

EFFECT OF CEPHALOTHIN ON GROWTH PATTERNS OF
MICRO-ORGANISMS

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Isolates of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* were incubated in the presence of inhibitory concentrations of cephalothin. After destruction of the antibiotic, there was a lag phase before *S. aureus* began to proliferate again. When similar experiments were conducted with *E. coli*, *K. pneumoniae*, and *P. mirabilis*, no lag phase was observed. This data suggests that the inhibitory activity of cephalosporins may be different for gram-positive cocci and gram-negative bacilli.

Early experiments studied the effects of exposure to penicillin G on the subsequent growth characteristics of gram-positive cocci^{2,3}. Organisms were exposed to penicillin G for several hours and then the drug was destroyed by penicillinase. Subsequently, there was a several-hour delay before the remaining organisms began to proliferate again. Similar experiments have been conducted recently with isolates of *Pseudomonas aeruginosa* exposed to carbenicillin⁴. Unlike the experience with gram-positive cocci, *P. aeruginosa* begins to proliferate very quickly after carbenicillin is destroyed by penicillinase. This difference in recovery rate between gram-positive cocci and gram-negative bacilli led us to investigate the effects of cephalothin on the growth of organisms *in vitro*. Our studies indicate that gram-negative bacilli recover more rapidly after exposure to cephalothin than gram-positive cocci.

Materials and Methods

Two isolates each of *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli* and *Proteus mirabilis* were used in these experiments. All of the isolates were cultured from blood specimens obtained from infected patients at this institution. The minimum inhibitory concentration (MIC) of cephalothin required for each organism was determined by the serial tube dilution technique⁵. The following procedure was performed with each of the 6 isolates. The organisms were incubated in MUELLER-HINTON broth at 37°C for 18 hours and a 2-ml aliquot of a 10⁻⁸ dilution was inoculated into 3 flasks containing a total volume of 20 ml MUELLER-HINTON broth per flask. One flask served as a control and cephalothin was added to the other 2 flasks so that the final concentration equalled 10 times the MIC for that isolate. After inoculation, all 3 flasks were incubated at 37°C for 6 hours. A 0.1-ml aliquot was removed from each flask at 0, 60, 90, 120, 140, 160, 180, 240, and 300 minutes and the number of colony-forming units (CFU) was determined in duplicate by plating these aliquots on Trypticase Soy agar. With the isolates of *P. mirabilis*, aliquots were removed at 120, 150 and 180 minutes rather than 120, 140, 160 and 180 minutes to simplify the procedure. β -Lactamase was prepared from a strain of *Bacillus cereus* and was added to one of the flasks containing cephalothin. Previous experiments determined the amount of β -lactamase necessary to destroy all of the cephalothin using a microbiological method. This was accomplished by ascertaining that amount of β -lactamase which resulted

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in maximum rate of growth of the organisms after exposure to cephalothin. The duration of exposure to β -lactamase was 15 minutes. β -Lactamase alone did not inhibit the growth of these organisms.

Results

The MICs for the isolates used in these experiments were: *S. aureus* 0.2, 0.2 $\mu\text{g/ml}$; *E. coli* 0.78, 1.56 $\mu\text{g/ml}$; *K. pneumoniae* 0.78, 1.56 $\mu\text{g/ml}$; and *P. mirabilis* 0.78, 3.12 $\mu\text{g/ml}$. During the 6-hour period, the concentration of organisms in the control flasks increased by 3~5 logs (Figs. 1~3). In the tubes containing 10 times the MIC, there was a progressive reduction in the concentration of organisms. Cephalothin was more active against *P. mirabilis* than the other organisms and at the end of the 6-hour period no viable organisms could be detected. After β -lactamase was added to destroy the cephalothin, one isolate of *S. aureus* failed to regain its ability to proliferate and the concentration of organisms remained stable during the remaining 210-minute period (Fig. 2). The other isolate of *S. aureus* recovered exponential growth only after a considerable delay (Fig. 1). The gram-negative bacilli recovered more quickly after β -lactamase was added and 3 isolates had recovered already when the next sample was obtained (Figs. 1~3). At the end of the 6-hour period, the concentrations of both isolates of *K. pneumoniae* and both isolates of *E. coli* in these latter flasks were within 1 log of the concentrations in the control flasks. Since cephalothin had a greater cidal activity against the isolates of *P. mirabilis* during the first 90-minute period, these organisms did not reach the concentration of organisms in the control flasks although their growth curves paralleled the growth curves of the controls.

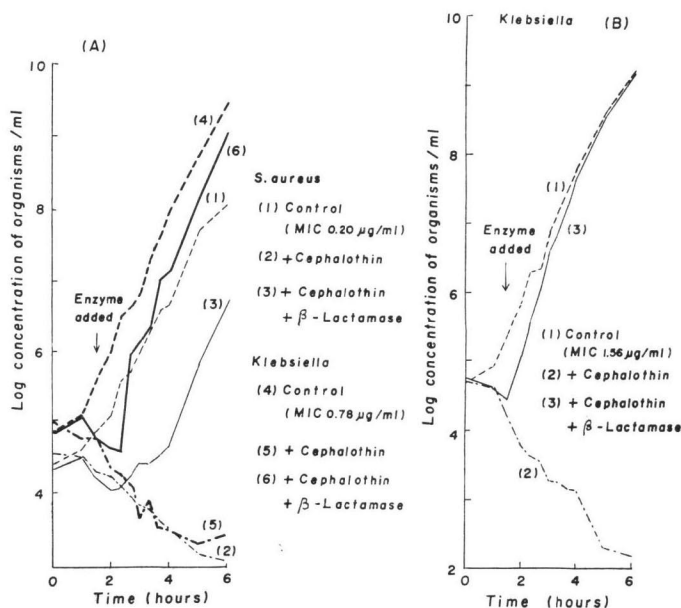
Discussion

This study indicates that the pattern of recovery after exposure to cephalothin is different for gram-positive cocci than for gram-negative bacilli. The results with gram-positive cocci are similar to those obtained by others with penicillin G^{2,5}. Likewise, the results with gram-negative bacilli are similar to those obtained with carbenicillin against *P. aeruginosa*.

These *in vitro* results may have clinical applications. It has been suggested that the delayed recovery of gram-positive cocci after exposure to penicillin G might explain its efficacy when administered at infrequent intervals for the treatment of pneumococcal pneumonia⁷. However, ROLINSON demonstrated that carbenicillin failed to eradicate experimental *Pseudomonas* infection when

Fig. 1. Growth characteristics of 2 isolates of *Klebsiella pneumoniae* exposed to cephalothin.

Fig. 1 (A) includes an isolate of *Staphylococcus aureus* for comparison.



the drug was administered by an intermittent schedule which resulted in intervals when the serum concentration was inadequate³⁾.

Schedule of antibiotic administration may be of critical importance in the treatment of gram-negative bacillary infections, especially in neutropenic patients. Since these patients lack host defenses to combat infection, recovery depends upon effective antibiotic activity. The ability of gram-negative bacilli to recover rapidly after exposure to these antibiotics may prevent the cure of these infections unless adequate serum concentrations are maintained continuously. Carbenicillin has been very effective in curing *Pseudomonas* infections in neutro-

penic patients, but the customary schedule of administration maintains adequate serum concentrations against most isolates of *P. aeruginosa*¹⁾. The cephalosporins have been considerably less effective in neutropenic patients⁴⁾. However, the usual schedule of administration of cephalothin results in periods when the serum concentrations may be inadequate. It is possible that continuous infusions of cephalothin might be more effective in these patients. Clinical studies of this method of drug administration are in progress.

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Fig. 2. Growth characteristics of 2 isolates of *Escherichia coli* exposed to cephalothin.

Fig. 2(A) includes a second isolate of *Staphylococcus aureus* for comparison.

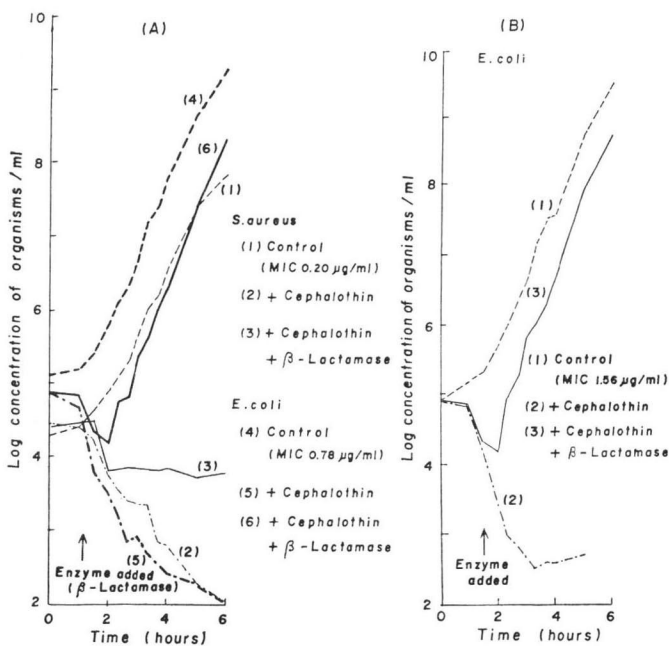
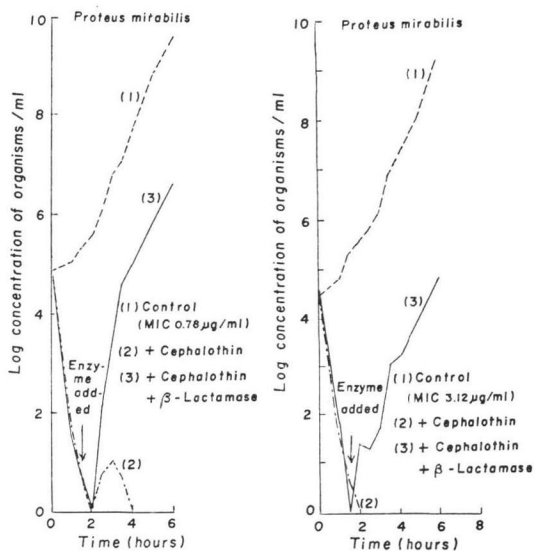


Fig. 3. Growth characteristics of 2 isolates of *Proteus mirabilis* exposed to cephalothin.



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