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MAJOR ARTICLES

Synergism of Ampicillin and Gentamicin against Obstructive Pyelonephritis Due to *Escherichia coli* in Rats

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Rats with obstructive pyelonephritis due to *Escherichia coli* were treated for different intervals with ampicillin and gentamicin either alone or in combination. The combination of ampicillin and gentamicin was synergistic in vitro and significantly more effective in vivo than was either drug alone. After treatment for 10 days, the combination of ampicillin and gentamicin was the only regimen that sterilized all of the pyelonephritic kidneys. The importance of achieving sterility was illustrated by the observation that severe infection and acute pyelonephritis recurred after religation of the ureter in 12 (71%) of 17 ampicillin-treated animals that had harbored as few as 46 organisms per kidney before ligation. A synergistic combination of antibiotics rapidly sterilizes obstructed pyelonephritic kidneys. In view of the particular risk of renal infection in the presence of urinary obstruction in humans, synergistic antibiotic combinations may be useful in the treatment of obstructive pyelonephritis in humans.

The emphasis in recent years on noninfectious causes of interstitial nephritis has minimized the importance of urinary tract infections in the presence of obstruction as a cause of serious kidney disease. Nevertheless, infectious pyelonephritis in obstructive renal disease is the second most common cause of end-stage kidney failure, may be responsible for 9%–18% of patients who require dialysis or transplantation [1, 2], and produces innumerable other cases of unilateral or severe bilateral kidney damage. We have experimental evidence that destruction of renal parenchyma results from injury due to inflammation and suppuration during acute pyelonephritis [3]. It is therefore of considerable importance to study the way in which antibiotics are most effective in preventing destruction of the obstructed infected kidney.

Previous studies of the effectiveness of antibiotic therapy in the treatment of experimentally induced retrograde kidney infections were not performed in the presence of renal obstruction, which is the most important factor in severe human kidney infections. Furthermore, these studies were not designed to determine a therapeutic regimen that would sterilize the infected kidneys, and antibiotics were not administered in dosages that mimicked treatment of human obstructive pyelonephritis [4–10]. Accordingly, this study was undertaken to investigate the treatment of acute pyelonephritis due to *Escherichia coli* in the rat with the antibiotics, dosages, and routes of administration commonly used in the treatment of pyelonephritis in humans.

For these studies, we used a model of acute experimental pyelonephritis in the rat, which resembled severe human pyelonephritis in that infection of the kidney was acquired by the retrograde route in the presence of partial obstruction [11]. Ampicillin and gentamicin were given alone

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and in combination to see whether in vitro synergism would be reflected in an in vivo response.

Materials and Methods

Bacteria and in vitro susceptibility tests. The strain of *E. coli* O6 (Williams) used to infect the rats has been previously described [11]. It is inhibited by 2.5 μg of ampicillin/ml or 1.2 μg of gentamicin/ml. Bacterial killing was studied in the following manner: an overnight broth culture was appropriately diluted in Mueller-Hinton broth (MHB; Baltimore Biological Laboratories [BBL], Cockeysville, Md.) and added to 50 ml of MHB to give a final concentration of 10^6 organisms/ml. Various amounts of ampicillin, gentamicin, or a combination of the two drugs were added to the flasks of MHB before the bacteria were inoculated. Samples were removed at different intervals and appropriately diluted in MHB without antibiotics before 0.5 ml of each dilution was plated onto Mueller-Hinton agar plates (BBL) containing 1,000 units of penicillinase/ml.

Production of pyelonephritis. Acute retrograde pyelonephritis was produced in male Wistar-Lewis rats weighing 225–250 g (Charles River Breeding Laboratories, Wilmington, Mass.). Acute retrograde pyelonephritis was induced as described previously [11] with slight modification. In brief, after the rats were deprived of water for 18 hr and placed under ether anesthesia, a midline incision of the abdominal wall was made. A 1.0-ml bacterial inoculum of 2×10^3 organisms/ml of an overnight culture of *E. coli* O6 (Williams) in trypticase soy broth (TSB; BBL) was gently infused into the bladder. Reflux consistently occurred when urine appeared at the external urethral meatus. The remainder of the 1.0-ml volume was passed externally through the urethra without causing further bladder distension. After the infusion a silk ligature around the left ureter was loosely tied through the left flank, and the abdominal wall was closed. The ligature was carefully removed from the outside 20 hr later. The incidence of gross pyelonephritis 30 hr after ligation was ~75% as judged by the appearance of numerous small abscesses on the surface of the kidney. Some degree of ureteral and pyelic dilatation as well as residual hydronephrosis remained throughout the course of the disease [12].

Treatment with antibiotics. Sodium ampicillin (Bristol Laboratories, Syracuse, N.Y.) (10 mg in a volume of 0.1 ml or 160 mg/kg per day) was injected im in alternate thighs at 6-hr intervals. Gentamicin (Schering, Kenilworth, N.J.) (1 mg in a volume of 0.1 ml or 8 mg/kg per day) was given at 12-hr intervals since it accumulates in the kidney [13]. Preliminary studies showed that these dosages would have an approximately equivalent antimicrobial effect in vivo. Serum levels were determined in five rats after im injection of a single dose of either drug. Blood was obtained from the tail by the "farmer's wife" method [14]. Serum levels were measured in a standard manner [15]. The mean levels (μg/ml) ± SD obtained with ampicillin were 35.5 ± 17.8 at 30 min, 6.5 ± 2.9 at 1 hr, and 1.2 ± 0.5 at 2 hr. The mean levels (μg/ml) ± SD of gentamicin were 8.0 ± 2.1 at 30 min, 4.6 ± 1.8 at 1 hr, and 1.9 ± 0.9 at 2 hr. For treatment with both antibiotics combined, the same dosages were used as when each drug was given alone. Control animals were given 0.1 ml of saline im four times daily. To determine the importance of the duration of treatment, the following schedules were used, each beginning 28–30 hr after ligation (numbers in parentheses are the number of times each drug was given; Amp = ampicillin and Gm = gentamicin): (*1*) over 1.5 days, Amp (six), Gm (three), Amp (six) plus Gm (three), saline (six), (*2*) over 3.5 days, Amp (14), Gm (seven), Amp (14) plus Gm (seven), saline (14); (*3*) over 5.5 days, Amp (22), Gm (11), Amp (22) plus Gm (11), saline (22); and (*4*) over 10 days, Amp (40), Gm (20), Amp (40) plus Gm (20), saline (40).

Between 24 and 36 rats were operated on at one time; they were divided into four groups and treated with the various regimens for various intervals. Each experiment was performed three times. A few rats in control groups (receiving saline) died as a result of septicemia between days 3 and 5 after infection, but none of the treated animals died.

Effect of treatment with antibiotics on kidney infection. The effectiveness of therapy was assessed on the basis both of the number of bacteria present in the kidney and of the severity of pyelonephritis as measured indirectly by kidney weight

(see below). Only rats showing macroscopic evidence of pyelonephritis (75% of all ligated rats) were selected for evaluation of the effectiveness of antibiotic treatment since kidneys without gross pyelonephritis were uniformly sterile at each time studied.

At the end of each treatment schedule, the rats were killed, and the kidneys were removed aseptically, weighed, and homogenized in 1 ml of TSB. Tenfold dilutions of the homogenates were made, and 0.5-ml volumes of the diluted homogenates were plated on trypticase soy agar plates containing 1,000 units of penicillinase/ml. The plates were incubated overnight aerobically at 37 C, and the number of cfu in each plate was counted. Cultures from animals treated with gentamicin were incubated in anaerobic jars (GasPak® catalyst; BBL) for 48 hr to decrease the activity of gentamicin [16].

Effect of treatment on the course of pyelonephritis. Previous studies using this model of experimentally induced pyelonephritis have shown that kidney weight provides the best quantitative assay of severity of pyelonephritis [12]. During the acute phase, kidney weight increased in proportion to the degree of inflammation and of exudation occurring in the renal parenchyma. To minimize the normal variation in kidney weights, the ratio of the weight of the left kidney to that of the right kidney (L/R) was used for comparison of the severity of pyelonephritis. The mean ratio is ~ 1 in normal animals and varies very little (mean ± SD, 0.978 ± 0.03).

Relapse after religation of the left ureter. A test of residual infection after treatment in the absence of persistent antibiotic. As demonstrated by Guze and Beeson [17], bacteria in a nonpyelonephritic kidney can cause destructive pyelonephritis if the ureter is ligated. This phenomenon was used to test the virulence of the few surviving organisms after therapy with ampicillin or gentamicin. Groups of 16–20 rats were operated on and treated for 10 days with one of the two antibiotics. Thirty-six hours after completion of therapy, the animals were either killed (controls) or operated on again. After rats were anesthetized with ether, the left kidney was exposed through a paramedial incision and inspected for the presence of pyelonephritic cortical lesions. The left ureter was religated by the procedure used in the initial ligation. After the abdomen was closed, the ligature was left in place for 72 hr. Animals were killed five days after religation of the left ureter and examined for kidney infection and pyelonephritis as described above.

Statistics. The differences observed in kidney weights and the number of viable bacteria (cfu)/g of kidney of treated and control animals were compared for significance by Student's unpaired *t*-test. Statistical comparison of the incidence of sterilization between the treated groups was done by the χ^2 method with Yates's correction.

Results

Bacterial killing by ampicillin and gentamicin in vitro. As shown in figure 1, the combination of ampicillin and gentamicin was more effective and more rapid in killing *E. coli* O6 (Williams) than was either drug alone, a finding demonstrating an in vitro synergistic effect. Among 12 fresh isolates of *E. coli* from human urine, results almost identical to those in figure 1 were obtained with 11 strains. These 11 strains were also killed through the synergistic action of ampicillin and

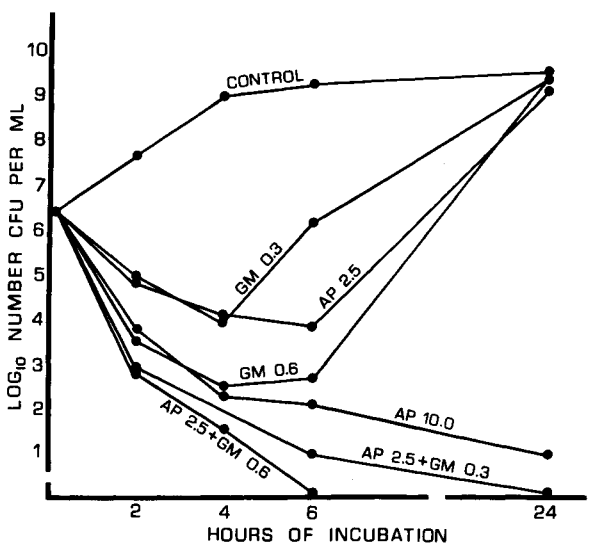

Figure 1. Bacterial killing of *Escherichia coli* O6 (Williams) by ampicillin (AP) and gentamicin (GM). Data are given as $\log_{10}$ number of cfu of *E. coli* O6 (Williams) found after exposure of the bacteria to various concentrations (μg/ml; indicated on the curves) of AP, GM, or a combination of the two drugs (AP + GM).

gentamicin when examined by the method used in figure 1.

Effect of treatment on kidney infection. Figure 2 shows the number of bacteria surviving in the homogenates of left kidney tissue after treatment of the rats for various intervals with ampicillin and gentamicin either alone or in combination. The combination of ampicillin and gentamicin was more effective than either drug alone; the number of bacteria/g of kidney after treatment for 3.5, 5.5, and 10 days with the combination of antibiotics was significantly lower than with either drug alone. There was no difference in the in vivo antimicrobial activity of ampicillin or gentamicin except for a slightly greater activity of gentamicin at 3.5 days.

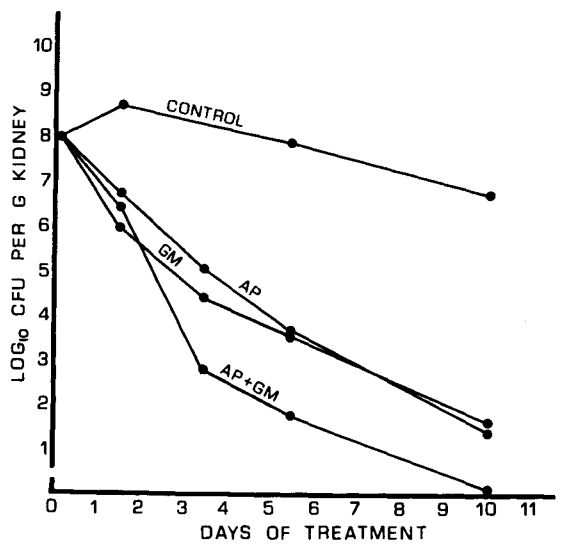

Figure 2. Effect of antibiotic treatment on rat kidney infection. The data are given as $\log_{10}$ number of cfu recovered/g of kidney after im treatment with ampicillin (AP), gentamicin (GM), or a combination of the two (AP + GM). Therapy was begun 28–30 hr after infection with *Escherichia coli* O6 (Williams) (day 0) and continued for 1.5, 3.5, 5.5, and 10 days. Each point represents the mean value for determinations in nine to 14 rats. Control animals received saline im. Statistical analysis for significance was done with Student's unpaired *t*-test, and the differences between therapy with different drugs were as follows. After 1.5 days: AP vs. GM, AP vs. AP + GM, and GM vs. AP + GM, *P* not significant. After 3.5 days: AP vs. GM, $P < 0.05$; AP vs. AP + GM, $P = 0.01$; and GM vs. AP + GM, $P < 0.05$. After 5.5 days: AP vs. GM, *P* not significant; AP vs. AP + GM, $P < 0.05$; and GM vs. AP + GM, $P < 0.05$. After 10 days: AP vs. GM, *P* not significant; AP vs. AP + GM, $P < 0.025$; and GM vs. AP + GM, $P < 0.05$.

More important, the combination of ampicillin and gentamicin was more effective in sterilizing kidneys than was either drug alone ($P < 0.05$; table 1). This was also seen if the sum of the effect of each individual drug was compared with the effect of therapy with the combined drugs after 5.5 or 10 days. After 10 days, combined therapy sterilized all kidneys. As early as 5.5 days after infection, when only one of eight and none of 13 kidneys were sterilized by gentamicin and ampicillin, respectively, treatment with the combination of antibiotics sterilized six of nine kidneys (not significant when compared to treatment with gentamicin alone; $P < 0.01$ when compared to treatment with ampicillin alone).

For further investigation of whether combined therapy was effective because of an additive effect of each drug or because of a synergistic effect of the combination, groups of 10 rats were treated with double dosages of ampicillin (320 mg/kg per day) for 5.5 and 10 days. The mean cfu (± SD)/g recovered from these kidneys was similar to that found after treatment with a single dosage of ampicillin (160 mg/kg per day): by 5.5 days of treatment, $3.0 \pm 1.5 \times 10^4$ vs. $4.7 \pm 2.6 \times 10^3$, respectively, and by 10 days, $1.2 \pm 0.7 \times 10^2$ vs. $6.4 \pm 6.7 \times 10$). There was no increased in-

Table 1. Comparison of the effect of therapy with a single drug (ampicillin [Amp] or gentamicin [Gm]) with that of therapy with the two antibiotics in combination on the outcome of rat kidney infection due to *Escherichia coli* O6 (Williams).

	Kidney condition after treatment for			
	5.5 days		10 days	
Treatment	Infected	Sterile	Infected	Sterile
Gm	8	1	5†	4
Amp	13*	0	10†	1
Sum of Gm and Amp	21*	1	15†	5
Amp plus Gm (combined)	3	6	0	8

NOTE. Data are number of rats. See Materials and Methods for antibiotic dosages used. Statistical analyses were done with the χ^2 method with Yates's correction. Except as noted, relevant comparisons were not significant.

*$P \leq 0.05$ compared with combined treatment for 5.5 days.

†$P \leq 0.05$ compared with combined treatment for 10 days.

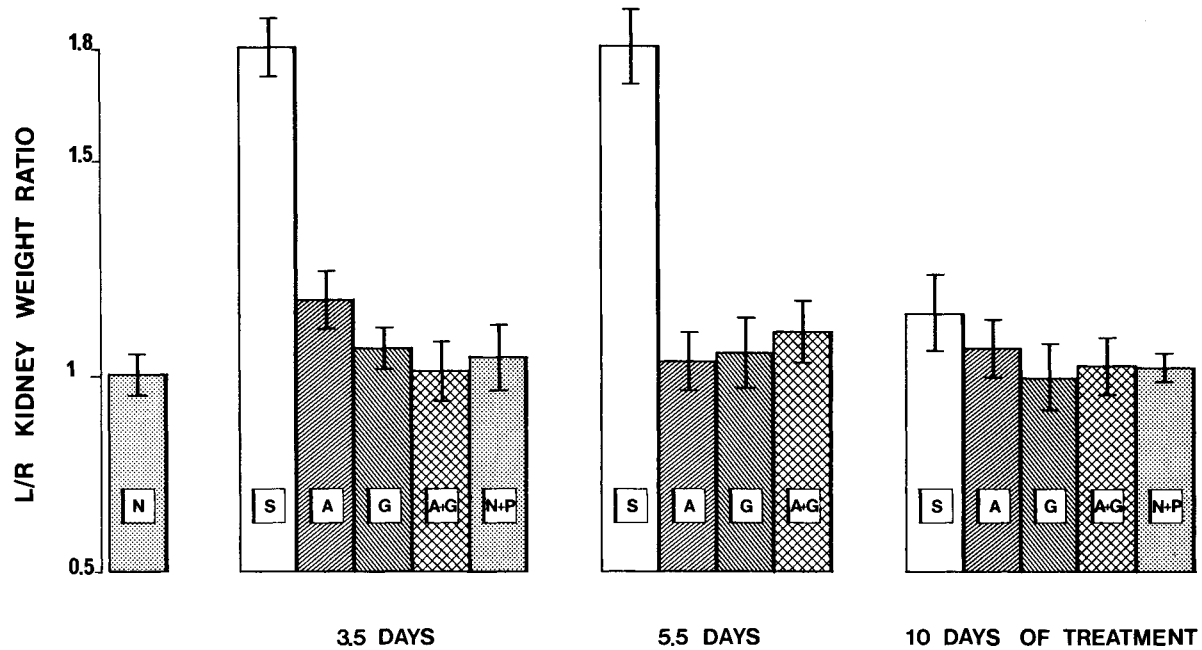

Figure 3. Effect of antibiotic treatment for various intervals on the severity of pyelonephritis as measured by the ratio of the weight of the left kidney to that of the right (L/R). N = L/R in normal rats; S = L/R in saline-treated controls; A = L/R in ampicillin-treated rats; G = L/R in gentamicin-treated rats; A + G = L/R in rats treated with ampicillin and gentamicin combined; and NP = no pyelonephritis, i.e., L/R in rats that did not develop pyelonephritis after ligation. The brackets over each column indicate the 95% confidence limit.

cidence of sterile kidneys. If an additive effect of ampicillin and gentamicin in combination were likely, one might have expected that doubling the dosage of a single drug (ampicillin) would have resulted in a similar therapeutic advantage. As this was not the case, the observation was therefore interpreted as an indirect argument for in vivo synergy of ampicillin and gentamicin.

The bacteria surviving after treatment for 10 days with either ampicillin or gentamicin alone were tested in several instances for acquired resistance to antibiotics. No loss of sensitivity to these antibiotics was demonstrated.

Effect of treatment on the severity of pyelonephritis. Both the degree of kidney infection and the severity of renal lesions were profoundly reduced by treatment. Figure 3 demonstrates the effect of antibiotic treatment on the course of the disease. By 3.5 days all three antibiotic regimens significantly reduced the L/R ratios, although gentamicin alone and the combination of gentamicin and ampicillin were slightly but significantly better than ampicillin alone ($P < 0.01$; table 2). After treatment for 5.5 and 10 days, the mean L/R ratio of all treated animals was not different from that of nonpyelonephritic controls, and no difference between the three antibiotic regimens was observed.

Religation of the left ureter after therapy for 10 days. To evaluate the significance of the small numbers of bacteria recovered from the kidneys of antibiotic-treated rats, ureters were religated 36 hr after the completion of antibiotic therapy, and bacteriologic studies were carried out five days later. At the time of religation, the kidneys were examined for macroscopic evidence of pyelonephritis. In each experiment one group of animals was studied bacteriologically at the time of religation of the ureter in the test group. Kidneys without macroscopic inflammatory lesions were sterile and remained so even after religation of the ureter.

Thirty-six hours after completion of treatment, the pyelonephritic kidneys in eight rats with lesions treated with ampicillin for 10 days were cultured and had a mean count (± SD) of 46 ± 69 cfu. Religation of the ureter in 17 pyelonephritic animals similarly treated resulted in a recurrence of acute infection in 12 animals (71%) five days later. The mean bacterial col-

Table 2. Severity of pyelonephritis due to *Escherichia coli* O6 (Williams) in rats after treatment with antibiotics for 3.5 days.

Treatment	No. of rats	Mean L/R ratio ± SD	Difference between treatment groups	Difference from pyelonephritic controls
Saline (controls)				
Animals without lesions*	7	1.024 ± 0.088		...
Pyelonephritic animals	18	1.767 ± 0.242	$P<0.0005$	...
Gentamicin	14	1.041 ± 0.089†		0.726 ($P<0.0005$)
Ampicillin	12	1.159 ± 0.126	$P<0.01$	0.608 ($P<0.0005$)
Ampicillin plus gentamicin	12	0.984 ± 0.085†	$P<0.0025$	0.783 ($P<0.0005$)

NOTE. See Materials and Methods for the dosages used in treatment of the rats with antibiotics. The severity of pyelonephritis in the left kidney is expressed as the ratio of the mean weight of the left kidney to that of the right kidney (L/R). Statistical analysis for significance was done with Student's unpaired *t*-test.

*Animals without lesions are those which did not develop pyelonephritis after ligation; the L/R ratio for this group is not significantly different from that of rats treated with gentamicin or ampicillin plus gentamicin.

†Difference not significant.

ony count (± SD) was $3.0 \pm 7.1 \times 10^8$ in the relapsing kidneys ($P < 0.0001$ when compared with controls killed at the time of religation). Thus the small number of bacteria remaining in the kidney after 10 days of ampicillin treatment was pathogenic in the reobstructed kidney.

It was of considerable interest that 36 hr after the completion of 10 days of gentamicin treatment, five of nine rats with lesions showed infection persisting in their pyelonephritic kidneys. Small numbers of bacteria (48 ± 66 cfu) were also recovered, a finding comparable to the number recovered from the kidneys of ampicillin-treated rats. In contrast to the ampicillin-treated rats, however, religation of the ureter did not rekindle the infection, either morphologically or bacteriologically, in eight rats with pyelonephritis. Since gentamicin is known to accumulate and persist in the kidney, this phenomenon was considered evidence of persistent antimicrobial activity. This phenomenon has been examined in detail and is the subject of a separate publication [18]. Because of this observation and because pyelonephritic kidneys treated with combined antibiotic therapy were all sterile, religation was not studied after treatment of the rats with ampicillin and gentamicin in combination.

Discussion

This study demonstrated that a combination of antibiotics is superior to either drug alone in the treatment of obstructive pyelonephritis when the two drugs are synergistic in vitro against the infecting organism. There is some evidence that this advantage is due to an in vivo synergism rather than to an additive effect because therapy with the combination of ampicillin and gentamicin was more effective than the sum of the effect of the drugs administered singly and because doubling the dosage of a single drug did not improve its efficacy in vivo.

Although evidence for the superiority of antibiotic combinations in vivo has been obtained in experimentally induced infections when the combination was synergistic in vitro [19–22], only the studies of Sapico et al. [10] on hematogenous enterococcal infection of the nonobstructed kidney have demonstrated such superiority in the treatment of pyelonephritis. McCabe and Jackson [23] found that among patients with pyelonephritis, urine was sterile in 87% of those treated with a combination of antimicrobial agents synergistic in vitro against the organism isolated from the urine, in 67% when the combination was additive, and in 14% when the combination was antagonistic. Such results, however, are not useful in judging the antimicrobial effect on the kidney parenchyma since these patients suffered frequent relapses [23].

The importance of sterilizing the infection in the presence of urinary tract obstruction was shown by the ease with which reinfection occurred in rat kidneys containing as few as 40–50 residual bacterial cfu after treatment with ampicillin.

The inability of small numbers of bacteria to reinfect gentamicin-treated animals is interesting and suggests that the known renal accumulation of gentamicin is accompanied by persistent antibacterial activity. If this is true, then this phenomenon is unlikely to last since there is continuous elimination of the stored gentamicin by the urine [13], thus emphasizing the need for sterilization.

Gentamicin was slightly superior to ampicillin in the eradication of bacteria after treatment for 3.5 days and was as effective as ampicillin after 5.5 and 10 days of treatment, even though gentamicin was administered only two times in 24 hr compared with four times in 24 hr for ampicillin. These observations provide additional evidence that accumulation and persistence of gentamicin in the renal parenchyma [13] are responsible for the therapeutic advantage of this drug over an antibiotic that does not accumulate in the treatment of renal parenchymal infection.

This study also demonstrated that antibiotic treatment not only affects the number of bacteria but also reduces the severity of renal inflammation as determined by a significant decrease in the L/R ratios. The ability of antibiotics to inhibit suppuration was as evident 5.5 or 10 days after treatment with a single drug as it was with the combination of antibiotics. In other words, the in vivo superiority of the antimicrobial combination was not expressed as an anti-inflammatory advantage, presumably because the bacterial load after therapy with a single drug was below the suppurative level.

These observations of the rat model system show that sterilization of obstructive pyelonephritis is best achieved with a combination of antibiotics that is synergistic in vitro. Many strains of *Pseudomonas* [19] and *Proteus mirabilis* [24] are susceptible to synergistic inhibition by penicillin and aminoglycoside antibiotics. Using the technique described for testing *E. coli* O6 (Williams), we found that 11 of 12 strains of *E. coli* isolated from the urine of patients were synergistically killed by ampicillin and gentamicin. This synergism could be of importance in the treatment of patients with obstructive pyelonephritis in whom prevention of relapse depends upon sterility.

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