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β-Lactamase Stability and Antibacterial Activity of Cefmenoxime (SCE-1365), a Novel Cephalosporin

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Cefmenoxime, a new cephalosporin antibiotic, has been shown to be stable to a *Staphylococcus aureus* penicillinase and R plasmid-mediated type I and type IV penicillinases. It was also resistant to hydrolysis by most cephalosporinases, but was susceptible to hydrolysis by a *Proteus vulgaris* β-lactamase. Cefmenoxime was active against cephaloridine-resistant species, except *Pseudomonas aeruginosa*, which was moderately resistant to cefmenoxime. Cefmenoxime was an inducer of *P. vulgaris* β-lactamase biosynthesis, but 1 μg or more of the drug per ml, which inhibits most of the clinical isolates of *P. vulgaris*, was required for the production of detectable amounts of the enzyme. Cefmenoxime was a strong competitive inhibitor of β-lactamases of *Enterobacter cloacae*, *Citrobacter freundii*, *P. aeruginosa*, and *Serratia marcescens*, but it did not inhibit penicillinases in spite of its resistance to hydrolysis.

Cephalosporin antibiotics are important chemotherapeutic agents because of their broad antibacterial spectrum. Older cephalosporins, however, have certain limitations in their antibacterial activities, derived from their poor intrinsic antibacterial activities (the abilities to reach the target site and to cause the antibacterial effect) or susceptibilities to β-lactamases from gram-negative organisms (1, 16, 21). To overcome these defects, many cephalosporins have been developed, but further improvements are desirable. For example, cefotiam (9, 10, 19) has a potent intrinsic antibacterial activity but is hydrolyzed by certain β-lactamases. Cefuroxime (12) and cefoxitin (14) are resistant to hydrolysis by the β-lactamases of various organisms, but their intrinsic antibacterial activities are not so high as that of cefotiam.

Cefmenoxime, 7β-[2-(2-aminothiazol-4-yl)-(*Z*)-2-methoxyiminoacetamido]-3-[(1-methyl-1*H*-tetrazol-5-yl)thiomethyl]ceph-3-em-4-carboxylic acid (Fig. 1; M. Ochiai, A. Morimoto, T. Okada, Y. Matsushita, O. Aki, M. Kida, and K. Okonogi, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 18th, Atlanta, Ga., abstr. no. 150, 1978) has a potent antibacterial activity against gram-positive and gram-negative organisms including β-lactamase producers.

The present paper describes the β-lactamase stability and antibacterial activity of cefmenoxime in comparison with those of cefuroxime, cefotaxime, and cefoxitin.

MATERIALS AND METHODS

Organisms. All strains except *Proteus vulgaris* CS2533 were clinical isolates. *P. vulgaris* CS2533 was a mutant obtained from *P. vulgaris* GN4413 by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, which was a constitutive producer of β-lactamase.

Antibiotics. Cefmenoxime, cefotiam, cefuroxime, and cefotaxime were prepared in our research division. Other antibiotics were obtained from the following sources: ampicillin from Takeda Chemical Industries, Ltd., Japan; benzylpenicillin from Meiji Seika Kaisha, Ltd., Japan; dicloxacillin from Banyu Pharmaceutical Co., Ltd., Japan; cefoxitin from Daiichi Seiyaku Co., Ltd., Japan; cephaloridine and cephalothin from Shionogi & Co., Ltd., Japan; and cefazolin from Fujisawa Pharmaceutical Co., Ltd., Japan.

Determination of antibacterial activity. The minimum inhibitory concentrations (MICs) were determined by a standard twofold serial dilution method with Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.). About 5 μl of bacterial suspension containing about 10^6 colony-forming units per ml was inoculated onto agar plates with a multiple inoculator (Sakuma, Tokyo). Plates were incubated at 37°C for 18 h, and the MIC was taken as the lowest concentration of the drug that inhibited the visible growth of bacteria.

Preparation of β-lactamases. The β-lactamases of *Escherichia coli* TN713 and *Pseudomonas aeruginosa* GN3407 are constitutive enzymes mediated by plasmids, and that of *Klebsiella pneumoniae* TN1698 is also constitutive. *Staphylococcus aureus* 1840 β-lactamase was induced by 0.5 μg of dicloxacillin per ml. The β-lactamases of *Citrobacter freundii* GN1706, *P. aeruginosa* U31, *Serratia marcescens* TN81, *En-*

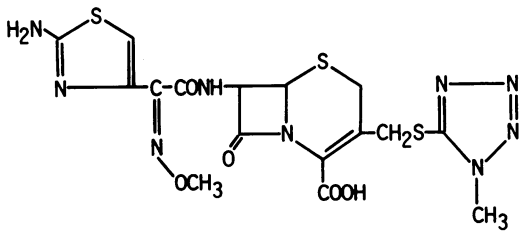

FIG. 1. *Chemical structure of cefmenoxime.*

terobacter cloacae TN1282, and *Proteus vulgaris* GN4413 were induced by 1 mg of benzylpenicillin per ml.

These enzymes were prepared as follows. An overnight culture was diluted 10-fold into 250 ml of Trypticase soy broth in a 1,000-ml flask, and the flask was shaken for 5 h on a rotary shaker at 37°C. When induction was required for the production of the enzyme, an inducer was added after 2 h of cultivation. The cells were harvested from 5 liters of cultured broth by centrifugation, suspended in 500 ml of 0.05 M phosphate buffer (pH 6.9), and disrupted with a ultrasonic oscillator (Kaijo Denki Co., Ltd.) in an ice bath. Cell debris was removed by centrifugation, and the enzyme was purified from the supernatant fluid. The β-lactamase of *S. aureus* 1840 was purified by phospho-cellulose column chromatography as described by Richmond (15); those of *P. vulgaris* GN4413 and *E. cloacae* TN1282 were purified by carboxymethyl Sephadex C-50 column chromatography and Sephadex G-100 gel filtration by the method of Hennessey and Richmond (4); those of *E. coli* TN713 and *P. aeruginosa* GN3407 were purified by diethylaminoethyl cellulose column chromatography and Sephadex G-100 gel filtration as described by Ogawara et al. (13). The enzymes of *K. pneumoniae* TN1698, *C. freundii* GN1706, *P. aeruginosa* U31, and *S. marcescens* TN81 were purified in the same way as that for the *E. coli* TN713 enzyme, with carboxymethyl cellulose instead of diethylaminoethyl cellulose.

Induction experiment of *P. vulgaris* β-lactamase. An overnight culture was diluted 20-fold into 6 ml of Trypticase soy broth, and *P. vulgaris* was grown with shaking for 2 h at 37°C. As an inducer, various amounts of cefmenoxime or benzylpenicillin were added, and the incubation was continued for a further 40 min. The cells were harvested by centrifugation, suspended in 3 ml of 0.05 M phosphate buffer (pH 6.9), and sonically disrupted in an ice bath. The cell debris was removed by centrifugation, and the β-lactamase activity in the resulting supernatant was measured.

Assay of β-lactamase activity. β-Lactamase activity was determined by the spectrophotometric method (11) or the modification of the microiodometric method of Novick (8).

(i) Spectrophotometric method. Four milliliters of the reaction mixture containing 0.2 mM substrate and 0.05 M phosphate buffer (pH 6.9) were incubated at 30°C in a Gilford spectrophotometer. The decrease of optical density at 265 nm or 275 nm was recorded after the addition of 0.2 ml of enzyme. The differences in molar extinction (liters per mole per centimeter) between cephalosporins and the corresponding hydrolyzed compounds were as follows: cefmenoxime (275 nm) 8,050; cefotiam (275 nm) 5,510; cefotaxime (265 nm) 6,250; cefuroxime (265 nm) 6,670; cefazolin (265 nm) 8,170; cephalothin (265 nm) 7,450; cephaloridine (265 nm) 9.080.

(ii) Microiodometric method. One milliliter of the reaction mixture contained 0.2 mM substrate, 0.05 M phosphate buffer (pH 6.9), and 0.05 ml of enzyme. After incubation for 10 min at 30°C, the reaction was stopped by the addition of 0.5 ml of sodium tungstate reagent (8). Each sample had a control in which the enzyme was added after the addition of the tungstate. Then 1.5 ml of an iodine-starch solution (8) was added. After standing for 15 min or more at room temperature, the absorbance at 620 nm was read in a Hitachi spectrophotometer. The enzyme activity was calculated from the difference in absorbance between control and experiment; 1 absorbance unit was equivalent to the following amounts of substrate hydrolyzed (nanomoles): cefmenoxime, 25.8; cefotaxime, 51.1; cefuroxime, 53.3; cefoxitin, 49.5; cefazolin, 22.9; cephalothin, 47.5; cephaloridine, 48.1; penicillins, 30.0.

The inhibition constant (K_i) was determined by assessing the ability of an antibiotic to inhibit the hydrolysis of ampicillin or cephalothin by a penicillinase or a cephalosporinase, respectively. Kinetic parameters were estimated from Lineweaver-Burk plots (5). One unit of β-lactamase was defined as the amount of enzyme that hydrolyzed 1 μmol of benzylpenicillin (penicillinase) or cephaloridine (cephalosporinase) per min at 30°C.

RESULTS

Susceptibility of strains. The susceptibilities of strains used are given in Table 1. *S. aureus* 1840 was penicillin resistant, and all other strains were cephaloridine resistant. Cefmenoxime was highly active against all the strains except *P. aeruginosa* strains, which were moderately resistant to cefmenoxime.

β-Lactamase stability of cefmenoxime. Table 2 shows that cefmenoxime, as well as cefoxitin, cefuroxime, and cefotaxime, was resistant to hydrolysis by β-lactamases from *E. cloacae* TN1282, *C. freundii* GN1706, *P. aeruginosa* U31, and *S. marcescens* TN81. Cefazolin and cephaloridine were the best substrates for these enzymes. Cefmenoxime, cefotaxime, cefuroxime, and cefoxitin were also resistant to hydrolysis by penicillinases from *S. aureus* 1840, *E. coli* TN713 (type I [17], TEM-1 [7]) and *P. aeruginosa* GN3407 (type IV [17], PSE-1 [7]). All cephalosporins except cefoxitin were good substrates for the *P. vulgaris* GN4413 β-lactamase (Table 2). For this enzyme, cefmenoxime, cefotaxime, and cefuroxime had K_m values of 170, 230, and 180 μM, respectively.

Inhibition of β-lactamase by cefmenoxime. As cefoxitin, cefuroxime, and cefotaxime, which are resistant to β-lactamase hydrolysis,

TABLE 1. *Antibacterial activities of cephalosporin antibiotics*

Strain	MIC (μg/ml) of:						
	Cefmenoxime	Cefotaxime	Cefuroxime	Cefoxitin	Cefotiam	Cefazolin	Cephaloridine
E. cloacae TN1282	0.78	0.78	50	>1,600	25	1,600	1,600
C. freundii GN1706	0.20	0.20	6.25	200	1.56	200	200
P. aeruginosa U31	50	100	800	>1,600	>1,600	>1,600	>1,600
S. marcescens TN81	1.56	3.13	800	400	1,600	>1,600	>1,600
P. vulgaris GN4413	1.56	12.5	>1,600	12.5	800	1,600	>1,600
S. aureus 1840	3.13	3.13	3.13	3.13	1.56	0.78	0.20
E. coli TN713	0.10	0.10	6.25	6.25	0.20	6.25	25
K. pneumoniae TN1698	0.20	0.10	12.5	1.56	1.56	100	50
P. aeruginosa GN3407	12.5	12.5	400	1,600	>1,600	>1,600	>1,600

TABLE 2. *Hydrolysis of cefmenoxime and other cephalosporins by β-lactamases*

Source of enzyme	Relative rate of hydrolysis[a]							
	Cefmenoxime	Cefotaxime	Cefuroxime	Cefoxitin	Cefotiam	Cefazolin	Cephaloridine	Benzylpenicillin
E. cloacae TN1282	0.1	0.1	0.1	0.3	41.4	67.0	100	28.5
C. freundii GN1706	0.1	0.1	0.1	0.2	4.8	96.1	100	7.9
P. aeruginosa U31	0.3	0.4	0.3	0.2	40.9	127	100	64.5
S. marcescens TN81	0.3	0.9	1.1	<0.1	53.0	279	100	11.4
P. vulgaris GN4413	44.9	38.5	232	<0.1	174	467	100	16.7
S. aureus 1840	<0.1	<0.1	<0.1	<0.1	<0.1	0.2	0.1	100
E. coli TN713	0.1	<0.1	0.1	<0.1	1.5	3.1	23.6	100
K. pneumoniae TN1698	1.6	0.7	2.6	<0.1	1.1	12.0	43.3	100
P. aeruginosa GN3407	<0.1	<0.1	<0.1	<0.1	0.4	0.7	5.7	100

[a] The enzyme activity was determined by the spectrophotometric method or the microiodometric method using a 0.2 mM concentration of each substrate and was expressed in relative rate of hydrolysis, taking the rate for benzylpenicillin (penicillinase) or cephaloridine (cephalosporinase) as 100.

inhibit β-lactamase (2, 3, 6), we investigated the β-lactamase-inhibitory activity of cefmenoxime. Figure 2 shows that cefmenoxime inhibited the β-lactamases of *E. cloacae* and *C. freundii* competitively. It was also a competitive inhibitor of the β-lactamases of *P. aeruginosa* U31 and *S. marcescens* TN81 (Table 3). For these enzymes, cefmenoxime had K_i values from 9.8×10^{-9} to 1.8×10^{-6} M, which were comparable with those of cefotaxime and cefuroxime. Although cefmenoxime was resistant to hydrolysis by penicillinases of *S. aureus* 1840, *E. coli* TN713, and *P. aeruginosa* GN3407, it scarcely inhibited these enzymes.

Antibacterial activity of cefmenoxime against *P. vulgaris*. Although cefmenoxime was hydrolyzed significantly by the *P. vulgaris* β-lactamase, 70% of the clinical isolates of *P. vulgaris* were inhibited by 0.1 μg of cefmenoxime per ml, and 90% were inhibited by less than 1 μg/ml (20). Therefore, we investigated whether the β-lactamase affected the antibacterial activity of cefmenoxime against *P. vulgaris* or not.

Three strains of *P. vulgaris* were tested. Each continued to produce β-lactamase constantly for at least 40 min after the addition of an inducer.

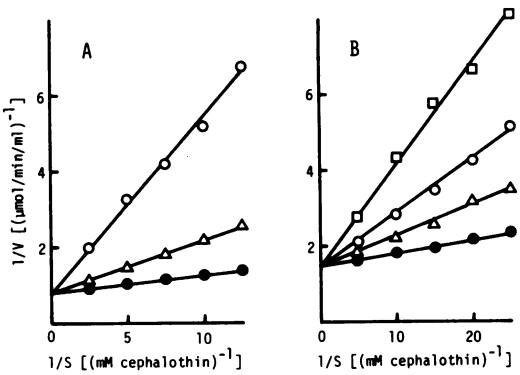

FIG. 2. *(A) Competitive inhibition of E. cloacae TN1282 β-lactamase hydrolysis of cephalothin by cefmenoxime. Symbols: ○, 1.0 μM; △, 0.2 μM; ●, control. (B) Competitive inhibition of C. freundii GN1706 β-lactamase hydrolysis of cephalothin by 0.04 μM cefmenoxime (○), 0.01 μM cefotaxime (△), and 0.05 μM cefuroxime (□); ●, control.*

Therefore, the organisms were incubated with various amounts of cefmenoxime or benzylpenicillin for 40 min (Fig. 3). The ability of cefmenoxime to induce the β-lactamase was almost

TABLE 3. *Inhibition of β-lactamases by cefmenoxime, cefotaxime, cefuroxime, and cefoxitin*

Source of enzyme	K_i (M)[a]			
	Cefmenoxime	Cefotaxime	Cefuroxime	Cefoxitin
E. cloacae TN1282	9.8×10^{-8}	7.6×10^{-8}	5.2×10^{-8}	1.3×10^{-6}
C. freundii GN1706	9.8×10^{-9}	5.3×10^{-9}	7.1×10^{-9}	7.6×10^{-7}
P. aeruginosa U31	3.0×10^{-7}	2.2×10^{-7}	4.9×10^{-8}	3.5×10^{-7}
S. marcescens TN81	1.8×10^{-6}	7.7×10^{-6}	1.6×10^{-6}	3.8×10^{-7}

[a] K_i was determined by assessing the ability of antibiotics to inhibit the hydrolysis of cephalothin.

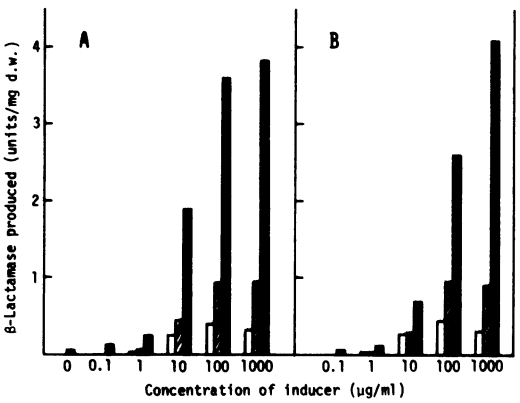

FIG. 3. *Induction of P. vulgaris β-lactamase. P. vulgaris strains GN4731 (unshaded), GN4413 (hatched), and TN1945 (black) were grown to the logarithmic phase, and β-lactamase was induced for 40 min by the addition of the indicated amounts of cefmenoxime (A) or benzylpenicillin (B).*

the same as that of benzylpenicillin. *P. vulgaris* strains GN4731 and GN4413 did not produce detectable amounts of β-lactamase even in the presence of 0.1 μg of the inducer per ml, whereas *P. vulgaris* TN1945 produced a small amount of β-lactamase without the inducer. The activity increased with the concentration of the inducer. But some of the strains lysed with 1 mg of the inducer per ml, and a part of the enzyme activity was found extracellularly. The amount of the enzyme produced by *P. vulgaris* TN1945 was the highest, and that produced by *P. vulgaris* GN4731 was the lowest at any concentrations of the inducer.

Next, we examined the relationship between the β-lactamase activity and the susceptibility of *P. vulgaris* to cefmenoxime. Table 4 shows a part of the results obtained with 24 clinical isolates chosen for their different susceptibilities to cefmenoxime and with a β-lactamase-constitutive mutant, CS2533. Among 25 strains tested, 2 strains, including *P. vulgaris* GN5297, produced a very low level of β-lactamase and were susceptible to 0.05 μg of cefmenoxime per ml. Sixteen strains produced from 0.1 to 0.6 U of β-lactamase per mg of bacterial dry cell weight when induced

TABLE 4. *β-Lactamase activity and antibiotic susceptibility of P. vulgaris*

Strain	β-Lactamase activity[a] (U/mg of dry wt)		MIC (μg/ml) of:		
	Without induction	With induction	Cefmenoxime	Cefuroxime	Cefoxitin
GN5297	<0.01	<0.01	0.05	3.13	6.25
GN4731	<0.01	0.39	0.10	100	3.13
GN4413	<0.01	1.08	1.56	>1,600	12.5
TN1945	0.02	2.43	6.25	>1,600	6.25
CS2533	2.90	2.92	50	>1,600	6.25

[a] Induction was carried out with 0.1 mg of benzylpenicillin per ml for 40 min.

by 0.1 mg of benzylpenicillin per ml, but were susceptible to 0.05 to 0.2 μg of cefmenoxime per ml. Those strains producing more than 1.0 U of β-lactamase showed slightly higher MICs (1.56 to 12.5 μg/ml). *P. vulgaris* CS2533, a β-lactamase-constitutive mutant, had the highest MIC.

Cefuroxime, which was hydrolyzed five times more easily than cefmenoxime, had no antibacterial activity against β-lactamase producers. On the other hand, the MICs of cefoxitin were not affected by the presence of the β-lactamase.

DISCUSSION

Cefmenoxime, as well as cefuroxime and cefotaxime, has been shown to be resistant to hydrolysis by various β-lactamases, except that of *P. vulgaris*. It showed increased β-lactamase stability compared with cefotiam, which is structurally similar to cefmenoxime but has no methoxyimino group in the 7-acyl side chain. The methoxyimino group of cefmenoxime may contribute to the stability to β-lactamases, especially to cephalosporinases, as does the 7α-methoxy group of cephamycin (14). The high antibacterial activity of cefmenoxime against cephaloridine-resistant organisms, as well as susceptible one, can be regarded as due to this β-lactamase stability.

Cefmenoxime was a potent competitive inhibitor of cephalosporinases except that of *P. vulgaris*, as were cefuroxime and cefotaxime. Fu

and Neu reported that cefotaxime inhibited the *C. freundii* β-lactamase noncompetitively (3), but our results with purified enzyme indicated that cefmenoxime, cefuroxime, cefoxitin, and cefotaxime were competitive inhibitors of the *C. freundii* GN1706 β-lactamase.

Cefmenoxime, cefuroxime, and cefotaxime have common properties: they are resistant to hydrolysis by most β-lactamases, susceptible only to *P. vulgaris* β-lactamase, and strongly active in inhibiting cephalosporinases. Shannon et al. reported that Ro 13-9904 was also resistant to hydrolysis by most β-lactamases, except those from *P. vulgaris, Bacteroides fragilis,* and one strain of *E. cloacae* (18). The only common structure, besides the cephalosporin nucleus, among these four cephalosporins is the presence of a methoxyimino group in the 7-acyl side chain. Consequently, the common properties among these four cephalosporins may be attributable to this methoxyimino moiety.

Cefmenoxime was highly active against most of the clinical isolates of *P. vulgaris,* in spite of its susceptibility to hydrolysis by the β-lactamase of this species. This paradoxical phenomenon can be explained by a greater affinity of cefmenoxime for its target site in the cell than for β-lactamase induction. Cefmenoxime scarcely induced β-lactamase formation by *P. vulgaris* at 0.1 μg/ml or less, although it was an active inducer at higher concentrations. The antibiotic may have a higher affinity for the antibacterial target site(s) than for the β-lactamase repressor and thus may inhibit the growth of *P. vulgaris* strains before it can act as a β-lactamase inducer.

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LITERATURE CITED

1. **Blumberg, P. M., and J. L. Strominger.** 1974. Interaction of penicillin with the bacterial cell: penicillin-binding proteins and penicillin-sensitive enzymes. Bacteriol. Rev. **38**:291–335.
2. **Darland, G., and J. Birnbaum.** 1977. Cefoxitin resistance to beta-lactamase: a major factor for susceptibility of *Bacteroides fragilis* to the antibiotic. Antimicrob. Agents Chemother. **11**:725–734.
3. **Fu, K. P., and H. C. Neu.** 1978. Beta-lactamase stability of HR756, a novel cephalosporin, compared to that of cefuroxime and cefoxitin. Antimicrob. Agents Chemother. **14**:322–326.
4. **Hennessey, T. D., and M. H. Richmond.** 1968. The purification and some properties of a β-lactamase (cephalosporinase) synthesized by *Enterobacter cloacae.* Biochem. J. **109**:469–473.
5. **Lineweaver, H., and D. Burk.** 1934. The determination of enzyme dissociation constants. J. Am. Chem. Soc. **56**:658–666.
6. **Mahoney, D. F., G. A. Koppel, and J. R. Turner.** 1976. Substrate inhibition of beta-lactamases, a method for predicting enzymatic stability of cephalosporins. Antimicrob. Agents Chemother. **10**:470–475.
7. **Matthew, M.** 1979. Plasmid-mediated β-lactamases of gram-negative bacteria: properties and distribution. J. Antimicrob. Chemother. **5**:349–358.
8. **Novick, R. P.** 1963. Analysis by transduction of mutations affecting penicillinase formation in *Staphylococcus aureus.* J. Gen. Microbiol. **33**:121–136.
9. **Nozaki, Y., A. Imada, and M. Yoneda.** 1979. SCE-963, a new potent cephalosporin with high affinity for penicillin-binding proteins 1 and 3 of *Escherichia coli.* Antimicrob. Agents Chemother. **15**:20–27.
10. **Numata, M., I. Minamida, M. Yamaoka, M. Shiraishi, T. Miyawaki, H. Akimoto, K. Naito, and M. Kida.** 1978. A new cephalosporin. SCE-963: 7-[2-(2-aminothiazol-4-yl)-acetamido]-3-[[[1-(2-dimethylaminoethyl)-1H-tetrazol-5-yl]thio]methyl]ceph-3-em-4-carboxylic acid. J. Antibiot. **31**:1262–1271.
11. **O'Callaghan, C. H., P. W. Muggleton, and G. W. Ross.** 1969. Effects of β-lactamase from gram-negative organisms on cephalosporins and penicillins, p. 57–63. Antimicrob. Agents Chemother. 1968.
12. **O'Callaghan, C. H., R. B. Sykes, D. M. Ryan, R. D. Foord, and P. W. Muggleton.** 1976. Cefuroxime—a new cephalosporin antibiotic. J. Antibiot. **29**:29–37.
13. **Ogawara, H., K. Maeda, and H. Umezawa.** 1972. A β-lactamase of *Escherichia coli.* Biochim. Biophys. Acta **289**:203–211.
14. **Onishi, H. R., D. R. Daoust, S. B. Zimmerman, D. Hendlin, and E. O. Stapley.** 1974. Cefoxitin, a semisynthetic cephamycin antibiotic: resistance to beta-lactamase inactivation. Antimicrob. Agents Chemother. **5**: 38–48.
15. **Richmond, M. H.** 1963. Purification and properties of the exopenicillinase from *Staphylococcus aureus.* Biochem. J. **88**:452–459.
16. **Richmond, M. H., and R. B. Sykes.** 1973. The β-lactamases of gram-negative bacteria and their possible physiological role. Adv. Microbiol. Physiol. **9**:31–88.
17. **Sawada, Y., S. Yaginuma, M. Tai, S. Iyobe, and S. Mitsuhashi.** 1976. Plasmid-mediated penicillin beta-lactamases in *Pseudomonas aeruginosa.* Antimicrob. Agents Chemother. **9**:55–60.
18. **Shannon, K., A. King, C. Warren, and I. Phillips.** 1980. In vitro antibacterial activity and susceptibility of the cephalosporin Ro 13-9904 to beta-lactamases. Antimicrob. Agents Chemother. **18**:292–298.
19. **Tsuchiya, K., M. Kida, M. Kondo, H. Ono, M. Takeuchi, and T. Nishi.** 1978. SCE-963, a new broad-spectrum cephalosporin: *in vitro* and *in vivo* antibacterial activities. Antimicrob. Agents Chemother. **14**: 557–568.
20. **Tsuchiya, K., M. Kondo, M. Kida, M. Nakao, T. Iwahi, T. Nishi, Y. Noji, M. Takeuchi, and Y. Nozaki.** 1981. Cefmenoxime (SCE-1365), a novel broad-spectrum cephalosporin: *in vitro* and *in vivo* antibacterial activities. Antimicrob. Agents Chemother. **19**:56–65.
21. **Zimmermann, W., and A. Rosselet.** 1977. Function of the outer membrane of *Escherichia coli* as a permeability barrier to beta-lactam antibiotics. Antimicrob. Agents Chemother. **12**:368–372.