CEPHAMYCIN DERIVATIVES: COMPARISON OF THE *IN VITRO* AND *IN VIVO* ANTIBACTERIAL ACTIVITIES OF SQ 14,359, CS-1170, AND CEFOXITIN

HANS H. GADEBUSCH, R. SCHWIND, P. LUKASZOW, R. WHITNEY and R. J. MCRIPLEY

The Squibb Institute for Medical Research, Princeton, N.J. 08540, U.S.A.

(Received for publication July 7, 1978)

SQ 14,359 is a new cephamycin-type (7α-OCH_3) antibiotic belonging to a series containing a 7α-ureidoacetyl substituent. The compound is the most potent extended spectrum derivative of this type yet reported, surpassing CS-1170 and cefoxitin by a wide margin. This activity *in vitro* which extends throughout the Enterobacteriaceae is particularly prominent against Gram-negative organisms that are producers of "cephalosporinase-type" β-lactamases such as *Enterobacter, Serratia, Citrobacter* and indole-positive *Proteus* species. Superior activity also is demonstrated *in vitro* against streptococci, β-lactamase-producing staphylococci, *Haemophilus influenzae, Neisseria gonorrhoeae*, and many Gram-negative pathogens resistant to aminoglycoside antibiotics. Experimental chemotherapeutic studies have confirmed these observations in wound and selected systemic infections in mice as well as acute pyelonephritis and meningitis in rats. The pharmacokinetics for each drug including antibiotic bound to serum was similar in both mice and rats. The pharmacokinetic profile in blood and cerebrospinal fluid favored SQ 14,359.

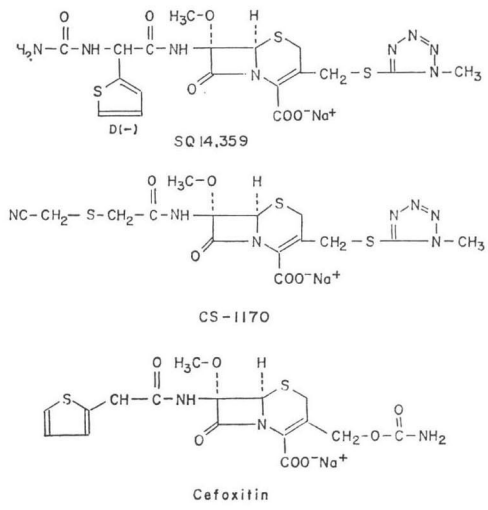
The synthesis of SQ 14,359, a 7β-thienylureido-acetyl-7α-methoxy cephalosporin derivative bearing a methyl tetrazolyl-thiomethyl substituent in the 3-position, recently has been reported[1]. Based on preliminary studies, the compound appears to be one of the most potent cephalosporins yet identified[2]. It is the purpose of this report to compare the antibiotic activity *in vitro* and *in vivo*, as well as the pharmacokinetics of this antibiotic (Fig. 1) with that of two other 7α-OCH_3 cephalosporins, CS-1170[3] and cefoxitin[4~6].

Materials and Methods

Antibiotics.

Standard laboratory powders of SQ 14,359; CS-1170; and cefoxitin were kindly provided by E. R. Squibb and Sons, Princeton, N.J.; Sankyo Company, Tokyo, Japan; and Merck Sharp and Dohme, Rahway, N.J., respectively. All of the batches used were at least 90% pure and all antimicrobial data were corrected for purity of the sample as stated by the suppliers. Samples of aminoglycosides representative of marketed material were obtained through the courtesy of Bristol Laboratories, Syracuse, N.Y. (amikacin, kanamycin), Meiji Seika Kaisha, Ltd., Tokyo, Japan (dibekacin), Schering Corporation, Bloomfield, N. J. (gentamicin, netilmicin, sisomicin), Eli Lilly Co., Indianapolis, Ind. (tobramycin), and E. R. Squibb and Sons, New Brunswick, N. J. (neomycin, streptomycin). Purity varied

Fig. 1. Structure of SQ 14,359, CS-1170, and cefoxitin.



from *ca.* 60% to 90%.

Bacterial cultures.

All test organisms were recent clinical isolates obtained from human sources. *Bacteroides fragilis* subspecies were received from D. LAMBE (Emory Univ., Atlanta, Ga.) and T. WILKINS (Virginia Polytechnic Institute, Blacksburg, Va.). Aminoglycoside-resistant cultures were obtained through the courtesy of D. SCHABERG (Center for Disease Control, Atlanta, Ga.), M. SANDE (Univ. of Virginia, Charlottesville, Va.), C. M. NOLAN (Univ. of Arkansas, Little Rock, Ark.), and M. RICHMOND (Univ. of Bristol, Bristol, England). *Escherichia coli* W3110 and *Klebsiella aerogenes* 1082E also were received from M. RICHMOND. All ampicillin-resistant isolates of *Haemophilus influenzae* were received from A. L. HARDING (Children's Hospital, Boston, Mass.).

Animals.

Female, white, Swiss mice of the CF-1 strain (Carworth Farms), average weight 18~20 g, and Holtzman rats (*ca.* 200 g) were used in all systemic and urinary tract efficacy trials. Outbred, albino, Sprague Dawley COBS/CD infant rats of both sexes (Charles River Labs.) at 5 days of age were used for all meningitis trials. All pharmacokinetic experiments were conducted with animals of the same type, weight, age, and sex as for chemotherapeutic studies. Animals were maintained in a temperature (22 ± 1°C)- and humidity (50 ± 2 R.H.)-controlled environment.

Broth-dilution susceptibility tests.

A few colonies of each organism were seeded into Brain-Heart Infusion (BBL) broth (BHI) and after overnight incubation, an amount of diluted culture was added to each antibiotic series in MUELLER-HINTON (MH), BHI, and/or Trypticase Soy (TS) broth to provide an initial number of colony-forming units (CFU) appropriate for the test. Incubation was at 35°C for 18~24 hours. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of antibiotic that suppressed visible growth. The minimum bactericidal concentration (MBC) was determined by subculturing all tubes without visible growth to antibiotic-free agar medium. The subculture endpoints were read after 48 hours at 35°C, and the MBC defined as the lowest concentration of antibiotic that gave no visible growth on subculture.

Agar-dilution susceptibility tests.

MIC's were determined in MH agar that was supplemented with 2% rabbit blood for Group A streptococci and *Streptococcus pneumoniae*, or with 5% "chocolatized" rabbit blood for *Haemophilus* and *Neisseria*. Stock solutions of the test substances were made in 0.05 M phosphate buffer (pH 7.0) to an initial concentration of 1 mg/ml. Further dilutions of each antibiotic were prepared in MH broth (pH 7.4); 1 ml amounts added to 90-mm Petri dishes, mixed with 9.0 ml of MH agar and allowed to harden. Test cultures grown in appropriate broth media for *ca.* 18~24 hours at 35°C were deposited on the solidified surface of the agar using one automated multiple-pronged replicator designed to deposit 0.03 ml of culture containing 10^4 CFU on the agar surface. *Bacteroides* were grown initially in chopped meat glucose broth (Scott) for 18~24 hours at which time inoculum levels had reached *ca.* 10^8 CFU/ml. For the test, antibiotics were diluted in MH agar containing 5% sheep blood. All plates were incubated aerobically at 35°C except those containing blood which were incubated under reduced oxygen tension in Gas Pak Jars (BBL).

Microbiological assays for antibiotic activity.

Both serum and urine samples from mice and adult rats were assayed on the day of collection using a disc diffusion assay[7]. Whole heparinized blood and cerebrospinal fluid (CSF) from neonatal rats was assayed in the same manner except that *ca.* 3.5 mm wells cut into the seeded agar were used to receive the body fluids. *Micrococcus luteus* ATCC 9341 served as the assay organism for SQ 14,359 and CS-1170, whereas *Staphylococcus aureus* MB-2786[5] was used for cefoxitin. Representative samples were also examined for evidence of metabolism using thin-layer chromatography and high voltage electrophoresis.

Preparation of β-lactamases.

Crude sonicates of *Enterobacter cloacae* SC 9965, *K. aerogenes* 1082E, and *E. coli* W-3110 were

prepared by methods described previously[2]. The enzymes from *Serratia marcescens* SC 9782 and *Proteus* species were prepared as described by FARRAR and O'DELL[8]. *Serratia* was induced with cephalothin; *Proteus* species with benzylpenicillin. Activity of β-lactamases was determined spectrophotometrically using the cephalosporin substrate 87/132 as described by O'CALLAGHAN[9]. Enzyme class was verified with the published literature[10] by determining the substrate profile for 5 antibiotics using the iodometric method of PERRET[11].

Enzymatic hydrolysis of cephalosporins.

Susceptibility of the 7α-OCH_3 cephalosporins to hydrolysis by various β-lactamases was determined spectrophotometrically[9] and related to the hydrolysis of cephalothin.

Antibiotic binding to serum protein.

Sera for this study were obtained from normal donors, the serum pool was frozen after collection and kept frozen at -20°C until used. The procedure for determining protein binding by ultra-filtration was identical to one previously described[12].

Chemotherapeutic trials.

Systemic infections: Experimental infections were produced by the intraperitoneal injection into mice of suitably diluted cultures of *S. aureus* SC 10,935 suspended in 5% sterile brewer's yeast and 9 different pathogens (in 7% gastric mucin) from the family Enterobacteriaceae. In each case the challenge dose constituted *ca.* 100 LD_{50} and, under these conditions, all untreated control animals died within 72 hours. Medication was given subcutaneously in divided doses at 1 and 5 hours after infection. At least 3 different doses of the test antibiotics were used; each dose group consisted of 10 mice. All animals were observed for a period of 6 days after which the median effective dose (ED_{50}) from duplicate experiments was calculated by the method of REED and MUENCH[13].

Wound infections: Experimental wound infections due to *S. aureus* SC 2406 (a penicillinase-producing strain) were produced in mice as described[14] and drug treatment administered s.c. at 1 and 5 hours after infection. Fifty percent endpoints based on the number of mice showing 3-$\log_{10}$ reductions in *S. aureus* viable cell counts from wounds at 18 hours as a function of dose were calculated[13].

Meningitis: Bacteremia and meningitis due to *Haemophilus influenzae* (one strain each ampicillin-sensitive and -resistant) was induced as described by WHITNEY *et al.*[15] In brief, infant rats (5-days of age) were inoculated intranasally with the appropriate pathogen by a modification of previously described methods[16,17]. Polyethylene tubing (I.D. 0.001 in.; O.D. 0.024 in.) adapted to fit a blunt-end 25 ga.needle, was carefully introduced *ca.* 3 mm into the nares of each of the unanesthetized rats. An inoculum of 0.015 ml (*ca.* 1.5×10^8 CFU) of the stock suspension of each organism was instilled using a Hamilton microsyringe. All rats were weighed daily for the duration of the experiment. Forty-eight hours after inoculation, 0.01 ml samples of blood were drawn from the incised tail veins using sterile micropipettes and the sample plated on a special agar medium consisting of Proteose Peptone No. 3 agar (Difco) supplemented with 2% hemoglobin solution (1 : 1) and 1% IsoVitalex (BBL) and incubated at 35~37°C for 18~24 hours. Rats that were bacteremic ($>10^4$ CFU/ml) 48 hours after infection and with weight gains of less than 28% (24~72 hours after infection) were selected for therapy. Pups meeting these criteria were randomized among the lactating dams and given single bolus drug doses i.p. once daily for 2 days beginning on post-infection day 3. Fifty percent endpoints based on the number of surviving rats with negative blood culture on day 6 and negative cerebrospinal fluid (CSF) culture on day 7 as a function dose were calculated[13]. Untreated rats under these conditions had in previous experiments demonstrated meningitis (culturally-positive CSF) in 96~98% of animals.

Acute pyelonephritis: An ascending bilateral pyelonephritis was initiated by instillation of *ca.* 5×10^7 CFU of *Enterobacter cloacae* SC 9965 into the urinary bladder of rats *via* a catheter. After 7 days, when infection of the kidneys was well established, as evidenced by the recovery of more than 10^4 CFU of the pathogen per g of tissue from both kidneys in a series of control animals, rats were treated once daily for 5 days with varying doses of the test cephalosporins. The median curative dose (CD_{50}) based on yields of only an occasional organism from both kidneys was calculated from data obtained 3 days after the last drug dose[13].

Pharmacokinetics.

Mice: Groups of 20 mice were given a single 100 mg/kg dose of the test compounds by subcutaneous injection. At specified intervals, groups of 4 mice were bled from the orbital sinus; the serum separated by centrifugation, pooled, and stored at 4°C until assayed. In addition, groups of 6 animals were given 1 ml of water *per os*, followed by an identical dose of the same antibiotics given subcutaneously. All animals were housed in metabolism cages to permit the collection of the total urinary output during the 6-hour period after drug administration.

Infant Rats: Groups of neonatal rats that had been infected with an ampicillin-resistant strain of *H. influenzae* (SC 10,556) and that were judged to be meningitic based on bacteremia ($>10^4$ CFU/ml) and poor weight gains (less than 15%) when evaluated during the 72-hour period after infection (plus positive CSF cultures in more than 80% of a group of control animals) were injected intraperitoneally with a 100 mg/kg dose of each of the test drugs. At intervals thereafter, blood and CSF were collected and assayed for antibiotic content.

Mature Rats: Groups of 3 rats were given a single 25 mg/kg dose of the test compounds by subcutaneous injection. At intervals, the animals were bled, the serum separated, and stored at 4°C until assayed. Six groups of 4 animals were first given *ca.* 2 ml of water *per os* and then 1 hour later 25 mg/kg of test drug. Urine was collected for a period of 6 hours and both serum and urines assayed together.

Results and Discussion

Antibacterial Spectrum

Based on studies *in vitro* with more than 500 disease-producing strains of bacteria, which were tested for susceptibility to SQ 14,359 and two other 7α-OCH_3 cephalosporins, this antibiotic, like CS-1170 and cefoxitin, must be classified as a broad-spectrum agent (Table 1; Figs. 2~6).

In addition to the high resistance shown by SQ 14,359 towards representative β-lactamases (Table 2), a subject that will be discussed in detail later, attention must be directed to the high level of activity shown by the compound against many Enterobacteriaceae that were resistant to gentamicin and other aminoglycosides (Table 3). In most instances SQ 14,359 was substantially more active against these organisms than the other cephems tested. Quantitative differences in susceptibility to 7α-OCH_3 cephalo-

Table 1. Susceptibility of miscellaneous bacterial species to SQ 14,359, CS-1170, and cefoxitin.

Organism	No. of strains	MIC_{90} (μg/ml)*		
		SQ 14,359	CS-1170	Cefoxitin
Gram-positive bacteria				
Actinomyces israelii	1	<0.2	<0.2	12.5
Corynebacterium diphtheriae	1	0.3	0.3	0.4
Clostridium histolyticum	1	<0.2	<0.2	0.2
Erysipelothrix insidiosa	1	0.2	0.2	0.2
Listeria monocytogenes	1	> 50	> 50	> 50
Nocardia asteroides	1	4.7	6.3	12.5
Staphylococcus aureus (methicillin-resistant)	20	6.25	6.25	12.5
Streptococcus faecalis	20	>100	>100	>100
Gram-negative bacteria				
Acinetobacter sp.	21	100	>100	>100
Bacteroides fragilis (various subspecies)	10	1.9	3.1	3.1
Citrobacter sp.	14	0.6	0.6	0.6
Edwardsiella tarda	1	<0.2	0.6	0.3
Hafnia alvei	2	4.8	4.8	4.8
Neisseria gonorrhoeae (penicillin-resistant)	2	0.2	0.8	1.2
Neisseria meningitidis	1	0.1	0.2	0.4
Pasteurella multocida	1	<0.2	0.4	0.4
Proteus inconstans	14	1.6	3.2	6.4
Pseudomonas aeruginosa	21	>100	>100	>100
Serratia liquefaciens	4	18.8	18.8	25

* Lowest concentration inhibiting at least 90% of strains, arithmetic mean if less than 10 strains are involved, or single value.

sporins could not be correlated with the presence of aminoglycoside-inactivating enzymes elaborated by the organisms in question.

Comparison of Susceptibility of Clinical Isolates

A more comprehensive over-view of the antibacterial activity of the compounds was provided by comparing the susceptibility of *ca.* 20 recent clinical isolates, within each of 17 groups that represent clinically important genera of bacteria.

With respect to the Gram-positive bacteria, all 3 antibiotics were about equally effective in inhibiting strains of *Streptococcus pyogenes* and *S. pneumoniae*, while SQ 14,359 and CS-1170 were more active than cefoxitin against benzylpenicillin-sensitive and -resistant (both methicillin-sensitive and resistant) staphylococci (Table 1; Fig. 2).

Except for benzylpenicillin-sensitive *Neisseria gonorrhoeae* (Fig. 3) and various *B. fragilis* subspecies (Table 1) against which all 3 cephems were equally active, SQ 14,359 and CS-1170 were more active than cefoxitin against strains of *E. coli*, *Klebsiella* sp., *Shigella* sp., and *Salmonella* sp. (Fig. 4). SQ 14,359 was clearly the most active compound against both ampicillin-sensitive and -resistant *H. influenzae* (Fig. 3), 2 strains of penicillin-resistant *N. gonorrhoeae*, *Proteus inconstans* (Table 1); *Proteus* (indole-positive and -negative), *Enterobacter* (*aerogenes* and *cloacae*) (Fig. 5), *Serratia marcescens* and *Citrobacter freundii* (Fig. 6). Small numbers of assorted Gram-negative and Gram-positive bacteria, except for *Listeria monocytogenes*, were sensitive to concentrations of all 3 antibiotics at MIC's ranging from <0.2 to 25 μg/ml. None

Fig. 2. Growth inhibitory activity of SQ 14,359, CS-1170, and cefoxitin against various organisms.
The number in parentheses indicates the number of strains.

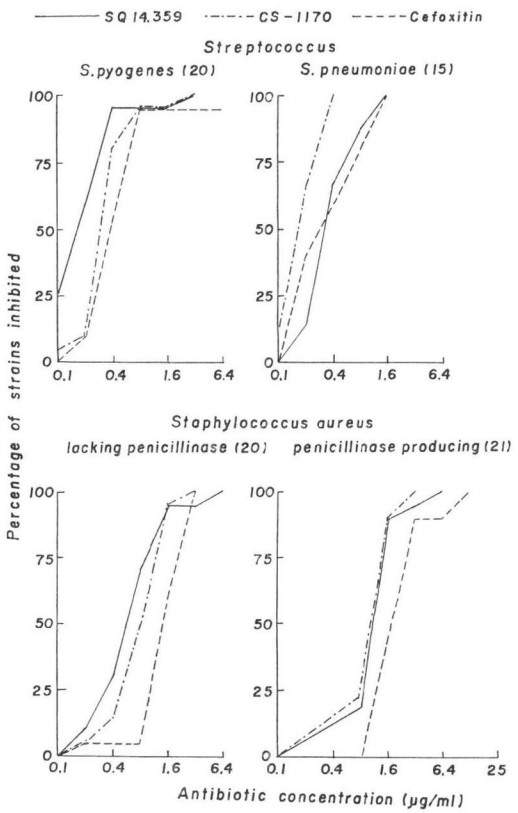


Fig. 3. Growth inhibitory activity of SQ 14,359, CS-1170, and cefoxitin against *Haemophilus influenzae* and *Neisseria gonorrhoeae* isolates.
The number in parentheses indicates the number of strains.

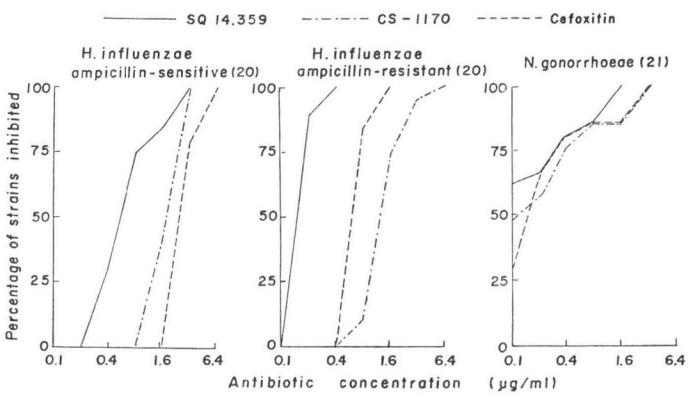


Fig. 4. Growth inhibitory activity of SQ 14,359, CS-1170, and cefoxitin against various Enterobacteriaceae.

The number in parentheses indicates the number of strains.

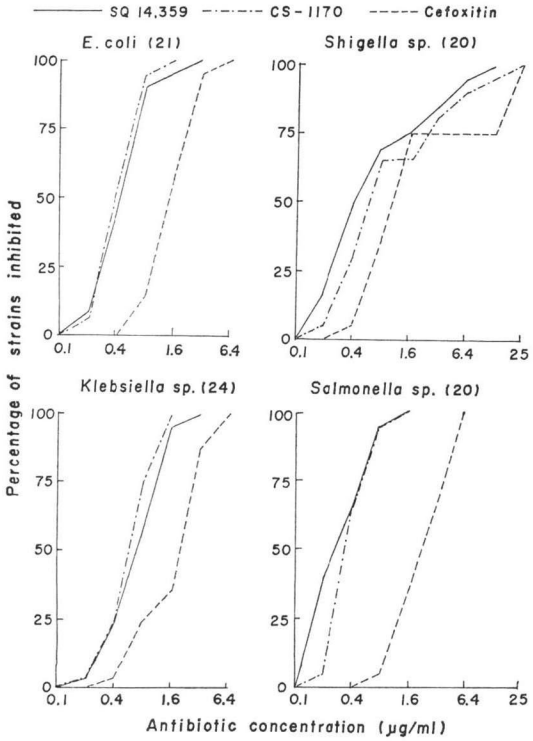
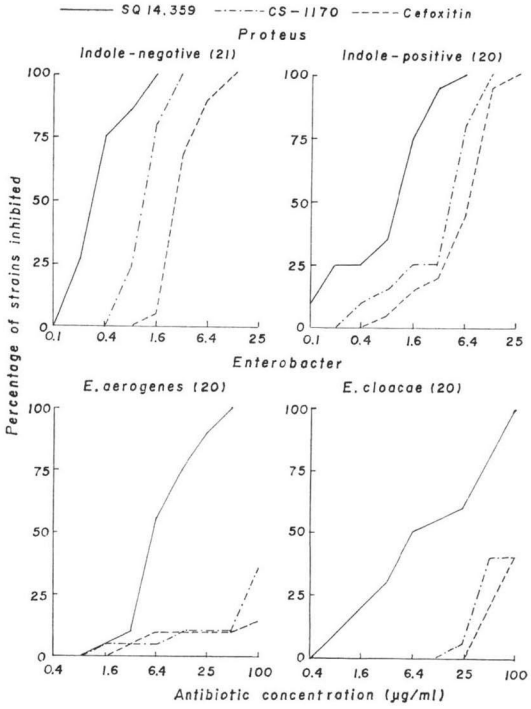


Fig. 5. Growth inhibitory activity of SQ 14,359, CS-1170, and cefoxitin against *Proteus* and *Enterobacter* species.

The number in parentheses indicates the number of strains.



of the compounds tested were active at 100 μg/ml against *Streptococcus faecalis, Pseudomonas aeruginosa*, or *Acinetobacter* sp. (Table 1).

Bactericidal Activity

All 3 cephalosporins, when tested in BHI, MH, and TS broth at inoculum levels of 10^3 and 10^6 CFU/ml, were bactericidal (never more than a 4-fold difference between MIC and MBC) against a panel of 8 sensitive organisms (data not shown). In order to gain more insight into this relationship 4 of the β-lactamase producing organisms shown in Table 2 were grown in the presence and absence of each antibiotic as proposed by GREENWOOD and O'GRADY[18] at inoculum levels even higher than those used earlier. These studies clearly showed (Fig. 7) that SQ 14,359 in each and every instance was quantitatively superior to the other antibiotics in suppressing the growth of the

Fig. 6. Growth inhibitory activity of SQ 14,359, CS-1170, and cefoxitin against strains of *Citrobacter freundii* and *Serratia marcescens*.

The number in parentheses indicates the number of strains.

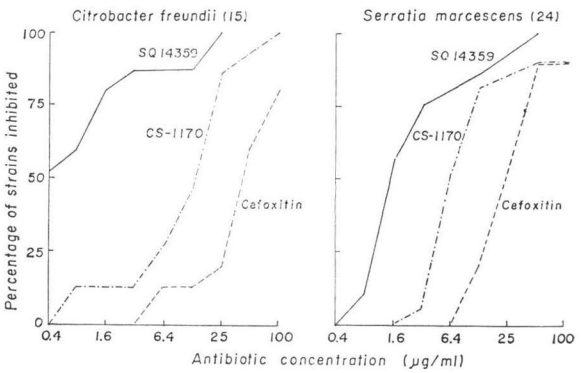
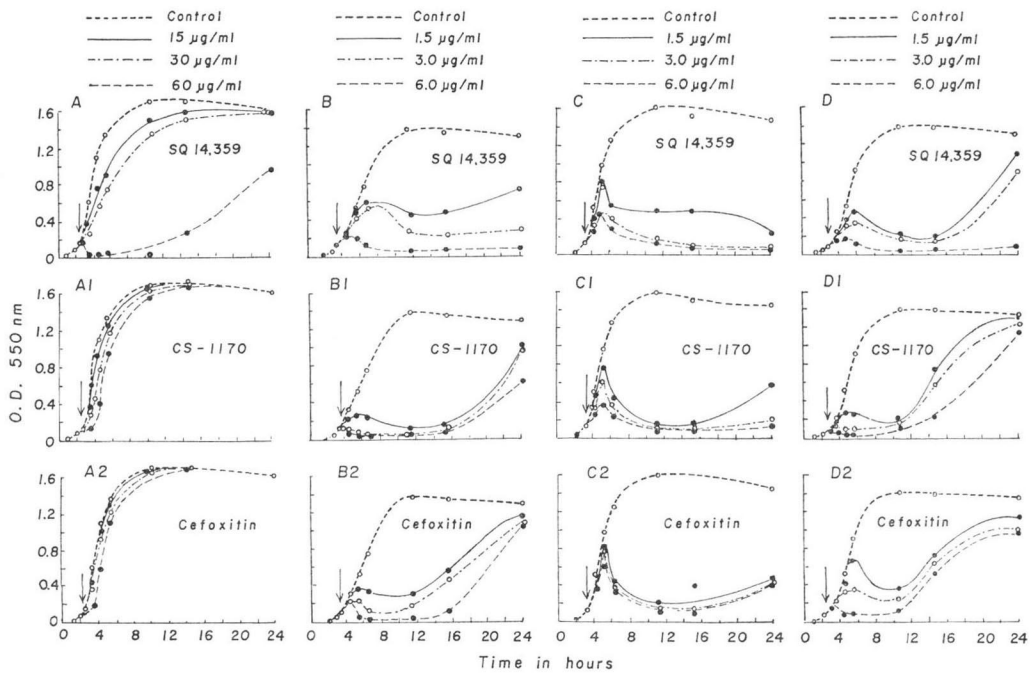


Fig. 7. Effect of varying concentrations of SQ 14,359, CS-1170, and cefoxitin on the growth curve of *Enterobacter cloacae* SC 9965 (A, A1, A2), *Escherichia coli* W 3110 (B, B1, B2), *Klebsiella pneumoniae* 1082E (C, C1, C2) and *Escherichia coli* SC 11,120 (D, D1, D2), respectively.
Arrow indicates addition of antibiotic. Approximate viable cell concentration at that point was *ca.* 5×10^7 CFU/ml for all organisms.



test strains; an observation that further supports the excellent stability of this antibiotic to the β-lactamases produced by them.

Effect of Inoculum Size on Antibacterial Activity/Resistance to β-Lactamases

As inoculum size (and also incubation time) are increased, small reductions that occur in biological activity are of no great concern. In the case of β-lactam antibiotics, significant reductions in antimicrobial activity can usually be attributed to lack of stability to extracellular β-lactamase(s) such as those produced by staphylococci. With Gram-negative bacteria the situation is somewhat different in that ability of the antibiotic to enter the cell in sufficient concentration and stability to intracellular enzyme(s) are both involved.

The results with the panel of organisms listed in Table 2 show that SQ 14,359 and in some cases one or both of the other cephems were very resistant to β-lactamases from a variety of classes, a property that no doubt was in part responsible for the high degree of antimicrobial activity shown by the subject cephems toward bacteria known to elaborate such enzymes. This observation was further confirmed by the stability of these cephems to hydrolysis by the enzymes isolated as crude sonicates from the whole cells. The stability of SQ 14,359, CS-1170, and cefoxitin to β-lactamase from *E. cloacae* SC 9965 (Table 2), in the absence of substantial antimicrobial activity against whole cells by the latter 2 compounds, suggests the presence of a permeability barrier in these cases.

Table 2. Antibacterial activity against β-lactamase-producing organisms and enzyme stability of SQ 14,359, CS-1170, cefoxitin, and cephalothin.

Organism	SC #	Enzyme class[a] produced	MIC (μg/ml) at inoculum level (CFU/ml)[b]								Relative rate of hydrolysis (%)[c]		
			SQ 14,359		CS-1170		Cefoxitin		Cephalothin				
			10^3	10^6	10^3	10^6	10^3	10^6	10^3	10^6	SQ 14,359	CS-1170	Cefoxitin
Enterobacter cloacae	9965	I	6.25	25	100	>100	>100	>100	>100	>100	<1	<1	<1
Serratia marcescens	9782	I	1.56	6.25	3.13	12.5	6.25	25	>100	>100	<1	<1	<1
Proteus morganii	9774	I	1.56	25	12.5	25	25	25	>100	>100	<1	3	2
P. rettgeri	8217	I	3.13	6.25	12.5	100	12.5	25	>100	>100	<1	1.5	<1
Escherichia coli (TEM+) W3110	10404	III	0.4	1.56	0.4	0.78	1.56	1.56	6.3	12.5	<1	<1	<1
Klebsiella aerogenes 1082E	10436	IV	0.4	1.56	0.4	1.56	1.56	1.56	>100	>100	<1	<1	<1
Escherichia coli	11120	V	0.8	3.13	0.8	2.4	1.56	3.13	4.7	37.5	2.2	2.2	0.6

[a] Enzyme classification according to RICHMOND and SYKES[8]. [b] Colony-forming units. [c] Value of 100 was arbitrarily assigned for cephalothin.

Table 3. Susceptibility of aminoglycoside-resistant Enterobacteriaceae to 7α-OCH_3 cephalosporins.

Organism	SC #	Minimum inhibitory concentration (μg/ml)																	
		Amikacin		Dibekacin		Gentamicin[a]		Netilmicin		Sisomicin		Tobramycin		SQ 14,359		CS-1170		Cefoxitin	
		10^4[b]	10^6[b]	10^4	10^6	10^4	10^6	10^4	10^6	10^4	10^6	10^4	10^6	10^4	10^6	10^4	10^6	10^4	10^6
Escherichia coli	11,079	12.5	25	100	>100	100	>100	1.6	3.2	>100	>100	100	>100	0.2	0.4	0.8	0.8	1.6	1.6
Klebsiella pneumoniae	11,066	6.25	6.25	6.25	6.25	25	50	1.6	3.2	25	50	3.2	3.2	1.6	3.2	1.6	1.6	6.25	6.25
Proteus rettgeri	11,104	1.6	1.6	>100	>100	>100	>100	>100	>100	>100	>100	100	>100	12.5	12.5	25	50	50	50
Enterobacter cloacae	11,078	1.6	1.6	25	50	12.5	12.5	0.2	0.4	6.25	6.25	12.5	25	25	50	100	>100	>100	>100
Serratia liquefaciens	11,077	1.6	1.6	25	50	12.5	25	0.8	0.8	6.25	6.25	12.5	25	1.6	3.2	12.5	25	12.5	50
S. marcescens	11,107	1.6	3.2	50	100	>100	>100	12.5	25	100	>100	25	25	3.2	6.25	12.5	50	25	50
S. marcescens	11,108	1.6	3.2	25	100	100	>100	12.5	25	50	>100	12.5	25	0.8	1.6	3.2	12.5	12.5	12.5
S. marcescens	11,109	1.6	1.6	25	25	>100	>100	12.5	25	100	>100	12.5	25	0.8	1.6	3.2	12.5	6.25	12.5
S. marcescens	11,111	12.5	12.5	25	50	6.25	6.25	6.25	6.25	1.6	3.2	12.5	25	25	25	100	100	>100	>100
S. marcescens	11,112	50	50	100	>100	25	50	100	>100	50	100	100	100	25	25	100	100	100	>100
S. marcescens	11,113	0.8	1.6	100	>100	>100	>100	0.8	0.8	100	>100	100	>100	0.4	0.8	3.1	6.25	3.1	12.5
S. marcescens	11,114	3.2	3.2	100	>100	50	100	0.8	6.25	25	25	50	50	0.8	0.8	6.25	12.5	12.5	12.5
S. marcescens	11,115	1.6	1.6	>100	>100	50	100	1.6	3.2	25	50	50	100	25	50	100	>100	>100	>100

[a] All organisms were potent β-lactamase-producers; gentamicin-resistant cultures (MIC$\geq$6.25 μg/ml) were also resistant to neomycin and kanamycin.
[b] colony-forming units.

Table 4. Comparison of the therapeutic efficacy of SQ 14,359, CS-1170, and cefoxitin in experimental infections in mice.

Pathogen[a]		SQ 14,359		CS-1170		Cefoxitin	
		MIC[b]	ED_{50}[c]	MIC	ED_{50}	MIC	ED_{50}
Staphylococcus aureus	SC 10935	0.8	6.9	3.1	9.8	3.1	20
Escherichia coli	SC 8294	0.8	2.0	1.6	2.0	3.1	4.0
Salmonella schottmuelleri	SC 3850	0.4	0.6	0.4	4.0	1.6	11.0
Klebsiella pneumoniae	SC 8340	0.8	1.7	1.6	2.6	3.1	6.0
Proteus morganii	SC 9774	6.3	55	12.5	13.4	25	25
P. rettgeri	SC 11104	12.5	55	25	111	25	78
Citrobacter freundii	SC 10204	12.5	89	25	80	50	186
Serratia marcescens	SC 9782	6.3	7.4	12.5	16	12.5	26
S. liquefaciens	SC 9068	1.6	10.5	12.5	41	12.5	91
Enterobacter cloacae	SC 9965	12.5	76	100	182	200	193

[a] All organisms are β-lactamase producers, except *S. schottmuelleri*; *P. rettgeri* SC 11104 is resistant to gentamicin and chloramphenicol.
[b] Minimum inhibitory concentration in μg/ml at 10^6 CFU applied to the agar surface with a multipoint inoculator.
[c] Median effective dose in mg/kg calculated from mice infected with *ca.* 100 LD_{50} i.p. and treated s.c. in divided doses at 1 and 5 hours after infection.

Table 5. Comparative efficacy of SQ 14,359, CS-1170, and cefoxitin in parenteral therapy of experimental wound infection due to *Staphylococcus aureus* in mice.

Pathogen	Test drug	MIC[a] (μg/ml)	MBC (μg/ml)	ED_{50}[b] (mg/kg)
S. aureus SC 2406 (ampicillin, streptomycin-resistant)	SQ 14,359	0.6	1.6	12 (10~13)
	CS-1170	1.2	3.1	15 (14~16)
	Cefoxitin	3.1	6.3	32[c]

[a] MIC determined in a broth dilution assay at an initial inoculum of 10^6 CFU/ml.
[b] Median effective dose after treatment of mice s.c. in divided doses at 1 and 5 hours after infection (95% confidence limits).
[c] This value was obtained in each of two separate experiments.

Table 6. Comparative efficacy of SQ 14,359, CS-1170, and cefoxitin in the parenteral therapy of experimental meningitis due to *Haemophilus influenzae* in neonatal rats.

Pathogen	Test drug	MIC[a] (μg/ml)	MBC (μg/ml)	CD_{50}[b] (mg/kg)
H. influenzae SC 10,556 (ampicillin-resistant)	SQ 14,359	0.5	0.5	16 (16~17)
	CS-1170	1.2	3.1	>200[c]
	Cefoxitin	1.2	3.1	230 (152~349)
H. influenzae SC 9930 (ampicillin-sensitive)	SQ 14,359	0.1	0.6	39 (28~56)
	CS-1170	1.6	3.1	>400[c]
	Cefoxitin	1.2	6.3	340 (224~515)

[a] MIC determined in a broth dilution assay at an initial inoculum of 10^7 CFU/ml.
[b] Median effective dose after treatment of neonatal rats i.p. once daily for two days, starting 3 days after infection.
[c] Highest level tested.

Table 7. Comparative efficacy of SQ 14,359, CS-1170, and cefoxitin in experimental acute pyelonephritis in rats.

Pathogen	Test drug	MIC[a] (μg/ml)	CD_{50}[b] (mg/kg)
E. cloacae SC 9965 (ampicillin/ cephalothin-resistant)	SQ 14,359	25	22
	CS-1170	100	72
	Cefoxitin	200	93

[a] Minimum inhibitory concentration at an inoculum of 10^6 CFU applied to the surface of the agar with a multipoint inoculator.
[b] Median curative dose after treatment once daily for 5 days.

Table 8. Antibiotic concentrations in mouse sera, antibiotic bound to serum and percent of dose excreted in urine.

Antibiotic[a]	Antibiotic conc. (μg/ml)[b] in mouse sera at:					Antibiotic (%) bound to serum[c]	Antibiotic (%)[b] detected in urine at 0.6 hr.
	0.3 hr.	0.6 hr.	1.0 hr.	3.0 hr.	6.0 hr.		
SQ 14,359	70 (63~78)	34 (27~40)	15 (8~27)	<1	<1	69	36 (28~44)
CS-1170	72 (57~87)	36 (28~46)	10 (6~15)	<1	<1	48	41 (38~44)
Cefoxitin	78 (66~89)	36 (25~51)	16 (12~21)	<1	<1	39	68 (61~85)

[a] A 100-mg amount of antibiotic/kg was given subcutaneously at 0 hour.
[b] Assays of bioactivity; geometric mean of 3 or more experiments; confidence limits (95%) in parentheses.
[c] Average of two determinations; antibiotic concentration equals 40 μg/ml.

Experimental Chemotherapy

Model infections which result in systemic disease in mice were established with the aid of Gram-positive and 9 Gram-negative organisms. All but one of these bacteria (*Salmonella schottmuelleri*) were potent producers of a variety of β-lactamases. Subcutaneous administration of the subject cephems have produced results which quantitatively favor SQ 14,359 in almost all cases (Table 4). The MIC was generally a good predictor of efficacy. None of the compounds tested were sufficiently well absorbed orally in this series to significantly modify the course of these infections (data not shown).

Parenteral therapy of an experimental surgical-wound infection in mice due to a penicillinase-producing *S. aureus* strain has shown each of the cephalosporins (Table 5) to be very effective in rapidly reducing the number of viable organisms contaminating such wounds. Quantitatively, SQ 14,359 possessed activity similar to that of CS-1170, and both were significantly more active than cefoxitin ($P < 0.05$) under these conditions.

Bacteremia and meningitis due to a representative ampicillin-sensitive and -resistant strain of *H. influenzae* in neonatal rats when treated parenterally with SQ 14,359 resulted in cure rates at relatively low doses of the drug (Table 6). Cefoxitin and CS-1170, on the other hand, were virtually inactive in both systems; intrinsic activity and pharmacokinetics (Table 9) appeared to play a role in the observed effects.

Acute pyelonephritis due to *E. cloacae* SC 9965 in adult rats was controlled successfully by the s.c. administration of each of the subject cephalosporins. In these therapeutic trials (Table 7) SQ 14,359 was substantially more active than either CS-1170 or cefoxitin as might be expected from the MIC. The lower urinary excretion rate for SQ 14,359 and CS-1170 in this species (Table 10) under these experimental conditions may have prevented the demonstration of even higher activity.

Table 9. Maximum whole blood or cerebrospinal fluid (CSF) concentrations (C_{max}), times to reach maximum concentrations (T_{max}), blood and CSF half-life values ($t_{1/2}$) of antimicrobial activity, and mean area under the blood and CSF concentration curve (AUC) of SQ 14,359, CS-1170 and cefoxitin in infant rats.

Antibiotic[a]	Source	Parameter			
		C_{max}[b] (μg/ml)	T_{max} (min)	$t_{1/2}$[c] (min)	AUC (μg-hr/ml)
SQ 14,359	Blood	120	20	41	129.3
	CSF	47	40	38	76
CS-1170	Blood	83	20	25	100.6
	CSF	15	40	44	53
Cefoxitin	Blood	110	20	20	49
	CSF	23	40	20	49

[a] A 100-mg amount of antibiotic/kg was given intraperitoneally at 0 hour.
[b] Expressed as microgram equivalent of administered drug.
[c] $t_{1/2}$ values were estimated from the terminal linear portion of the blood or CSF concentration *vs.* time curves, plotted semilogarithmically.

Table 10. Maximum serum concentration (C_{max}), times to reach maximum concentration (T_{max}), serum half-life value ($t_{1/2}$) of antimicrobial activity, mean area under the serum concentration curve (AUC), antibiotic bound to serum protein, and percent of dose of SQ 14,359, CS-1170, and cefoxitin excreted in urine of adult rats.

Antibiotic[a]	Serum					Antibiotic (%) detected in urine at 0~6 hr.
	C_{max}[b] (μg/ml)	T_{max} (min)	$t_{1/2}$[c] (min)	AUC (μg-hr/ml)	Bound (%)[d]	
SQ 14,359	15.5	15	14.5	8.6	62	27
CS-1170	18.6	15	16	10.0	42	29
Cefoxitin	24.6	15	16	13.5	37	86

[a] A 25-mg amount of antibiotic/kg was given subcutaneously at 0 hour.
[b] Expressed as microgram equivalents of administered drug.
[c] $t_{1/2}$ values were estimated from the terminal linear portions of the serum concentration *vs.* time curves, plotted semilogarithmically.
[d] Average of two determinations, antibiotic concentration equals 40 μg/ml.

Pharmacokinetics and Metabolism

After the subcutaneous administration to separate groups of mice of a single loading dose of *ca.* 100 mg/kg of each of the test cephems, peak blood levels ranging from 70~78 μg/ml were obtained in all instances at *ca.* 20 minutes (Table 8). The serum half-life of all compounds was less than 1 hour. Protein binding was highest for SQ 14,359 (69% at 40 μg/ml) and lowest for CS-1170 (48% at 40 μg/ml); cefoxitin binding was intermediate between these values. The urinary excretion rate during the 6 hour test period after drug dosing was highest for cefoxitin, and substantially lower for CS-1170 and SQ 14,359 (68%, 41%, and 35% of administered dose excreted, respectively).

Intraperitoneal injection of 100 mg of the subject cephems/kg of body weight to meningitic neonatal rats presumed to exhibit inflamed meninges produced a pharmacokinetic profile in blood and CSF [peak concentration (C_{max}), half-life ($t_{1/2}$) and mean area under the concentration curve (AUC)] that favored SQ 14,359 (Table 9). Time of peak concentration (T_{max}) in serum CSF (20 and 40 minutes, respectively) was the same for all drugs. Based on the relatively high levels of drug in the CSF of these animals, and in a similar group of non-infected controls (data not shown) which showed values

about one-fifth of those given, it must be concluded that the blood-brain barrier in infant rats at 8 days of age is incompletely formed. Nevertheless, structural features and/or physico-chemical properties found in SQ 14,359 seem to play a role in the facilitated uptake into the CSF of this drug, over that of CS-1170 and cefoxitin. These properties remain to be identified.

Adult rats given a single dose of *ca.* 25 mg/kg s.c. of each of the test compounds showed peak serum levels at *ca.* 15 minutes and serum half-lives of less than 30 minutes (Table 10). Cefoxitin showed the highest level (24.6 μg/ml) and SQ 14,359 the lowest (15.5 μg/ml); CS-1170 was intermediate. The AUC was lowest for SQ 14,359 (8.6 μg-hour/ml), somewhat higher for CS-1170 (10.0 μg-hour/ml) and highest for cefoxitin (13.5 μg-hour/ml). Protein binding in serum was similar to that in mice. Elimination kinetics based on urinary excretion in the rat were similar to those in the mouse.

All the test cephems were very stable in that only the test antibiotic and no metabolites were demonstrated in serum or urine of mice or rats after parenteral administration.

Acknowledgements

We are indebted to Mr. O. KOCY for providing analytical support throughout this study. The technical assistance of S. EISENBURGER, P. ERHARD, C. GOOD, B. MINASSIAN, B. REMSBURG and S. P. SCHWARTZ is gratefully acknowledged.

References

1) APPLEGATE, H. E.; C. M. CIMARUSTI, J. E. DOLFINI, W. H. KOSTER, M. A. ONDETTI, W. A. SLUSARCHYK, M. G. YOUNG, H. BREUER & V. D. TREUNER: Diastereomeric 7-ureidoacetyl cephalosporins. II. 7β-[[[(Aminocarbonyl)amino]-2-thienylacetyl]amino]-7-methoxy-3-[[(1-methyl-1H-tetrazol-5yl)thio]-methyl]-8-oxo-5-thia-1-azabicyclo[4.2.0]-oct-2-ene-2-carboxylic acid. J. Antibiotics 31: 561~569, 1978
2) GADEBUSCH, H. H.; H. I. BASCH, P. LUKASZOW, B. REMSBURG & R. SCHWIND: Diastereomeric 7-ureidoacetyl cephalosporins. III. Contribution of D-and L-isomers to the growth inhibiting activities of 7α-H and 7-α-OCH_3 derivatives for Gram-positive and Gram-negative bacteria. J. Antibiotics 31: 570~579, 1978
3) NAKAO, H.; H. YANAGISAWA, B. SHIMIZU, M. KANEKO, M. NAGANO & S. SUGAWARA: A new semisynthetic 7-α-methoxy-cephalosporin, CS-1170: 7β-[(cyanomethyl)-thio]acetamido]-7-α-methoxy-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic acid. J. Antibiotics 29: 554~558, 1976
4) WALLICK, H. & D. HENDLIN: Cefoxitin, a semisynthetic cephamycin antibiotic: Susceptibility studies. Antimicr. Agents & Chemoth. 5: 25~32, 1974
5) ONISHI, H. R.; D. R. DAOUST, S. B. ZIMMERMAN, D. HENDLIN & E. O. STAPLEY: Cefoxitin, a semisynthetic cephamycin antibiotic: Resistance to β-lactamase inactivation. Antimicr. Agents & Chemoth. 5: 38~48, 1974
6) MILLER, A. K.; E. CELOZZI, Y. KONG, B. A. PELAK, D. HENDLIN & E. O. STAPLEY: Cefoxitin, a semisynthetic antibiotic: *In vivo* evaluation. Antimicr. Agents & Chemoth. 5: 33~37, 1974
7) BASCH, H.; R. ERICKSON & H. GADEBUSCH: Epicillin: *In vitro* laboratory studies. Infec. Immun. **4**: 44~49, 1971
8) FARRAR, W. E. & N. M. O'DELL: β-Lactamases and resistance to penicillins and cephalosporins in *Serratia marcescens*. J. Infect. Dis. 134: 245~251, 1976
9) O'CALLAGHAN, C. H.; A. MORRIS, S. M. KIRBY & A. H. SHINGLER: Novel method for the detection of β-lactamases by using a chromogenic cephalosporin substrate. Antimicr. Agents & Chemoth. 1: 283~288, 1972
10) RICHMOND, M. H. & R. B. SYKES: The β-lactamases of gram-negative bacteria and their possible physiological role. *In* A. H. ROSE & W. E. TEMPEST (*Eds*): Advances in Microbial Physiology, Vol. 9, pp. 31~88, Academic Press, New York. 1973
11) PERRET, C. J.: Iodometric assay of penicillinase. Nature (London) 174: 1012~1013, 1954
12) SINGHVI, S. M.; A. F. HEALD, H. H. GADEBUSCH, M. E. RESNICK, L. T. DIFAZIO & M. A. LEITZ: Human serum protein binding of cephalosporin antibiotics *in vitro*. J. Lab. Clin. Med. 89: 414~420, 1977
13) REED, L. J. & H. MUENCH: A simple method of estimating fifty percent endpoints. Amer. J. Hyg. 27: 493~499, 1938

14) McRIPLEY, R. J. & R. R. WHITNEY: Characterization and quantitation of experimental surgical-wound infections used to evaluate topical antibacterial agents. Antimicr. Agents & Chemoth. 10: 38～44, 1976
15) WHITNEY, R. R.; R. J. McRIPLEY & H. H. GADEBUSCH.: Evaluation of chemotherapeutic agents in an experimental *Hemophilus influenzae* bacteremia and meningitis in infant rats. Abstracts Annual Meeting Am. Soc. Microbiol. A 19, 1977
16) MOXON, E. R.; A. L. SMITH, D. R. AVERILL & D. H. SMITH: *Haemophilus influenzae* meningitis in infant rats after intranasal inoculation. J. Infect. Dis. 129: 154～162, 1974
17) MOXON, E. R. & P. T. OSTROW: *Haemophilus influenzae* meningitis in infant rats: Rate of bacteremia in pathogenesis of age-dependent inflammatory responses in cerebrosprinal fluid. J. Infect. Dis. 135: 303～307, 1977
18) GREENWOOD, D. & F. O'GRADY: Comparison of the responses of *Escherichia coli* and *Proteus mirabilis* to seven β-lactam antibiotics. J. Infect. Dis. 128: 211～222, 1973