BACTERICIDAL ACTIVITY OF CEFADROXIL, CEPHALEXIN, AND CEPHRADINE IN AN *IN VITRO* PHARMACOKINETIC MODEL

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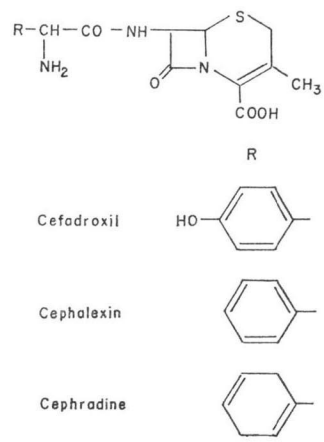
Cefadroxil (Duricef, Mead Johnson & Company), resembles cephalexin and cephradine in spectrum of antibacterial activity but differs in human pharmacokinetic properties. Whether the latter are likely to affect activity *in vivo* was assessed by determining bactericidal activity against clinical isolates under conditions simulating the variation of drug concentration in the blood stream after an oral dose of 500 mg to adults. In this kinetic model, cefadroxil was more active than cephalexin or cephradine against *Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae, Proteus mirabilis, Haemophilus influenzae* and one of two strains of *Escherichia coli*. The other strain of *E. coli* was virtually unaffected by the cephalosporins. *S. pyogenes* was equally susceptible to all three cephalosporins. Analysis of the results suggests that the pharmacokinetic properties of an antibiotic affect its activity in the blood stream, provided the susceptibility of the infecting organism is concentration-dependent within the range of drug concentrations occurring in serum.

Cefadroxil is a cephalosporin similar to cephalexin and cephradine in structure (Fig. 1) and spectrum of antibacterial activity[1,2], but different in human pharmacokinetic properties[2~4]. Given orally to adults, cefadroxil reaches the peak serum concentration later and has a longer half-life than cephalexin or cephradine.

Fig. 1. Structures of cefadroxil, cephalexin and cephradine.



Standard assays of antibiotic activity do not take into account the putative effect of pharmacokinetic parameters on activity. NISHIDA *et al.*[5] devised an assay procedure for bactericidal activity in which the concentration of antibiotic is modified periodically—by addition of a concentrated solution of drug or dilution with unmedicated medium—in order to simulate the variation of antibiotic concentration in the blood stream. We applied this *in vitro* pharmacokinetic model to a comparison of cefadroxil with cephalexin and cephradine.*

* A partial report on these studies was presented at the 10th International Congress of Chemotherapy, Zürich, Switzerland, 18~23, September 1977, and appeared, in the form of an extended abstract, in W. SIEGENTHALER and R. LÜTHY (*ed.*), Current Chemotherapy, Proceedings of the 10th International Congress of Chemotherapy, American Society for Microbiology, Washington, D. C., 1978, Vol. II, pp. 746~748.

Materials and Methods

Cephalosporins

Cefadroxil trihydrate was obtained from Laboratoires Allard (Paris), cefadroxil monohydrate from Bristol Italiana (Sud) S.p.A.; cephalexin monohydrate was a product of Eli Lilly & Company, and cephradine monohydrate of E. R. Squibb & Sons.

Bacteria

The bacterial strains were of clinical origin and typical for the respective species in their susceptibility to the cephalosporins as determined by standard assay procedures for growth inhibitory and bactericidal activity (Table 1).

Table 1. Susceptibility of selected bacterial strains to cefadroxil, cephalexin, and cephradine under standard assay conditions.

Organism		Minimum inhibitory concentration (μg/ml)			Minimum bactericidal concentration (μg/ml)		
		Cefadroxil	Cephalexin	Cephradine	Cefadroxil	Cephalexin	Cephradine
Staphylococcus aureus	A 20395	4	8	8	–	–	–
	A 20405	4	8	8	32	63	63
Streptococcus pneumoniae	A 21851	2	4	2	2	4	4
	A 9585	2	2	2	2	4	4
Streptococcus pyogenes	A 9604	0.25	0.5	0.5	8	16	8
	A 20203	0.25	0.5	0.5	4	32	8
Escherichia coli	A 15119	16	8	16	16	8	16
	A 20108	8	16	16	63	32	63
Klebsiella pneumoniae	A 15130	16	16	16	16	16	16
	A 9662	16	16	16	16	16	16
Proteus mirabilis	A 20046	16	16	16	32	16	32
	A 20121	16	16	16	16	32	32
Haemophilus influenzae	A 21685	32	8	32	63	32	32
	A 21523	32	8	16	63	32	32

Growth inhibitory and bactericidal activity was assayed in MUELLER-HINTON broth, supplemented with 4% defibrinated sheep blood for streptococci and 5% inactivated human serum plus 1% IsoVitaleX for *Haemophilus*. Activity was determined after 21 hours of incubation at 37°C. Initial cell density ranged from $1 \sim 8 \times 10^5$ colony-forming units/ml. The antibiotic concentration inducing a 99.9% loss of viability was defined as minimum bactericidal concentration.

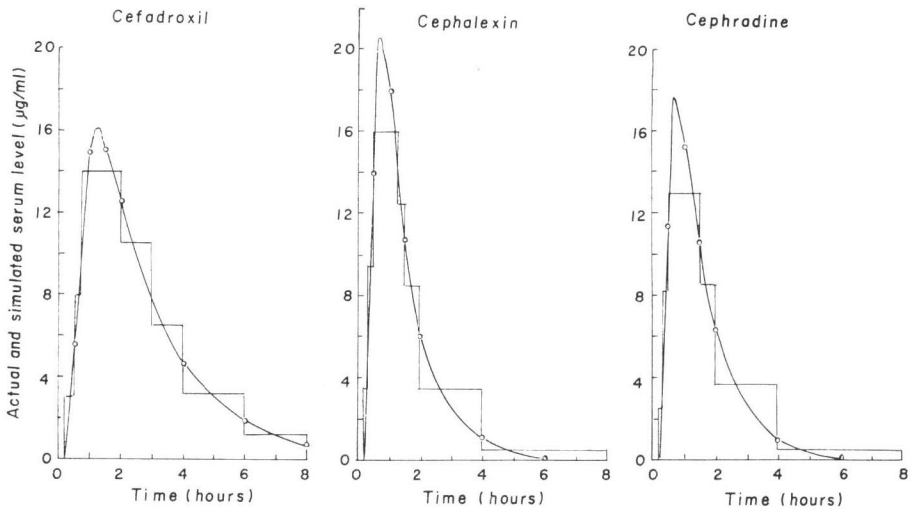
Medium

Experiments were performed in 95% pooled human serum with a 5% supplement specific for the requirements of the individual organism. For most strains the supplement, MUELLER-HINTON broth (Difco), was at normal strength, but for *Proteus mirabilis* and *Haemophilus influenzae* it was at 10-fold normal strength. The medium for *Haemophilus* contained, in addition, 0.5% IsoVitalex (BBL). For *Proteus* and *Haemophilus* the serum had to be inactivated beforehand by heating at 56°C for 30 minutes. The medium was adjusted to pH 7.4 with HCl and sterilized by filtration with Nalgene filters of 0.45 μm pore size.

Bactericidal activity by the kinetic model

Stock cultures were grown for 16~20 hours at 37°C in the appropriate medium. The inoculum was obtained by diluting the culture with the same medium to a cell density of approximately 5×10^5 colony-forming units (CFU) per ml. Aliquots of 9.7 ml inoculum were transferred to test tubes (25 × 200 mm), one for a growth control and one for each cephalosporin to be tested. The tubes were placed in a water bath at 37°C and 10 minutes later 0.1 ml of medium containing 100-fold the desired initial concentration of cephalosporin was added. Drug concentration was adjusted periodically by adding

Fig. 2. Actual and simulated serum levels of cefadroxil, cephalexin, and cephradine after oral administration of a 500 mg dose to humans. (Data according to study HL-75-47 by PFEFFER *et al.*[4]).



antibiotic-containing or unmedicated medium, according to the pattern of a simulated blood level curve determined by drawing a step-wise graph based on actual average serum levels in humans after an oral dose of 500 mg (Fig. 2). Areas under the simulated and actual serum level curves were about the same. The procedure for adjusting drug concentrations is exemplified in Table 2 by the protocol for cefadroxil. The control culture was not diluted and its growth was therefore limited by medium, whereas the antibiotic-containing cultures were able to grow continuously once drug concentration was below inhibitory levels.

At intervals (0, 1.5, 2.5, 3.5, 4.5, 6 and 8 hours) samples were removed, diluted in saline, and plated for viable cell count determination. Cell densities, adjusted for the intermittent dilutions of the culture, were plotted as a function of time.

Table 2. Schedule of concentration adjustments for cefadroxil in the kinetic model

Time	Change in volume (ml)	Added antibiotic (μg)	Volume (ml)	Antibiotic concentration (μg/ml)
0			9.7	0
10 min.	0.1	30	9.8	3
30	0.1	50	9.9	8
45	0.1	60	10	14
1.5hrs.	−4		6	14
2	2	0	8	10.5
2.5	−1.8		6.2	10.5
3	3.8	0	10	6.5
3.5	−2		8	6.5
4	8.2	0	16.2	3.2
4.5	−1.2		15	3.2
6	−5		10	3.2
6	16.7	0	26.7	1.2
6	−15		11.7	1.2
8	−5		6.7	1.2

Bactericidal activity at fixed antibiotic concentrations

Conventional kill curves were obtained with three of the organisms. Conditions of these tests were identical with those of the kinetic model, except that antibiotic concentration was kept constant throughout the test.

Stability in serum

Stability of the cephalosporins at 37°C in test medium was determined in the absence of organisms over a period of 24 hours. The initial cephalosporin concentration was 50μg/ml. Residual antibiotic activity was measured periodically by an agar diffusion technique with *Bacillus subtilis* ATCC 6633 as assay organism. Since the three cephalosporins were relatively stable under these conditions ($t_{1/2}$ of

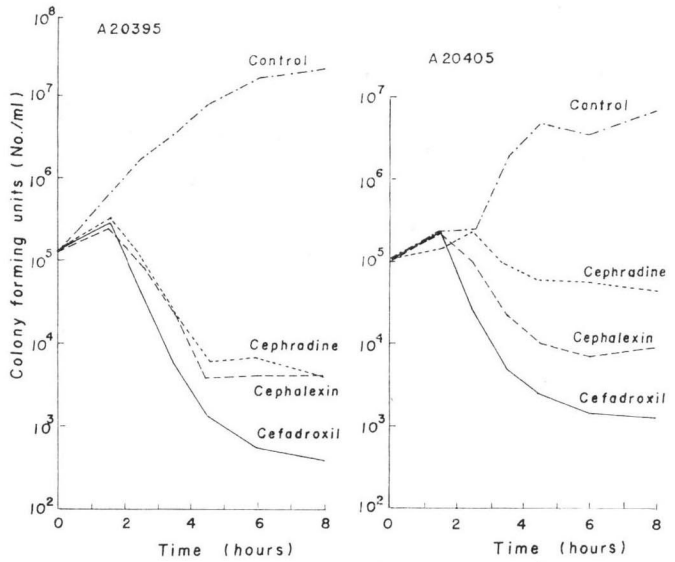
23 hours for cefadroxil and >24 hours for cephalexin and cephradine) corrections for loss of activity during bactericidal activity determinations were not deemed necessary.

Results and Discussion

Bactericidal activity by the kinetic model was determined for two strains each of the following bacterial species: *Staphylococcus aureus*, *Streptococcus pneumoniae*, *S. pyogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *P. mirabilis*, and *H. influenzae*. The data presented are mean values of two or three experiments.

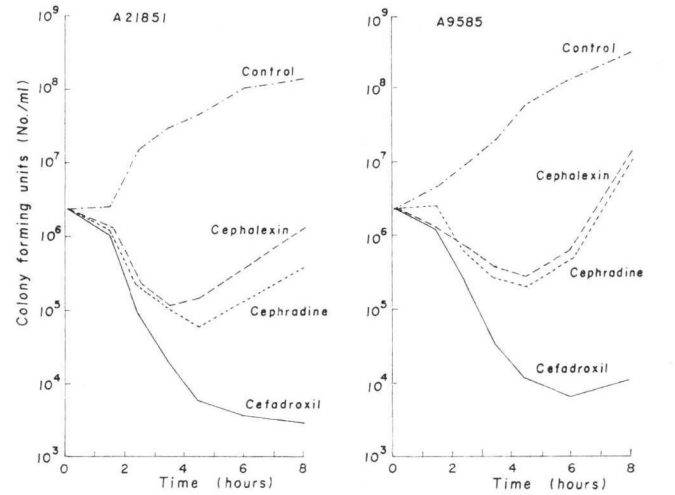
Against two strains of *S. aureus*, A20395 which lacks β-lactamase and A20405, a β-lactamase producer, cefadroxil was the most active of the three cephalosporins (Fig. 3). Indeed, the number of CFU after 8 hours of incubation was 10~40 times lower in the presence of cefadroxil than of either cephalexin or cephradine.

Fig. 3. Bactericidal activity of cefadroxil, cephalexin, and cephradine against two strains of *Staphylococcus aureus* determined by the kinetic model.



Even more pronounced differences in activity occurred against *S. pneumoniae* (Fig. 4). Whereas with cephalexin and cephradine a decline, during the first 4.5 hours of incubation, was followed by a marked increase in the number of CFU, with cefadroxil the decline continued for 6 hours, and no significant regrowth occurred during the remainder of the experiment. At the end of 8 hours, there were 10^2~10^3 times fewer CFU in the cultures containing cefadroxil than in those containing cephalexin or cephradine.

Fig. 4. Bactericidal activity of cefadroxil, cephalexin, and cephradine against two strains of *Streptococcus pneumoniae* determined by the kinetic model.



S. pyogenes, in contrast to *S. pneumoniae*, was equally susceptible to all three cephalosporins (Fig. 5). The num-

ber of CFU declined slowly but steadily to about one-tenth of the initial value by the end of the experiment.

With all Gram-positive organisms, different strains of the same species gave similar sets of kill curves, a pattern that did not extend to Gram-negative organisms. The two strains of *E. coli* reacted differently to antibiotic treatment (Fig. 6). Cephalexin and cephradine prevented net growth of strain A15119 for 4.5 hours after which normal growth resumed. Cefadroxil was more effective, decreasing the number of CFU by 10-fold prior to resumption of normal growth after 6 hours. Conversely, cultures of strain A-20108 were virtually unaffected by the cephalosporins, although cefadroxil caused a brief delay of growth early in the experiment.

The two strains of *K. pneumoniae* also differed in their reaction to treatment with antibiotic (Fig. 7). Cefadroxil reduced the number of CFU of strain A15130 by nearly 99% before growth resumed after 6 hours of incubation. Cephalexin induced a lesser loss of viability with normal growth resuming after 4.5 hours. Cephradine caused no net loss in the number of viable cells, and full growth resumed after only 3.5 hours

Fig. 5. Bactericidal activity of cefadroxil, cephalexin, and cephradine against two strains of *Streptococcus pyogenes* determined by the kinetic model.

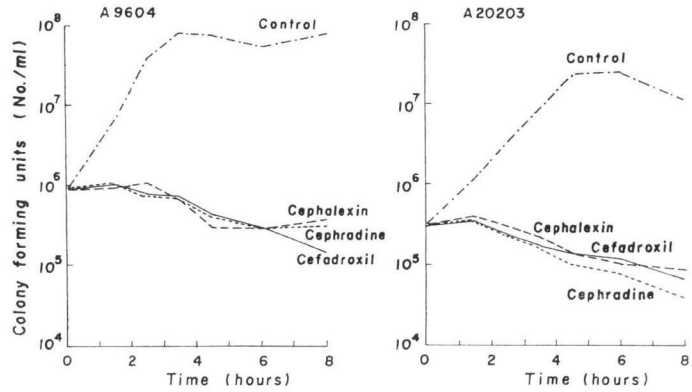


Fig. 6. Bactericidal activity of cefadroxil, cephalexin, and cephradine against two strains of *Escherichia coli* determined by the kinetic model.

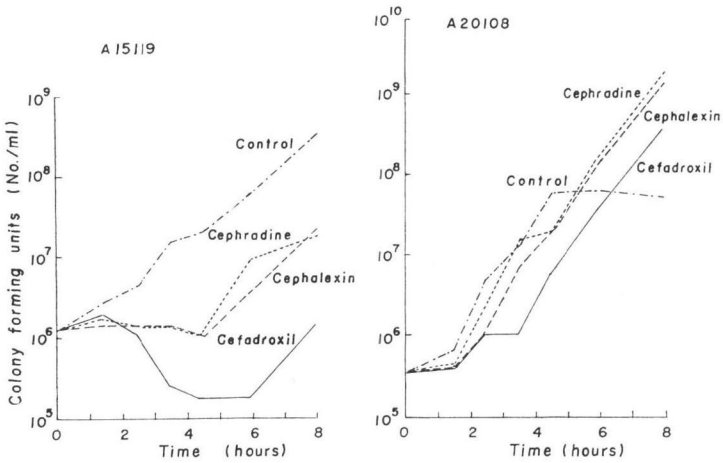
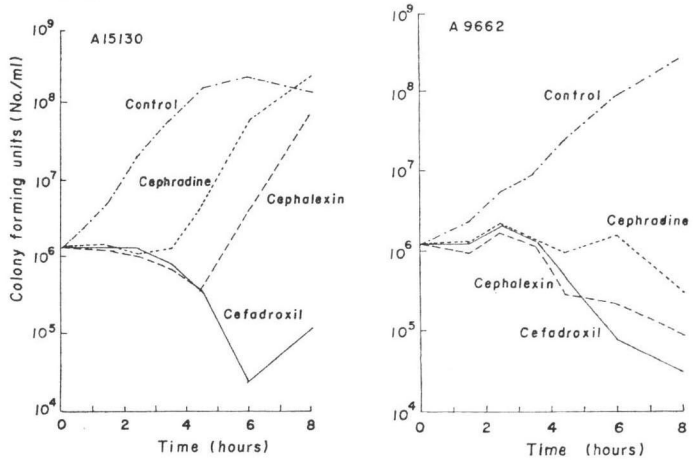


Fig. 7. Bactericidal activity of cefadroxil, cephalexin, and cephradine against two strains of *Klebsiella pneumoniae* determined by the kinetic model.



of incubation. With strain A9662, on the other hand, the number of CFU in all medicated media declined throughout the experiment. The decline was minimal with cephradine but more pronounced with cefadroxil and cephalexin.

After a generally sharp but brief drug-induced decline in viability, cultures of *P. mirabilis* resumed growth (Fig. 8). Resumption of normal growth was rapid with cephalexin and cephradine and gradual with cefadroxil. The growth pattern of *H. influenzae* was similar (Fig. 9), except that the decline in the number of CFU was more prolonged and resumption of full growth slower than with *P. mirabilis*.

A shortcoming of the kinetic model in its current form is the fact that reducing antibiotic concentration requires dilution of the culture and it has not been established that dilution is without effect on activity. GRASSO *et al.*[6] recently investigated the bactericidal activity of cephalosporins by analog computer. This procedure substitutes discreet, quasi continuous modifications of antibiotic concentration for the intermittent, abrupt changes of NISHIDA's model but, ipso facto, does not avoid dilution. An attempt we made to replace dilution by filtration with subsequent suspension in properly medicated medium was not successful, since recovery of cells, particularly at lower cell densities, was not altogether reliable. Although this technique has its own shortcomings, a comparison of the two procedures would have at least offered some indication whether dilution affects bactericidal activity. However, in this study, overall dilutions of the various medicated cultures differ by less than 3-fold; it is therefore reasonable to assume that if a dilution-effect does indeed occur, it should affect all cultures to a similar extent.

Not surprisingly, bactericidal activity under standard conditions (Table 1) was not predictive of activity by the kinetic model. Since the assay procedures differed in more than one respect—fixed

Fig. 8. Bactericidal activity of cefadroxil, cephalexin, and cephradine against two strains of *Proteus mirabilis* determined by the kinetic model.

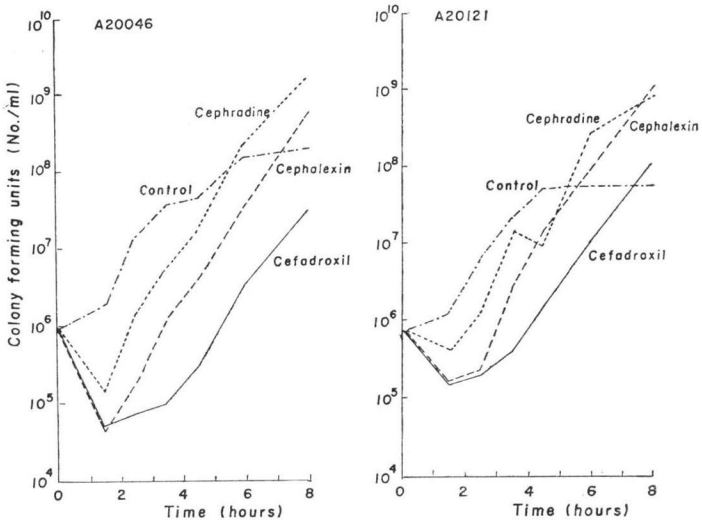
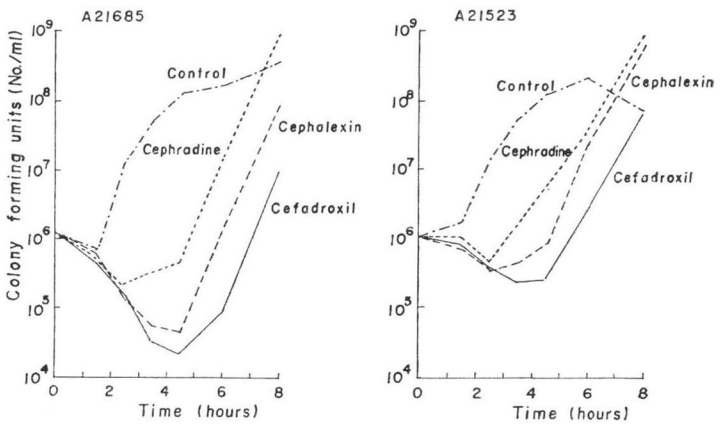


Fig. 9. Bactericidal activity of cefadroxil, cephalexin, and cephradine against two strains of *Haemophilus influenzae* determined by the kinetic model.



versus variable drug concentration, broth medium versus serum, and 21-hour sampling versus up to 8-hour sampling—the two sets of results give no clear indication of the effect incorporation of pharmacokinetic parameters into the experimental design has on bactericidal activity. To elucidate this question, kill curves for three selected organisms were determined using fixed antibiotic concentrations under conditions otherwise identical with those of the kinetic model. Under the assay conditions of the kinetic model, cefadroxil was considerably more active than cephalexin and cephradine against two of the organisms, *S. pneumoniae* A21851 (Fig. 4) and *K. pneumoniae* A15130 (Fig. 7), whereas against the third, *S. pyogenes* A9604, the three cephalosporins were equally active (Fig. 5).

Fig. 10. Bactericidal activity of cefadroxil, cephalexin and cephradine against *Streptococcus pneumoniae* A21851 at fixed antibiotic concentrations.

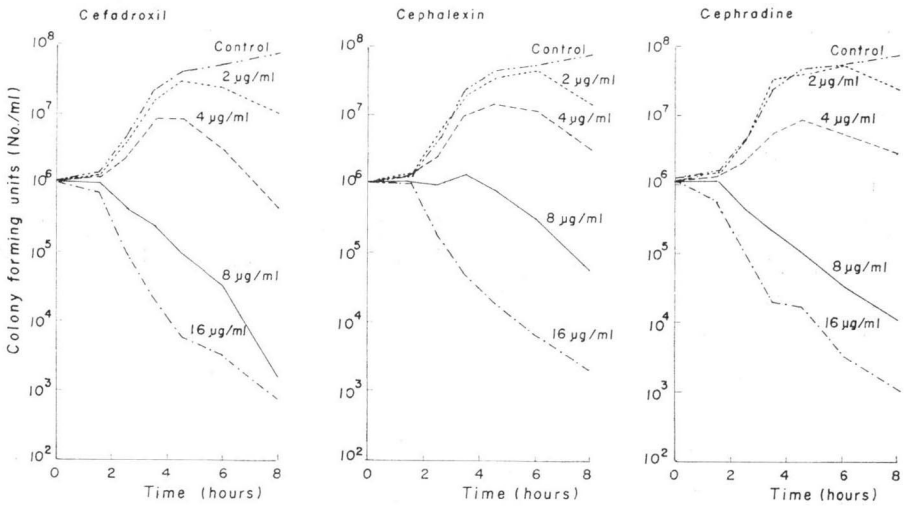


Fig. 11. Bactericidal activity of cefadroxil, cephalexin and cephradine against *Klebsiella pneumoniae* A15130 at fixed antibiotic concentrations.

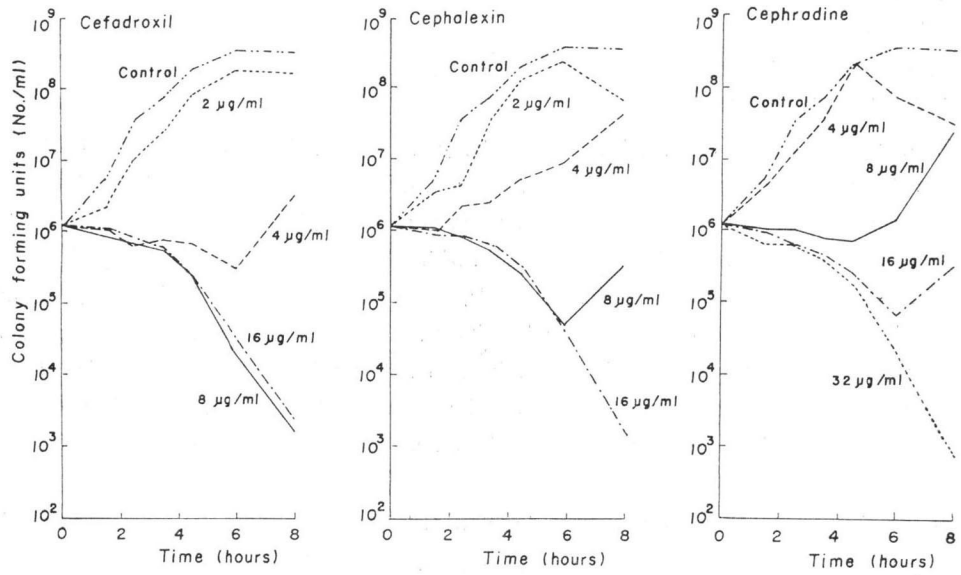
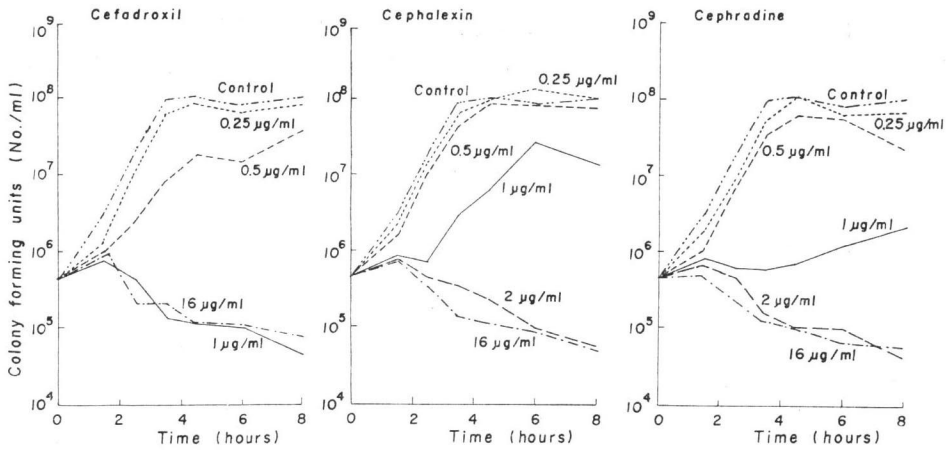


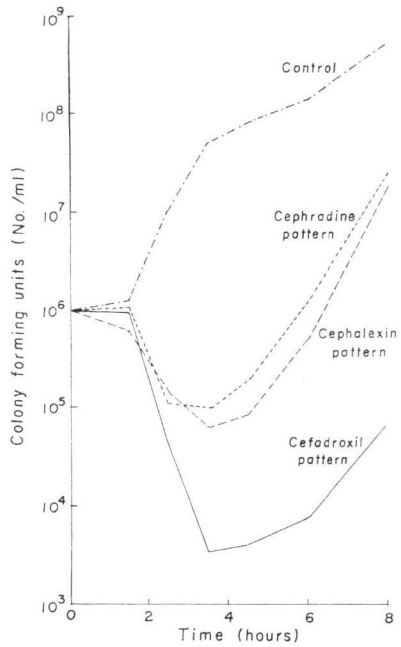
Fig. 12. Bactericidal activity of cefadroxil, cephalexin, and cephradine against *Streptococcus pyogenes* A9604 at fixed antibiotic concentrations. (For the sake of clarity, kill curves obtained with some intermediate antibiotic concentrations have been omitted).



At fixed concentrations in serum the cephalosporins did not differ in bactericidal activity against *S. pneumoniae* A21851 (Fig. 10). Within a range of concentrations coinciding with that used in the kinetic model, activity varied with concentration. Loss of viability was rapid at 16 μg of cephalosporin per ml, somewhat slower at 8 μg/ml; bactericidal activity was much reduced at 4 μg/ml and nearly absent at 2 μg/ml. The greater activity of cefadroxil by the kinetic model can therefore be attributed to the sharp dependence of bactericidal activity on cephalosporin concentration. Results were similar with *K. pneumoniae* A15130 except that cephradine was only about half as active as cefadroxil (Fig. 11). Against this organism, the greater effectiveness of cefadroxil by the kinetic model can also be largely ascribed to dependence of activity on antibiotic concentration. By contrast, maximum bactericidal activity against *S. pyogenes* A9604 was attained at low antibiotic concentration, *i.e.*, 1 μg/ml of cefadroxil and 2 μg/ml of cephalexin and cephradine (Fig. 12); consequently, above these levels, a higher concentration confers no further advantage. This fact accounts for a lack of differentiation between the cephalosporins by the kinetic model.

In view of the similar intrinsic bactericidal activities of the three cephalosporins against *S. pneumoniae* A21851 any one of these compounds would be expected to mimic the activity of another if the proper kinetic pattern is applied.

Fig. 13. Bactericidal activity of cephradine against *Streptococcus pneumoniae* A21851 at concentrations varied according to the kinetic pattern of cefadroxil, cephalexin, or cephradine.



As illustrated in Fig. 13 with cephradine, the activity of this compound was indeed markedly increased when concentration was varied according to the kinetic pattern of cefadroxil, but no significant change in activity occurred when the kinetic pattern of cephalexin was followed.

Results of this study suggest that in the blood stream, isoactive antibiotics with divergent pharmacokinetic properties may differ in efficacy, if the susceptibility of the infecting organism is concentration-dependent within the range of drug concentrations occurring in serum.

Acknowledgments

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