoculum roughly 1,000 times smaller than the ID_{50} for untreated mice and 1,000 times greater than the ID_{50} for treated animals. The mice were sacrificed 7 days later, and counts of *S. faecalis* in homogenates of various organs were determined. The results (Table 1) show that, except for the heart ($P = 0.15$), the proportion of animals harboring *S. faecalis* was significantly greater in the streptomycin-treated group than in controls ($P < 0.003$). The cecum, small intestine, spleen, and heart of 10% of untreated animals were colonized with *S. faecalis*. By comparison, the organism colonized the cecum and small intestine of 100% of streptomycin-treated mice and translocated to the mesentery, liver, and spleen of the majority of animals and to the heart of 40%. Mean population levels of *S. faecalis* were consistently greater in streptomycin-treated than in untreated animals; the differences were statistically significant ($P < 0.003$), except for the heart ($P = 0.10$).

TABLE 1. Localization of *S. faecalis* in the organs of mice[a]

Organ	Mean $\log_{10}$ viable counts recovered from colonized animals[b]	
	Untreated	Streptomycin treated
Cecum	4.00 (10)	8.30 ± 0.84^d (100)[d]
Small intestine	2.00 (10)	6.64 ± 1.06^d (100)[d]
Mesentery	N[c] (0)	3.54 ± 1.16^d (90)[d]
Liver	N (0)	2.74 ± 0.80^d (80)[d]
Spleen	1.70 (10)	1.98 ± 0.51^d (80)[d]
Heart	1.70 (10)	2.40 ± 0.74 (40)

[a] Challenge dose, 4.6×10^6 viable *S. faecalis* cells.
[b] Results expressed as CFU ± standard deviation per gram of organ (wet weight); numbers in parentheses represent percentages of mice harboring *S. faecalis* in the particular organ.
[c] N, Fewer than 50 CFU/g of homogenate.
[d] Significantly greater values than those obtained from untreated mice.

Prevention of infections with LY146032. The effectiveness of LY146032 in preventing extraintestinal infections by *S. faecalis* in streptomycin-treated mice was determined next. Two groups of 25 streptomycin-treated mice and a third untreated control group were challenged orogastrically with 5.5×10^5 viable *S. faecalis* cells. The mice of one of the streptomycin-treated groups were given 20 mg of LY146032 per kg of body weight at the time of challenge and daily thereafter for 7 days. All the mice were sacrificed 1 week after challenge, and the numbers of *S. faecalis* cells in their organs were determined (Table 2). With the exception of one mouse that harbored *S. faecalis* in the liver, untreated animals were not colonized with the challenge organism. Streptomycin-treated animals, on the other hand, had high populations of *S. faecalis* in the cecum and small intestine. Translocation occurred in a number of these animals as evidenced by the presence of *S. faecalis* in the mesentery, liver, spleen, and heart. Administration of LY146032 to streptomycin-treated mice resulted in a statistically significant decrease in the incidence of intestinal colonization by the organism (colon and small bowel, $P < 0.003$), a signifi-

TABLE 2. Prevention of *S. faecalis* infections by LY146032[a]

Organ	Mean $\log_{10}$ viable counts recovered from colonized animals[b]		
	Untreated	Streptomycin treated	Streptomycin treated + LY146032 at time of challenge
Cecum	N[c] (0)	6.31 ± 1.75 (80)	4.87 ± 2.01[d] (20)[d]
Small intestine	N (0)	4.30 ± 1.46 (68)	2.15 ± 0.74[d] (16)[d]
Mesentery	N (0)	2.96 ± 1.04 (40)	1.48[d] (4)
Liver	1.77 (4)	2.75 ± 1.11 (36)	N[d] (0)
Spleen	N (0)	2.18 (4)	N (0)
Heart	N (0)	3.46 ± 0.80 (16)	N[d] (0)

[a] Challenge dose, 5.5×10^5 viable *S. faecalis* cells.
[b] Results expressed as CFU ± standard deviation per gram of organ (wet weight); numbers in parentheses represent percentages of mice harboring *S. faecalis* in the particular organ.
[c] N, Fewer than 50 CFU/g of homogenate.
[d] Significantly lesser values than those obtained from streptomycin-treated mice.

cant reduction in the size of *S. faecalis* populations (colon, small bowel, mesentery, $P < 0.002$), and the absence of the organism in the liver ($P = 0.001$), spleen ($P = 0.32$), and heart ($P = 0.04$). There was no evidence that streptomycin enhanced the repressive activity of LY146032, i.e., there was no synergism. On the contrary, it diminished the repressive activity of LY146032 in vitro. The MIC of LY146032 for *S. faecalis* in Todd-Hewitt broth was 8 μg/ml in the presence of streptomycin, but only 2 μg/ml in its absence.

Amelioration of established infection with LY146032. The final phase of the study determined whether LY146032 could ameliorate established extraintestinal *S. faecalis* infections in mice. Two groups of 20 streptomycin-treated mice were challenged orogastrically with 6.6×10^6 viable *S. faecalis* cells. One week after challenge, mice of one of the groups were given 20 mg of LY146032 per kg of body weight per day for a total of 7 days. Two weeks after challenge, all mice were sacrificed and the number of *S. faecalis* cells in their organs were determined. The results (Table 3) show that the cecum, small intestine, mesentery, and liver of mice in both groups contained *S. faecalis*. However, no organisms were isolated from the spleen or heart in either group. Administration of LY146032 appeared to cause a moderate decrease in the incidence of colonization of the cecum ($P = 0.078$) and small intestine ($P = 0.048$) by *S. faecalis* and a small reduction in *S. faecalis* populations in these organs. However, only the decreases in small bowel populations were of statistical significance ($P = 0.036$). LY146032 had no significant effect on either infection rates ($P = 0.37$; $P = 0.23$) or population levels ($P = 0.49$; $P = 0.27$) of enterococci in the mesentery or liver, respectively.

DISCUSSION

This study demonstrated intestinal colonization and translocation of resistant *S. faecalis* in streptomycin-treated mice. The mechanisms by which enteric streptomycin administration facilitates intestinal colonization with *S. faecalis* are not known. In studies with *Salmonella typhimurium* and *Pseudomonas aeruginosa*, we found that streptomycin had little effect on cecal oxidation-reduction potential and protein and carbohydrate concentrations or on intestinal motility (13). However, it caused a significant increase in cecal water content and pH and a decrease in the concentrations of several volatile fatty acids. In vitro, normalization of the pH and volatile fatty acid concentrations of cecal contents restored resistance to the growth of both test organisms. It is possible that these or similar changes induced by streptomycin facilitate colonization with *S. faecalis* as well. Whatever the case, streptomycin pretreatment increased susceptibility to enterococcal colonization almost 10^6-fold and significantly increased the likelihood of the translocation of the organisms to extraintestinal sites.

Several studies have shown that the experimental lipopeptide LY146032 is bactericidal for enterococci (*S. faecalis*, *Streptococcus faecium*) (9, 10), including strains resistant to aminoglycosides (D. A. Preston, F. T. Counter, and M. Surprenant, 24th ICAAC, abstr. no. 1080, 1984). In our study, administration of LY146032 to streptomycin-treated mice begun at the time of *S. faecalis* challenge significantly reduced intestinal colonization by the organism (Table 2). Except for the spleen, translocation of *S. faecalis* to extraintestinal sites, including the blood, was also significantly reduced. Early treatment with LY146032 actually eliminated *S. faecalis* infections of the spleen completely, but the change was not significant because of the low rate of splenic infection (4%) in the streptomycin-treated controls. We have no explanation for this low rate since the spleens of up to 16% of other streptomycin-treated animals have yielded *S. faecalis* (data not shown).

How treatment with LY146032 reduces intestinal colonization and translocation by *S. faecalis* is not known. In both the prevention and amelioration experiments (Tables 2 and 3), it appeared to have a direct suppressive effect on multiplication of *S. faecalis* in the intestine. The fact that intestinal carriage of the organism was suppressed but not completely eliminated may be due to several factors. In the mouse, only about 7% of LY146032 is excreted in the bile (Eli Lilly & Co., personal communication), perhaps an amount too small to completely clear the gut of susceptible organisms. However, the drug is excreted unchanged, so the presence of less biologically active forms of it in the bile is undoubtedly not a factor. *S. faecalis* that has reached stable growth phase in the gut, as perhaps occurred in the amelioration studies before the administration of LY146032, may be less vulnerable to the drug. LY146032 does not appear to be bactericidal for nondividing *S. aureus* (N. Allen, W. Alborn, Jr., J. Hobbs, Jr., and H. Percifield, 24th ICAAC, abstr. no. 1081, 1984). For mice receiving early treatment with LY146032 (prevention studies), *S. faecalis* translocation may have been eliminated by killing organisms that

TABLE 3. Amelioration of established *S. faecalis* infections in mice by LY146032[a]

Organ	Mean $\log_{10}$ viable counts recovered from colonized animals[b]	
	Streptomycin treated	Streptomycin treated + LY146032 1 week postchallenge
Cecum	6.40 ± 1.13 (85)	5.91 ± 1.79 (60)
Small intestine	4.21 ± 1.18 (80)	3.81 ± 1.14[d] (50)
Mesentery	2.72 ± 0.90 (35)	2.64 ± 0.86 (25)
Liver	2.47 ± 0.44 (15)	2.62 ± 0.76 (30)
Spleen	N[c] (0)	N (0)
Heart	N (0)	N (0)

[a] Challenge dose, 3.75×10^6 viable *S. faecalis* cells.
[b] Results expressed as CFU ± standard deviation per gram of organ (wet weight); numbers in parentheses represent percentages of mice harboring *S. faecalis* in the particular organ.
[c] N, Fewer than 50 CFU/g of homogenate.
[d] Significantly lesser value than that obtained from streptomycin-treated mice.

crossed the intestinal wall, by suppressing *S. faecalis* populations in the gut, or by both mechanisms. Indeed, other studies have shown that bacterial translocation in the mouse model depends substantially on the presence of high concentrations of test organisms in the gut (1, 2, 14).

In contrast to the results obtained with early LY146032 treatment, therapy initiated 1 week after challenge with *S. faecalis* (amelioration studies) appeared to be relatively ineffective in reducing both intestinal colonization and translocation (Table 3). Intraphagocytic sequestration of *S. faecalis* may be the best explanation. Recent evidence suggests that translocation of intestinal bacteria occurs when organisms are phagocytosed by M cells, special absorptive cells in the epithelium that overlie Peyer's patches and other mucosal lymphoid follicles (12). The organisms are then transferred, apparently intact, via large vesicles and released into the underlying space where they are taken up by macrophages. Although *S. faecalis* is not by nature an intracellular pathogen, evidence suggests that it and other organisms survive within phagocytes. Wells et al. (18) cultured enterococci from experimental abscesses that had been produced with only *Bacteroides* sp. and *Escherichia coli* and suggested that translocated enterococci from the intestine were carried to the infected sites by macrophages. In other translocation studies, Wells et al. (17) recovered viable *E. coli* from macrophages in mesenteric lymphatics. Similarly, staphylococci can be found within phagocytes, and their survival in these cells has been implicated in the therapeutic failure or clinical recurrence of treated infections (16).

Once bacteria are sequestered within phagocytes, the effectiveness of antibiotics directed against them is often unpredictable even though the drugs may achieve high intraphagocytic concentrations. Thus, Hand and King-Thompson (7) found that clindamycin and erythromycin achieved high intracellular levels in neutrophils, but failed to produce a significant reduction in the numbers of viable intraphagocytic *Staphylococcus aureus* cells. Aminoglycosides are able to concentrate 1,000-fold in lysosomal granules, but intracellular pathogens such as *Mycobacterium tuberculosis* that inhibit lysosome-phagosome fusion are not affected by them (6). For LY146032, Van der Auwera et al. (P. Van der Auwera, G. Petrikkos, T. Matsumoto, and M. Husson, J. Antimicrob. Chemother., in press) have shown that the intracellular drug concentration in neutrophils may be as high as 60% or more of the extracellular concentration. Despite this, LY146032 does not appear to alter intracellular killing of *S. aureus*, even for susceptible organisms at drug levels equal to the mean inhibitory concentration. Thus, in our animals, viable *S. faecalis* cells sequestered within Kupffer cells and mesenteric macrophages during 7 days of translocation might have been relatively protected from LY146032 given after this time period. Indeed, during the amelioration experiments (Table 3), suppression of *S. faecalis* populations by LY146032 occurred in the gut, where the organisms are presumably not intracellular, but not in the mesentery or liver.

ACKNOWLEDGMENT

This study was supported by a grant from Eli Lilly & Co.

LITERATURE CITED

1. **Bohnhoff, M., B. L. Drake, and C. P. Miller.** 1954. Effect of streptomycin on susceptibility of intestinal tract to experimental *Salmonella* infection. Proc. Soc. Exp. Biol. Med. **86:**132–137.
2. **Bohnhoff, M., and C. P. Miller.** 1962. Enhanced susceptibility to *Salmonella* infection in streptomycin-treated mice. J. Infect. Dis. **111:**117–127.
3. **Chandrasekar, P. H., B. R. Smith, J. L. LeFrock, and B. Carr.** 1984. Enterococcal superinfection and colonization with aztreonam therapy. Antimicrob. Agents Chemother. **26:**280–282.
4. **Clumeck, N., Y. Van Laethem, B. Gordts, N. Jaspar, and J. P. Butzler.** 1983. Use of ceftazidime in the therapy of serious infections, including those due to multiresistant organisms. Antimicrob. Agents. Chemother. **24:**176–180.
5. **Dougherty, S. H., A. B. Flohr, and R. L. Simmons.** 1983. "Breakthrough" enterococcal septicemia in surgical patients: 19 cases and a review of the literature. Arch. Surg. **118:**232–237.
6. **Edelson, P. J.** 1982. Intracellular parasites and phagocytic cells: cell biology and pathophysiology. Rev. Infect. Dis. **4:**124–135.
7. **Hand, W. L., and N. L. King-Thompson.** 1986. Contrasts between phagocyte antibiotic uptake and subsequent intracellular bactericidal activity. Antimicrob. Agents Chemother. **29:**135–140.
8. **Hentges, D. J., A. J. Stein, S. W. Casey, and J. U. Que.** 1985. Protective role of intestinal flora against infection with *Pseudomonas aeruginosa* in mice. Influence of antibiotics on colonization resistance. Infect. Immun. **47:**118–122.
9. **Jorgensen, J. H., L. A. Maher, and J. S. Redding.** 1987. In vitro activity of LY146032 (daptomycin) against selected aerobic bacteria. Eur. J. Clin. Microbiol. **6:**91–96.
10. **Machka, K., and I. Braveny.** 1987. Comparative in vitro activity of LY146032 (daptomycin) against gram-positive cocci. Eur. J. Clin. Microbiol. **6:**96–99.
11. **Murphy, T. F., and M. Barza.** 1982. Treatment of intraabdominal infection with moxalactam. Rev. Infect. Dis. **4**(Suppl.): s670–s675.
12. **Owen, R. L., N. F. Pierce, and R. T. Apple.** 1986. M cell transport of *Vibrio cholerae* from the intestinal lumen into Peyer's patches. A mechanism for antigen sampling and for microbial transepithelial migration. J. Infect. Dis. **153:**1108–1118.
13. **Que, J. U., S. W. Casey, and D. J. Hentges.** 1986. Factors responsible for increased susceptibility of mice to intestinal colonization after treatment with streptomycin. Infect. Immun. **53:**116–123.
14. **Que, J. U., and D. J. Hentges.** 1985. Effect of streptomycin administration on colonization resistance to *Salmonella typhimurium* in mice. Infect. Immun. **48:**169–174.
15. **Reed, L. G., and H. Muench.** 1938. A simple method of estimating fifty percent end points. Am. J. Hyg. **27:**493–497.
16. **Rogers, D. E., and R. Tompsett.** 1952. The survival of staphylococci within human leukocytes. J. Exp. Med. **95:**209–230.
17. **Wells, C. L., M. A. Maddaus, and R. L. Simmons.** 1987. Role of the macrophage in the translocation of intestinal bacteria. Arch. Surg. **122:**48–53.
18. **Wells, C. L., O. D. Rotstein, T. L. Pruett, and R. L. Simmons.** 1986. Intestinal bacteria translocate into experimental intraabdominal abscesses. Arch. Surg. **121:**102–106.
19. **Wilson, W. R., N. K. Henry, T. F. Keys, J. P. Anhalt, F. R. Cockerill III, R. S. Edson, J. E. Geraci, P. E. Hermans, S. M. Muller, and J. E. Rosenblatt.** 1984. Empiric therapy with moxalactam alone in patients with bacteremia. Mayo Clin. Proc. **59:**318–326.
20. **Zervos, M. J., C. A. Kauffman, P. M. Therasse, A. G. Bergman, T. S. Mikesell, and D. R. Schaberg.** 1987. Nosocomial infection by gentamicin-resistant *Streptococcus faecalis*. An epidemiologic study. Ann. Intern. Med. **106:**687–691.