IN VITRO ANTIBACTERIAL ACTIVITY OF DISODIUM α-SULFOBENZYLPENICILLIN*

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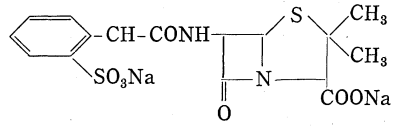
(Received for publication June 4, 1971)

Disodium α-sulfobenzylpenicillin (sulfocillin) shows a potent *in vitro* antibacterial activity against Gram-positive and Gram-negative bacteria. *In vitro* studies of sulfocillin were carried out using the microorganisms lately isolated from clinical source. Sulfocillin demonstrates antibacterial activity against penicillin G resistant strains of staphylococci as nearly strong as that against the sensitive ones. Against *Escherichia coli*, *Proteus vulgaris* and *P. mirabilis*, sulfocillin is also active, while being moderate against *P. morgani* and *Pseudomonas aeruginosa*. The antibacterial activity is somewhat influenced by the inoculum size, being strong in the case of a small inoculum at acidic pH. The activity is influenced neither by the presence of horse serum in the medium, nor by the difference of test media. Protein binding rate of the penicillin examined by cellophane bag dialysis is low. Sulfocillin is relatively stable against staphylococcal penicillinase. Bacterial resistance to sulfocillin is demonstrated stepwise by the serial transfer, as in the case of carbenicillin. Cross resistance is found between sulfocillin and other penicillins against *Staphylococcus aureus* FDA 209P and *Escherichia coli* NIHJ JC-1, which are made resistant to penicillins by serial subcultures in culture medium. Bactericidal and bacteriolytic examinations with *S. aureus* FDA 209P and *E. coli* NIHJ JC-1 reveals strong activities of sulfocillin. The paradoxical reduction of both activities at high concentrations of sulfocillin and carbenicillin was observed with *S. aureus* FDA 209P. Sulfocillin is relatively stable at pH 2.

Sulfocillin is a new semisynthetic penicillin. The physicochemical characteristics of this penicillin are described by NOMURA *et al.*[1] The chemical structure of sulfocillin is as follows:



Disodium α-Sulfobenzylpenicillin

This communication is concerned mainly with the *in vitro* antimicrobial activities, such as the antimicrobial spectrum, sensitivity distribution of microorganisms isolated from clinical source, influence of inoculum size, medium pH and the addition of serum, protein binding, stability against penicillinase, development of resistance, cross

* Generic name of disodium sulfobenzylpenicillin is recommended to be disodium sulfocillin by Committee of Japanese Accepted Names.

resistance, bactericidal and bacteriolytic activity and chemical stability.

Materials and Methods

Penicillin: Disodium sulfocillin, disodium carbenicillin and benzylpenicillin were prepared by Takeda Chemical Industries, Lted., Osaka, Japan, and sodium ampicillin was supplied by Wyeth Laboratories, U.S.A.

Antimicrobial test: The minimum inhibitory concentration of the penicillins was determined by a two-fold serial dilution using Trypticase soy agar (TSA) (BBL) or nutrient agar supplemented with 10 % beef blood (blood-TSA) as culture medium. The test organisms were cultivated for 18~24 hours on TSA or blood-TSA, and one loopful of a suspension containing about 10^8 viable units per ml of test organism was streaked on each assay plate. The plates were incubated at 37°C in the presence of the test penicillins, and the antibacterial readings were determined routinely at the 18 th hour. The minimal inhibitory concentration of the penicillin is defined as the lowest concentration whereby the visible growth of the test organism is completely prevented.

Development of resistance: The development of bacterial resistance against the penicillin was studied with *Staphylococcus aureus* FDA 209P or *Escherichia coli* NIHJ JC-1 cultivated in Trypticase soy broth (TSB) (BBL). Resistant strains were made in such a way that the organism, grown in drug-containing medium as same as the control is transferred successively every 48 hours into the next series of broth tubes containing the same or higher concentrations of penicillin.

Bactericidal activity: The viability of the microorganism in the presence of the drug was determined by the plate count technique. An 18-hour culture of *S. aureus* FDA 209P or *E. coli* NIHJ JC-1 was suspended in 10^3 volumes of TSB, and the antibiotic was added to give concentrations of 0.1, 1, 10 or 100 mcg/ml. Aliquots were withdrawn from each tube prior to incubation and at 2, 4, 6 and 8 hours of incubation at 37°C. Platings were made in duplicate at several dilutions to ensure reliable counts. Colony counts were made 48 hours thereafter.

Bacteriolytic activity: The bacteriolytic effect of the penicillin was observed by optical density measurements. As 18-hour culture of *S. aureus* FDA 209P or *E. coli* NIHJ JC-1 suspended in 10^2 volumes of TSB were cultivated at 37°C under shaking. The penicillin was added to give concentrations of 0.1, 1, 10, 100 or 1,000 mcg/ml at the logarithmic growth phase. The optical density in the presence of the drug was determined by the use of a Coleman Universal Spectrophotometer at 650 mμ.

Protein binding: Five ml of horse serum in a cellophane bag of Visking 20/32 was dialysed against 10 ml of M/15 phosphate buffer (pH 7.2) containing 5 or 10 mcg/ml of the penicillin for 48 hours at 4°C. The percentage binding of the penicillin was calculated as follows:

$$\frac{(a-b)V}{aV} \times 100$$

V=Total volume.
a=Concentration of penicillin in buffer.
b=Concentration of penicillin horse serum.

Inactivation of the penicillin by staphylococcal penicillinase: Ten ml of CY medium devised by NOVIK[2] in T-type tube inoculated with 0.1 ml of 18-hour culture of *S. aureus* 1840, resistance to 400 mcg/ml of benzylpenicillin, was shaked for 16 hours at 37°C. The contents of the tubes were pooled and centrifuged. The supernatant was sterilized by filtration through the Millipore filter of MF 50. The crude enzyme present in the supernatant was separated according to RICHMOND's method[3]. One unit of the enzyme inactivated completely 1 μmole of benzylpenicillin in 1 hour at 37°C (as determined by hydroxylamine method). One and half ml of reaction mixture containing 5 μmole of the substrate

in 8 μmole of tris-HCl buffer (pH 7.5) and also 30 units of enzyme was placed in a water bath at 37°C for assay at 5 minutes intervals. Thus, residual penicillin was assayed following the hydroxylamine method.

Results

Antibacterial Spectrum

The antibacterial activities of sulfocillin and carbenicillin against representative bacterial species is summarized in Table 1.

Sulfocillin and carbenicillin exhibited similar spectra. Both penicillins showed a strong antibacterial activity against Gram-positive bacteria except benzylpenicillin-resistant *Staphylococcus aureus* 1840. *Streptococcus viridans* was moderately sensitive. A strong antibacterial activity against *Neisseria gonorrhoeae* was also noted with both penicillins. Also, the penicillins were active against Gram-negative bacilli including *Pseudomonas aeruginosa*.

Table 1. Antibacterial spectrum of sulfocillin and carbenicillin

Organism	Medium	MIC in mcg/ml	
		Sulfocillin	Carbenicillin
Staphylococcus aureus FDA 209 P	Trypticase soy agar	3.125	3.125
〃 〃 Heatley	〃	3.125	3.125
〃 〃 308 A-1	〃	3.125	3.125
〃 〃 1840	〃	25	50
Streptococcus pyogenes E-14	Trypticase soy agar-10 % beef blood	0.78	0.78
〃 〃 Dick	〃	0.78	0.78
〃 〃 S-8	〃	0.78	0.78
〃 〃 NY-5	〃	0.78	0.39
Streptococcus viridans sp.	〃	12.5	12.5
Diplococcus pneumoniae Type I	〃	1.56	1.56
〃 〃 II	〃	1.56	0.78
〃 〃 III	〃	1.56	0.78
Corynebacterium diphtheriae	〃	6.25	1.56
Bacillus subtilis PCI-219	Trypticase soy agar	0.39	3.125
Neisseria gonorrhoeae	Trypticase soy agar-10 % beef blood	0.39	0.39
Shigella dysenteriae EW-1	Trypticase soy agar	0.78	6.25
Shigella flexneri EW-10	〃	12.5	12.5
〃 〃 EW-40	〃	6.25	3.125
Shigella sonnei EW-33	〃	6.25	6.25
Salmonella paratyphi A	〃	25	12.5
〃 〃 B	〃	6.25	6.25
〃 〃 C	〃	12.5	12.5
Salmonella typhosa Boxhill-53	〃	6.25	6.25
〃 〃 Watson	〃	6.25	6.25
Salmonella typhymurium	〃	6.25	6.25
Escherichia coli NIHJ JC-1	〃	12.5	12.5
〃 〃 NIHJ	〃	6.25	12.5
〃 〃 Umezawa	〃	12.5	12.5
〃 〃 K-12	〃	12.5	25
〃 〃 O-78	〃	12.5	12.5
〃 〃 O-111	〃	6.25	6.25
Vibrio colerae Inaba	〃	3.125	6.25
Klebsiella pneumoniae	〃	25	25
Proteus vulgaris	〃	1.56	1.56
Proteus morganii	〃	50	25
Proteus mirabilis	〃	6.25	6.25
Proteus OX-2	〃	12.5	25
〃 OX-19	〃	25	25
〃 OX-K	〃	6.25	50
Pseudomonas aeruginosa sp.	〃	50	50
〃 〃 U 31	〃	50	50
〃 〃 N 18	〃	3.125	3.125
〃 〃 D 36	〃	100	100
〃 〃 N 10	〃	50	50
〃 〃 P 8	〃	100	100

Inoculum size: One loopful of bacterial suspension (1 mg/ml).

Activity against Clinically-isolated Microorganisms

Recent clinical isolates of various bacteria were made available through the

Fig. 1. Sensitivity distribution of clinically-isolated bacteria against sulfocillin.

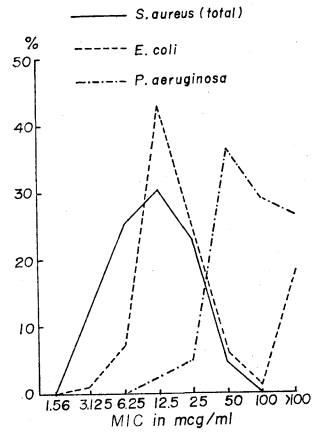


Fig. 2. Sensitivity distribution of clinically-isolated benzylpenicillin-resistant *Staphylococcus aureus* against sulfocillin, carbenicillin, ampicillin and benzylpenicillin.

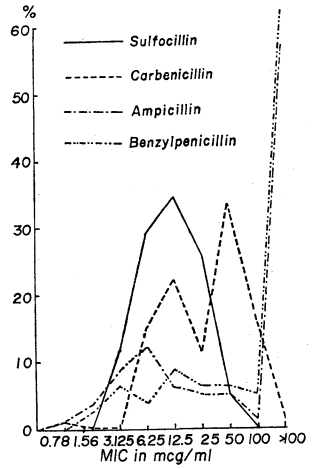
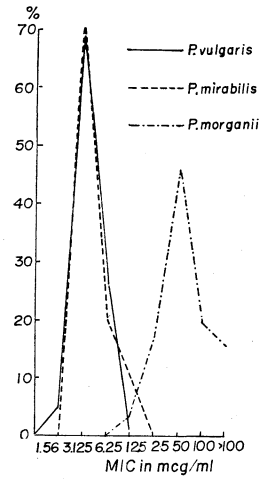


Fig. 3. Sensitivity distribution of clinically-isolated proteus group *Bacilli* against sulfocillin.



courtesy of Miss Y. SHIMIZU, Central Clinical Laboratory, Osaka University Hospital.

Sensitivity distribution of clinically isolated microorganisms, determined by the minimum inhibitory concentrations of sulfocillin is summarized in Table 2, and the percentage distribution of the minimum inhibitory concentrations is diagrammatically shown in Figs. 1 and 3. Sensitivity of the benzylpenicillin resistant staphylococci to sulfocillin is compared with those to carbenicillin, ampicillin and benzylpenicillin. The results are summarized in Table 3, and the percentage distribution of the sensitivity is also diagrammatically shown in Fig. 2.

Table 2. Sensitivity distribution or clinically isolated bacteria against sulfocillin

	MIC in mcg/ml							
	1.56	3.125	6.25	12.5	25	50	100	>100
Staph. aureus (Benzylpenicillin-sensitive)		7	3					
Staph. aureus (Benzylpenicillin-resistant)		5	23	28	21	4		
E. coli		1	6	36	20	5	1	15
Pr. vulgaris	3	41	16					
Pr. mirabilis	7	2	1					
Pr. morganii				1	4	12	5	4
Ps. aeruginosa				1	2	15	12	11

Table 3. Sensitivity distribution of clinically isolated benzylpenicillin resistant *Staph. aureus* against sulfocillin, carbenicillin, ampicillin and benzylpenicillin.

Penicillins	MIC in mcg/ml								
	0.78	1.56	3.125	6.25	12.5	25	50	100	>100
Sulfocillin			5	23	28	21	4		
Carbenicillin	1			12	18	9	27	13	1
Ampicillin	1	3	7	10	5	4	4	1	46
Penicillin G		2	5	3	7	5	5	4	50

1) Sensitivity of the benzylpenicillin-sensitive *S. aureus*: The data in Table 2 indicated that all 10 strains tested were inhibited at concentrations of 1.56~3.125 mcg/ml. These minimum in-

hibitory concentrations observed for clinically-isolated strains were almost identical with those obtained with standard laboratory strains.

2) Sensitivity of the benzylpenicillin resistant *S. aureus*: As shown in Table 3 and Fig. 2, 28 of 81 clinically-isolated benzylpenicillin-resistant strains (34.6 %) as well as the benzylpenicillin-sensitive strains were inhibited at the almost same concentration (3.125~6.25 mcg/ml) of sulfocillin. Most of the strains (49/81, 60.5 %) were inhibited at concentrations of 3.125~25 mcg/ml of this penicillin.

The distribution of minimum inhibitory concentrations of other penicillins were also compared. With carbenicillin, 39 of 81 strains (48.1 %) were inhibited at concentrations of 6.25~12.5 mcg/ml, 40 of 81 strains (49.4 %) at 6.25~12.5 mcg/ml, and 40 of 81 strains (49.4 %) at 50~100 mcg/ml. By respective ampicillin and benzylpenicillin, in contrast, 46 of 81 strains (56.8 %) and 50 of 81 strains (61.7 %) were not inhibited at the concentration of 100 mcg/ml.

3) Sensitive of *E. coli*: Sixty-three of 84 strains of *E. coli* (75 %) were inhibited by concentrations of sulfocillin below 25 mcg/ml. The inhibition of the standard laboratory strain of *E. coli* was noted at concentrations of 6.25~12.5 mcg/ml.

4) Sensitivity of *Proteus* group: Sixty strains of *P. vulgaris*, 10 strains of *P. mirabilis* and 26 strains of *P. morganii* were tested for their sensitivity. *P. vulgaris* and *P. mirabilis* showed similar distribution patterns in sensitivity. All strains of *P. vulgaris* were inhibited at concentrations of 1.56~6.25 mcg/ml of sulfocillin. Also, all strains of *P. mirabilis* were inhibited at concentrations of 3.125~12.5 mcg/ml. However, the sensitivity of *P. morganii* against sulfocillin was less than that of the two other *Proteus* groups. The strains of *P. morganii* were inhibited at concentrations of higher than 12.5 mcg/ml.

5) Sensitivity of *P. aeruginosa*: Thirty of 41 stratins were inhibited in their

Table 4. Effect of inoculum size on antibacterial activity of sulfocillin and carbenicillin

Organism	MIC in mcg/ml											
	Sulfocillin						Carbenicillin					
	10^3	10^4	10^5	10^6	10^7	10^8	10^3	10^4	10^5	10^6	10^7	10^8
Staph. aureus FDA 209P	0.39	0.39	0.78	1.56	3.125	3.125	0.1	0.2	0.39	0.78	1.56	3.125
〃 Heatley	0.1	0.2	0.39	0.39	1.56	3.125	0.05	0.1	0.2	0.39	0.78	1.56
〃 308 A-1	0.78	1.56	1.56	1.56	1.56	3.125	0.39	0.78	0.78	0.78	1.56	1.56
E. coli NIHJ JC-1	6.25	6.25	12.5	12.5	12.5	12.5	6.25	6.25	6.25	12.5	12.5	12.5
〃 NIHJ	1.56	1.56	3.125	3.125	6.25	6.25	1.56	1.56	3.125	3.125	6.25	6.25
〃 Umezawa	3.125	3.125	6.25	6.25	12.5	12.5	3.125	6.25	6.25	6.25	12.5	25
K. pneumoniae	1.56	1.56	3.125	6.25	6.25	12.5	1.56	1.56	1.56	6.25	6.25	12.5
Pr. vulgaris	0.78	0.78	0.78	0.78	1.56	3.125	0.2	0.78	0.78	0.78	0.78	0.78
Ps. aeruginosa U 31	25	25	25	50	100	100	25	25	50	50	100	100
〃 N 18	0.2	0.2	0.4	1.56	1.56	6.25	0.2	0.39	0.39	0.78	3.125	6.25
〃 D 36	25	25	25	50	50	100	50	50	50	50	100	100
〃 P 8	12.5	12.5	12.5	25	50	100	25	25	25	25	50	100
〃 sp.	12.5	25	50	50	100	100	25	25	25	50	50	100

Inoculum size: One loopful of bacterial suspension. Medium: Trypticase soy agar.

Table 5. Effect of medium pH on antibacterial activity of sulfocillin and carbenicillin

Organism	MIC in mcg/ml							
	Sulfocillin				Carbenicillin			
	pH 6.0	pH 7.0	pH 8.0	pH 9.0	pH 6.0	pH 7.0	pH 8.0	pH 9.0
Staph. aureus FDA 209P	0.78	3.125	3.125	3.125	0.78	3.125	3.125	3.125
〃 Heatley	0.39	1.56	1.56	3.125	0.39	1.56	1.56	3.125
〃 308 A-1	0.78	1.56	3.125	3.125	0.78	1.56	3.125	3.125
E. coli NIHJ JC-1	12.5	12.5	50	25	12.5	12.5	12.5	50
〃 NIHJ	3.125	6.25	12.5	6.25	3.125	3.125	3.125	6.25
〃 Umezawa	12.5	12.5	25	50	12.5	6.25	12.5	12.5
K. pneumoniae	12.5	12.5	12.5	25	12.5	12.5	25	25
Pr. vulgaris	0.78	1.56	1.56	3.125	0.39	0.78	1.56	1.56
Ps. aeruginosa U 31	50	100	100	100	100	200	200	200
〃 N 18	6.25	6.25	6.25	12.5	3.125	6.25	6.25	12.5
〃 D 36	100	100	100	100	100	100	200	200
〃 P 8	50	100	400	400	100	100	200	200
〃 sp.	50	50	100	100	50	100	100	100

Inoculum size: One loopful of bacterial suspension (10^8 V.U./ml). Medium: Trypticase soy agar.

Table 6. Effect of horse serum concentrations in medium on antibacterial activity of sulfocillin and carbenicillin

Organism	MIC in mcg/ml							
	Sulfocillin				Carbenicillin			
	0 %	10 %	20 %	50 %	0 %	10 %	20 %	50 %
Staph. aureus FDA 209P	3.125	3.125	3.125	3.125	1.56	1.56	1.56	1.56
〃 Heatley	1.56	1.56	3.125	3.125	0.39	0.78	1.56	3.125
〃 308 A-1	3.125	3.125	3.125	6.25	3.125	3.125	3.125	6.25
E. coli NIHJ JC-1	12.5	12.5	25	12.5	6.25	6.25	6.25	6.25
〃 NIHJ	6.25	6.25	3.125	3.125	3.125	3.125	3.125	3.125
〃 Umezawa	12.5	12.5	12.5	12.5	12.5	12.5	12.5	6.25
K. pneumoniae	12.5	12.5	12.5	12.5	12.5	12.5	6.25	1.56
Pr. vulgaris	1.56	3.125	3.125	3.125	1.56	3.125	0.78	0.78
Ps. aeruginosa U 31	200	200	200	200	100	100	50	100
〃 N 18	12.5	6.25	12.5	3.125	3.125	3.125	6.25	6.25
〃 D 36	200	200	100	200	200	100	100	50
〃 P 8	25	50	50	50	50	100	200	100
〃 sp.	100	100	100	100	100	100	100	50

Inoculum size: One loopful of bacterial suspension (10^8 V.U./ml). Medium: Trypticase soy broth.

growth at concentration of 12.5~100 mcg/ml of sulfocillin, and 11 of 41 strains were not inhibited at 100 mcg/ml. These minimum inhibitory concentrations were equivalent to those obtained with standard laboratory strains.

Influence of inoculum size, medium pH, addition of serum and difference in media on the activity of sulfocillin: The minimum inhibitory concentrations of sulfocillin against 3 strains of *S. aureus*, 3 strains of *E. coli*, 1 strain of *K. pneumoniae*, 1 strain of *P. vulgaris* and 5 strains of *P. aeruginosa* were observed with various inoculum size or conditions of media.

The results shown in Table 4 demonstrate that an increase in the minimum inhibitory concentration of the penicillin against test organisms occurred as the inoculum size was increased from 10^3 to 10^8. Increase in the minimum inhibitory concentration of the drug varied according to the bacterial species.

Table 5 demonstrates the influence of the pH of the medium on the minimum inhibitory concentration of the penicillin against test organisms. The antibacterial activity of sulfocillin at pH 6.0 was more than that at pH 9.0.

As shown in Table 6, the addition of 50 % horse serum to the medium did not influence the inhibitory activity.

The minimum inhibitory concentration of the drug in various media is shown in Table 7. The antibacterial activity of the penicillin was not affected by the media used.

Table 7. Effect of several medium on antibacterial activity of sulfocillin

Organism	MIC in mcg/ml				
	TSA*	NA*	NH*	HI*	BHI*
Staph. aureus FDA 209P	3.125	3.125	3.125	3.125	3.125
〃 Heatley	1.56	1.56	1.56	1.56	1.56
〃 308 A-1	3.125	3.125	3.125	3.125	3.125
E. coli NIHJ JC-1	12.5	12.5	12.5	12.5	12.5
〃 NIHJ	6.25	6.25	12.5	6.25	12.5
〃 Umezawa	12.5	12.5	12.5	12.5	12.5
K. pneumoniae	12.5	12.5	12.5	12.5	25
Pr. vulgaris	1.56	1.56	1.56	1.56	1.56
Ps. aeruginosa U 31	50	50	100	50	50
〃 N 18	3.125	3.125	3.125	6.25	3.125
〃 D 36	100	50	50	50	50
〃 P 8	50	50	50	50	100
〃 sp.	50	50	50	50	100

* TSA=Trypticase soy agar (BBL) HI=Heart infusion agar (Eiken).
NA=Nutrient agar (Eiken). BHI=Brain heart infusion agar (Eiken).
MH=MUELLER-HINTON medium (Eiken).

Table 8. Binding of sulfocillin and carbenicillin by horse serum

Penicillin	Concentration (mcg/ml) before dialyse	Concentration (mcg/ml) after dialysed to		Per cent bound	Mean per cent bound
		Horse serum	Buffer		
Sulfocillin	10	4.88	7.64	36.13	27.17
		4.1	5.3	22.64	
		3.83	4.95	22.73	
	5	2.81	4.3	34.67	28.19
		2.75	4.3	36.05	
		2.01	2.68	25.19	
Carbenicillin	10	5.4	7.5	28.0	18.55
		4.95	5.5	10.0	
		4.78	5.8	17.67	
	5	2.7	3.44	21.49	20.64
		3.09	4.3	28.26	
		2.73	3.35	18.66	
		2.83	3.3	14.25	

Binding with serum protein: The results of dialysis experiment are shown in Table 8. The variations of the binding capacity of protein between individual experiments were slight. Sulfocillin was found to bind to a low degree, *i.e.*, the protein binding rate was calculated to be 27.17 % at a concentration of 10 mcg/ml or 28.19 % at 5 mcg/ml.

Behavior of Sulfocillin against Staphylococcal Penicillinase

The rates of hydrolysis at 37°C of sulfocillin, carbenicillin, ampicillin, and benzylpenicillin in the presence of penicillinase obtained from *S. aureus* 1840 are plotted against the incubation time in Fig. 4. Fifty per cent inactivation of sulfocillin by

Fig. 4. Behavior of sulfocillin, carbenicillin, ampicillin and benzylpenicillin against penicillinase produced by *Staphylococcus aureus* 1840.

Five-tenth ml of reaction mixture which contained the substrate 5 μmoles, Tris-HCl buffer (pH 7.5) 8 μmoles and enzyme 30 units (for benzylpenicillin) were placed in a water bath at 37℃, and individual tubes were withdrawn for assay at 5 minutes intervals. Residual penicillin was calculated hydroxyl amine method.

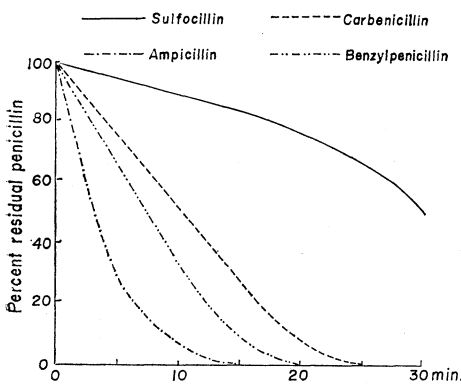
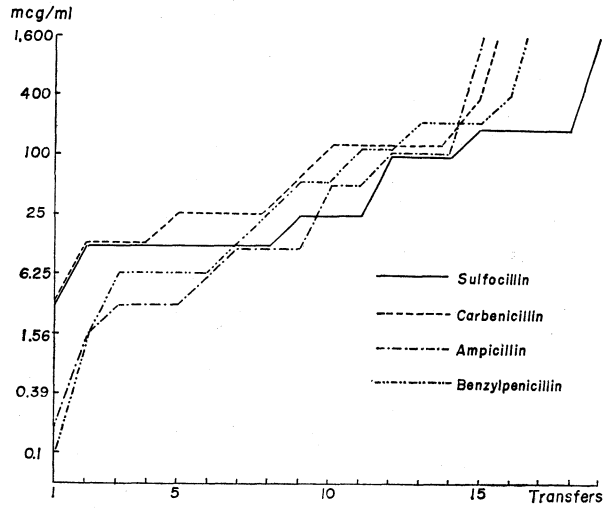


Fig. 5. Patterns of development of resistance of *S. aureus* FDA 209P to sulfocillin, carbenicillin, ampicillin and benzylpenicillin.



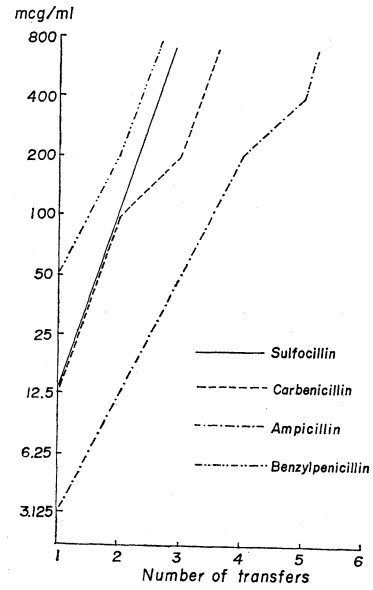
staphylococcal penicillinase was observed within 30 minutes. However, complete inactivation of ampicillin, benzylpenicillin and carbenicillin by staphylococcal penicillinase was observed within 15 minutes, 20 and 25 minutes, respectively.

Development of Resistance

The development of resistance of *S. aureus* FDA 209P and *E. coli* NIHJ JC-1 to sulfocillin, carbenicillin, ampicillin and benzylpenicillin was tested.

The rapidity and degree of the resistance of staphylococci developed *in vitro* to four penicillins are shown in Fig. 5. The resistance to sulfocillin and carbenicillin proceeded slowly, while those to ampicillin and benzylpenicillin were rapid during the 1st to 3rd passage, but slow during the 3rd to 13th passage. At the 15th to 19th transfer, however, resistance to all penicillins resulted in the minimum inhibitory concentration of 400 mcg/ml.

Fig. 6. Patterns of development of resistance of *E. coli* NIHJ JC-1 to sulfocillin, carbenicilin, ampicillin and benzylpenicillin.



As shown in Fig. 6, when *E. coli* is used as the test organism, the bacterial resistance to four penicillins appeared more rapid. At the 3rd to 6th passages, the acquired resistance to four penicillins resulted in the minimum inhibitory concentration of 400 mcg/ml.

Cross Resistance

Cross resistance was studied with *S. aureus* FDA 209P or *E. coli* NIHJ JC-1 which had been made resistant, respectively to sulfocillin, carbenicillin, ampicillin, and benzylpenicillin by serial subcultures in TSB containing penicillin. Each resistant strain was tested for their sensitivity against four penicillins by the agar dilution method. Mutual cross resistances of bacteria among the four penicillins was confirmed as shown in Table 9.

Table 9. Cross resistance test among sulfocillin, carbenicillin, ampicillin and benzylpenicillin

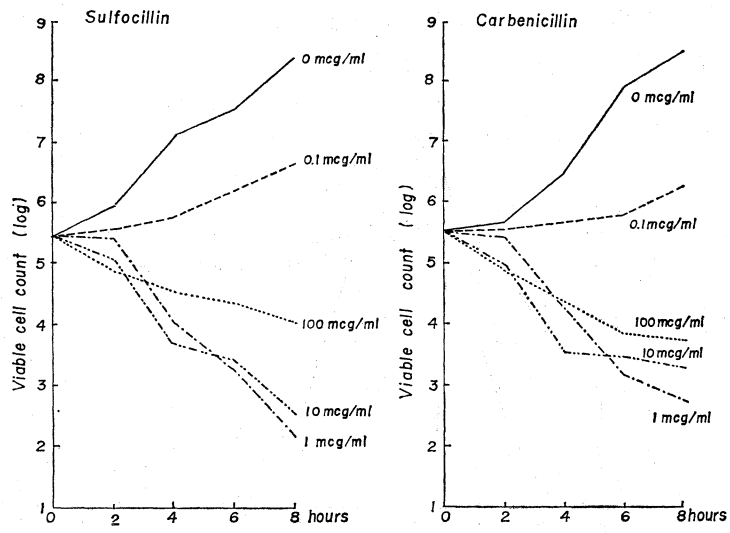
Organism	MIC in mcg/ml			
	Sulfocillin	Carbenicillin	Ampicillin	Benzyl-penicillin
Staph. aureus FDA 209P (parent)	3.125	3.125	0.05	0.025
R-Sulfocillin	>400	>400	100	200
R-Carbenicillin	>400	>400	400	>400
R-Ampicillin	>400	100	>400	200
R-Benzylpenicillin	400	50	200	200
E. coli NIHJ JC-1 (parent)	12.5	12.5	3.125	25
R-Sulfocillin	>400	>400	25	100
R-Carbenicillin	>400	>400	50	>400
R-Ampicillin	>400	400	>400	>400
R-Benzylpenicillin	400	100	100	400

Bactericidal Activity

The bactericidal effect of sulfocillin to *S. aureus* FDA 209P and *E. coli* NIHJ JC-1 are shown in Figs. 7 and 8. The viability of microorganisms cultivated in TSB with various amounts of the penicillin were determined by plate count. The viable counts in logarithmic scale are plotted against time of exposure to the antibiotic.

When the penicillin was added simultaneously, the bactericidal action on *S. aureus* FDA 209P was markedly demonstrated at the concentration of 1 mcg/ml or 10 mcg/ml of the sulfocillin. At the concentration of 0.1 mcg/ml, weak bactericidal activity was observed. The paradoxical reduction of the penicillin activity at 100 mcg/ml as reported by EAGLE and MUSSELMAN[4] was also demonstrated with sulfocillin at the same concentration.

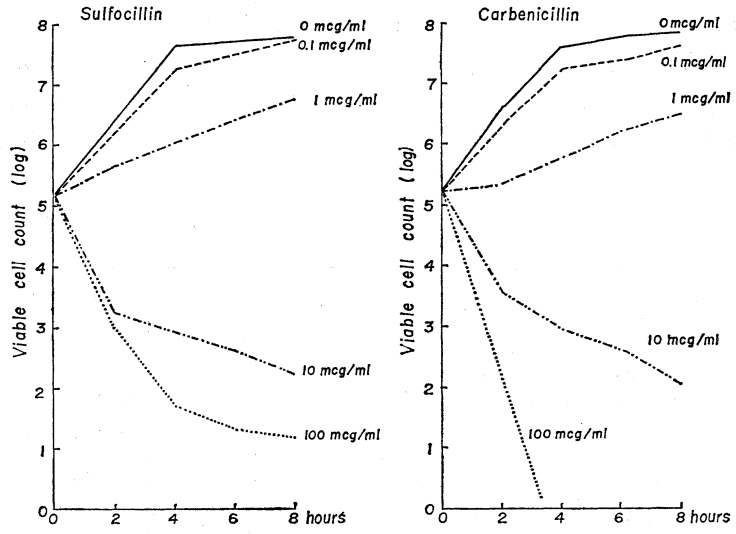
Fig. 7. Bactericidal activity of sulfocillin and carbenicillin on *S. aureus* FDA 209P.



Marked bactericidal action against *E. coli* NIHJ JC-1 was also

demonstrated at concentrations of 10 mcg/ml and 100 mcg/ml. At the concentration of 1 mcg/ml, the effect was weak, and at concentration of 0.1 mcg/ml the bactericidal activity disappeared (Fig. 8).

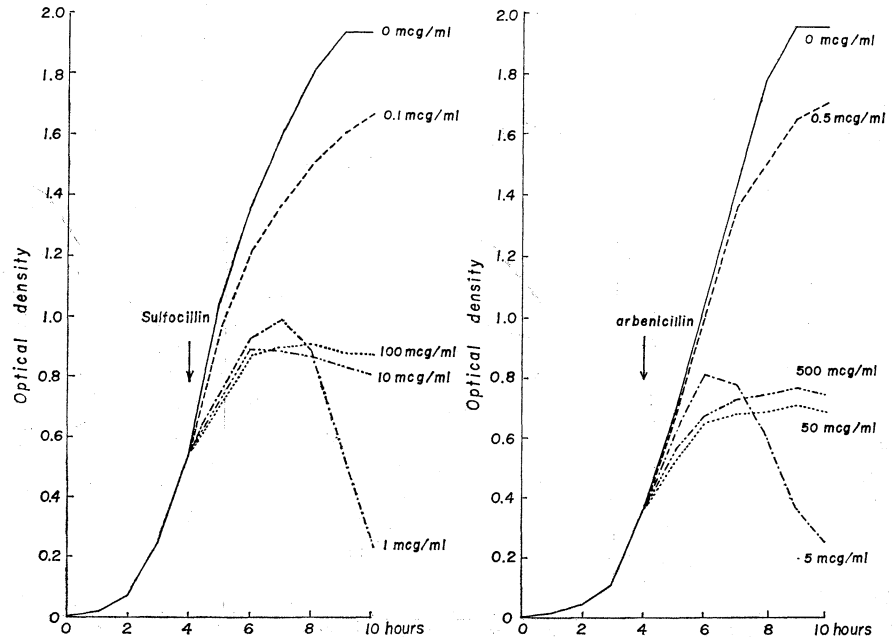
Fig. 8. Bactericidal activity of sulfocillin and carbenicillin on *E. coli* NIHJ JC-1.



Bacteriolytic Activity

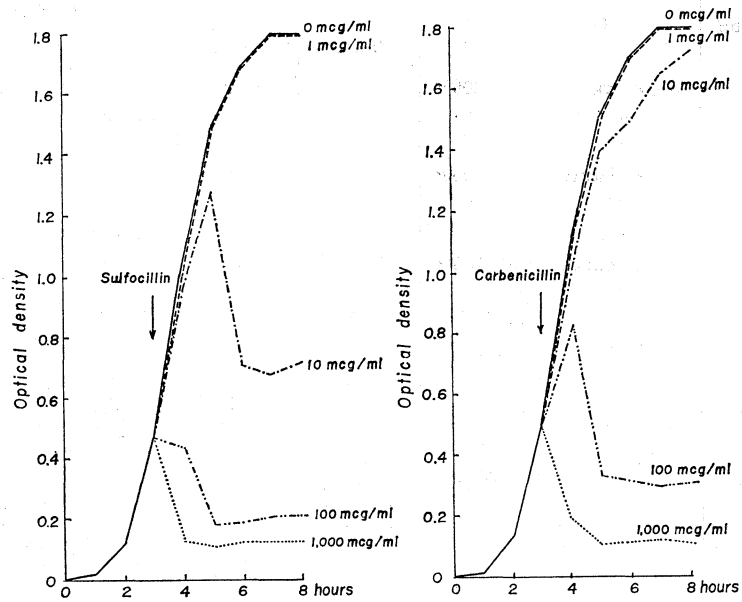
The bacteriolytic effect of sulfocillin to *S. aureus* FDA 209P and *E. coli* NIHJ JC-1 are shown in Figs. 9 and 10. The optical density of microorganisms cultivated in TSB with various concentrations of the penicillin were determined with a spectrophotometer. Four hours after the start of the shaking, the penicillin was added to the *S. aureus* culture. The lytic action of sulfocillin was marked at the concentration of 1 mcg/ml. The weak bacteriolytic effect was observed at the concentration of 0.1 mcg/ml. At concentrations of 10 mcg/ml and 100 mcg/ml, the paradoxical reduction of the activity of the penicillin was observed (Fig. 9).

Fig. 9. Bacteriolytic activity of sulfocillin and carbenicillin on *S. aureus* FDA 209P.



Three hours after the start of shaking, the penicillin was added to *E. coli* NIHJ JC-1 culture. The strongly lytic action was observed at concentrations of 100 mcg/ml or 1,000 mcg/ml of sulfocillin, though at a concentration of 10 mcg/ml lysis took place only to a more minor extent.

Fig. 10. Bacteriolytic activity of sulfocillin and carbenicillin on *E. coli* NIHJ JC-1.



Stability of Sulfocillin

Sulfocillin solutions at pH 2 (glycocol-HCl buffer), pH 4 (glycocol-HCl buffer), pH 7 (phosphate buffer) and pH 9 (glycocol-NaOH buffer) were kept at 4°C, 37°C and 100°C. The growth inhibitory activity against 3 strains of *S. aureus* was assayed at various lengths of time of standing.

Table 10 shows the stability of the penicillin solution at the various pH levels at 100°C. Sulfocillin lost antibacterial activity when kept at pH 2 for 5 minutes. However, at pH 4 to 9, the antibacterial activity remained without inactivation after 30 minutes.

Sulfocillin, as shown in Tables 11 and 12, is stable at 37°C and pH 2 for 5 hours, and no antibacterial activity was lost by incubation of 100 mcg/ml solution for one day. At pH 4 and 9, sulfocillin was moderately inactivated. The activity of the penicillin solution at pH 7 was not significantly affected after 7 days.

Table 10. Effect of pH on the antibacterial activity of sulfocillin in solution at 100°C

pH	Organism	MIC in mcg/ml				
		0 min.	5 min.	10 min.	20 min.	30 min.
2	*Staph. aureus* FDA 209P	3.125	>100	>100	>100	>100
	〃 Heatley	1.56	>100	>100	>100	>100
	〃 308 A-1	1.56	>100	>100	>100	>100
4	*Staph. aureus* FDA 209P	3.125	3.125	6.25	12.5	25
	〃 Heatley	1.56	1.56	3.125	12.5	12.5
	〃 308 A-1	1.56	1.56	3.125	6.25	12.5
7	*Staph. aureus* FDA 209P	3.125	3.125	3.125	6.25	6.25
	〃 Heatley	1.56	1.56	1.56	3.125	3.125
	〃 308 A-1	3.125	3.125	3.125	6.25	6.25
9	*Staph. aureus* FDA 209P	3.125	3.125	3.125	3.125	3.125
	〃 Heatley	1.56	1.56	1.56	1.56	1.56
	〃 308 A-1	3.125	3.125	3.125	3.125	3.125

Table 11. Effect of pH on the antibacterial activity of sulfocillin in solution at 37°C

pH	Organism	MIC in mcg/ml					
		0 hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.
2	*Staph. aureus* FDA 209P	3.125	3.125	3.125	6.25	6.25	6.25
	〃 Heatley	1.56	1.56	3.125	3.125	6.25	6.25
	〃 308 A-1	3.125	3.125	3.125	6.25	6.25	12.5
4	*Staph. aureus* FDA 209P	3.125	3.125	3.125	3.125	6.25	6.25
	〃 Heatley	1.56	3.125	3.125	3.125	3.125	3.125
	〃 308 A-1	3.125	3.125	3.125	3.125	3.125	6.25
7	*Staph. aureus* FDA 209P	3.125	3.125	3.125	3.125	3.125	3.125
	〃 Heatley	3.125	1.56	1.56	1.56	1.56	1.56
	〃 308 A-1	3.125	3.125	3.125	3.125	3.125	3.125
9	*Staph. aureus* FDA 209P	3.125	3.125	3.125	3.125	3.125	3.125
	〃 Heatley	1.56	1.56	1.56	1.56	1.56	1.56
	〃 308 A-1	3.125	3.125	3.125	3.125	3.125	3.125

Table 12. Effect of pH on the antibacterial activity of sulfocillin in solution at 37°C

pH	Organism	MIC in mcg/ml						
		0 day	1 day	2 days	3 days	4 days	5 days	7 days
2	*Staph. aureus* FDA 209P	3.125	100	100	100	100	100	100
	〃 Heatley	1.56	100	100	100	100	100	100
	〃 308 A-1	3.125	100	100	100	100	100	100
4	*Staph. aureus* FDA 209P	3.125	3.125	3.125	6.25	6.25	6.25	25
	〃 Heatley	1.56	1.56	1.56	3.125	3.125	3.125	12.5
	〃 308 A-1	3.125	3.125	3.125	6.25	6.25	6.25	25
7	*Staph. aureus* FDA 209P	3.125	3.125	3.125	3.125	3.125	3.125	3.125
	〃 Heatley	1.56	1.56	1.56	1.56	1.56	1.56	1.56
	〃 308 A-1	3.125	3.125	3.125	3.125	3.125	3.125	3.125
9	*Staph. aureus* FDA 209P	3.125	3.125	3.125	6.25	6.25	12.5	25
	〃 Heatley	1.56	1.56	1.56	3.125	3.125	12.5	12.5
	〃 308 A-1	3.125	3.125	3.125	3.125	6.25	12.5	12.5

Table 13. Effect of pH on the antibacterial activity of sulfocillin in solution at 4°C

pH	Organism	MIC in mcg/ml						
		0 day	1 day	2 days	3 days	4 days	5 days	7 days
2	*Staph. aureus* FDA 209P	3.125	3.125	3.125	3.125	3.125	3.125	6.25
	〃 Heatley	1.56	1.56	1.56	1.56	1.56	1.56	3.125
	〃 308 A-1	3.125	3.125	3.125	3.125	3.125	3.125	6.25
4	*Staph. aureus* FDA 209P	3.125	3.125	3.125	3.125	3.125	3.125	3.125
	〃 Heatley	1.56	1.56	1.56	1.56	1.56	1.56	1.56
	〃 308 A-1	3.125	3.125	3.125	3.125	3.125	3.125	3.125
7	*Staph. aureus* FDA 209P	3.125	3.125	3.125	3.125	3.125	3.125	3.125
	〃 Heatley	1.56	1.56	1.56	1.56	1.56	1.56	1.56
	〃 308 A-1	3.125	3.125	3.125	3.125	3.125	3.125	3.125
9	*Staph. aureus* FDA 209P	3.125	3.125	3.125	3.125	3.125	3.125	6.25
	〃 Heatley	1.56	1.56	1.56	1.56	1.56	1.56	3.125
	〃 308 A-1	3.125	3.125	3.125	3.125	3.125	3.125	6.25

As shown in Table 13, sulfocillin solution was stable at 4°C and at pH levels of 2~9 for 7 days.

Discussion

The antibacterial spectrum of sulfocillin was determined by the usual dilution methods. Sulfocillin is active against Gram-positive and Gram-negative bacteria. The activity of this penicillin against the benzylpenicillin-resistant staphylococci was approximately equivalent of that against sensitive staphylococci. This is partly derived from the stable nature of this penicillin against staphylococcal penicillinase. The most significant feature of the antibacterial spectrum of sulfocillin is the antibacterial activity against *P. aeruginosa*. In fact, 73.2 % of *P. aeruginosa* strains were inhibited at concentration of 12.5～100 mcg/ml of sulfocillin. Chemically, carbenicillin is reported to be unstable and is contaminated with a few percent of benzylpenicillin, while sulfocillin is stable and does not evaluated benzylpenicillin even after storage at high temperature. However, as far as the *in vitro* antibacterial activities are concerned, sulfocillin and carbenicillin is similar in activity.

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