Carbenicillin*: A New Semisynthetic Penicillin Active against *Pseudomonas pyocyanea*

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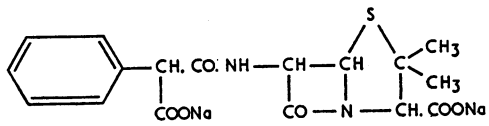
Carbenicillin (disodium α-carboxybenzylpenicillin; BRL 2064) is a new semisynthetic penicillin with a wide spectrum of activity against Gram-positive and Gram-negative bacteria. In particular, carbenicillin shows a certain level of activity against *Pseudomonas pyocyanea* (*Pseudomonas aeruginosa*), and the compound is also active against many strains of *Proteus vulgaris*, *Proteus rettgeri*, and *Proteus morganii*, which are typically resistant to ampicillin.

The structure of carbenicillin is as follows:



The compound, which should be stored in the refrigerator, is readily soluble in water, giving a clear neutral solution, which shows no significant loss of activity after 24 hours at room temperature and is stable in the refrigerator for at least seven days. In acid solution carbenicillin is relatively unstable, the half-life at *p*H 2 being approximately two hours at room temperature and approximately 30 minutes at 37° C.

Antibacterial Activity of Carbenicillin

The antibacterial spectrum of carbenicillin is shown in Table I. Minimum inhibitory concentrations were determined by serial dilution of the drug in agar, and one drop of an overnight broth culture of each organism was used as inoculum.

TABLE I.—*Antibacterial Spectrum of Carbenicillin*

	Minimum Inhibitory Concentration (μg./ml.)*
Escherichia coli	5·0
Klebsiella aerogenes	250
Salmonella typhi	12·5
Shigella flexneri	5·0
„ *sonnei*	5·0
Pseudomonas pyocyanea	50
Proteus mirabilis	2·5
„ *morganii*	5·0
„ *rettgeri*	2·5
„ *vulgaris*	5·0
Haemophilus influenzae	0·5
Staphylococcus aureus Oxford	0·5
„ *aureus*†	50
β-Haemolytic streptococcus	0·25
Streptococcus faecalis	25
„ *pneumoniae*	0·5
Bacillus subtilis	1·25
Sarcina lutea	0·5
Clostridium tetani	0·25
„ *welchii*	0·25

* Serial dilution in agar; inoculum, one drop of an overnight culture.
† Penicillinase-producing strain.

Carbenicillin is active against *Escherichia coli*, *Salmonella* species, *Shigella* species, *Proteus mirabilis*, and *Haemophilus influenzae*, and in this respect the spectrum of activity is similar to that of ampicillin, although carbenicillin is in general rather less active than ampicillin. Most strains of *Klebsiella aerogenes* are resistant to carbenicillin and the level of activity against enterococci (*Streptococcus faecalis*) is relatively low.

Carbenicillin is active against pyogenic streptococci, pneumococci, and penicillin-sensitive strains of staphylococci, although the compound is considerably less active in this respect than penicillin G or ampicillin. Carbenicillin is not stable to staphylococcal penicillinase and is not active against penicillin-resistant strains of staphylococci. The activity of carbenicillin against several strains of *Pr. vulgaris*, *Pr. rettgeri*, and *Pr. morganii* is shown in Table II. These species of proteus are characteristically resistant to ampicillin, and in the experiment shown in Table II all the strains required an ampicillin concentration of at least 50 μg./ml. to prevent growth. In contrast, many of these strains were relatively sensitive to carbenicillin and in some cases were inhibited by concentrations as low as 1.25 μg./ml. Strains of *Pr. mirabilis*, however, which are resistant to ampicillin are also resistant to carbenicillin. The reason for the activity which carbenicillin shows against certain ampicillin-resistant proteus appears to be due to greater stability of the compound towards the penicillinases produced by these organisms. On the other hand, penicillinase-producing strains of *Pr. mirabilis*, like those of *Kl. aerogenes*, inactivate both carbenicillin and ampicillin.

TABLE II.—*Activity of Carbenicillin Against Ampicillin-resistant Strains of Proteus*

		Minimum Inhibitory Concentration (μg./ml.)*	
		Carbenicillin	Ampicillin
Proteus morganii	A	1·25	50
	F	1·25	50
	G	1·25	50
rettgeri	A	1·25	50
	I	2·5	250
	B	12·5	250
vulgaris	I	2·5	250
	A	5·0	125
	E	5·0	250
	G	50	> 500
	H	250	> 500
„ *mirabilis*	8	> 500	> 500
	889	> 500	> 500

* Determined by serial dilution in agar. The inoculum was one drop of an overnight broth culture, and M.I.C. values were read after 18 hours at 37° C.

The most significant feature of the antibacterial spectrum of carbenicillin is the activity against *Ps. pyocyanea*. In terms of the minimum inhibitory concentration the activity against this pathogen is relatively low, since for most strains a concentration in the order of 50 μg./ml. is required to prevent growth. However, from the clinical point of view activity is essentially meaningful in relation to the concentration of drug which can be reached at the site of infection. Carbenicillin itself is remarkably free from toxic effects (Acred *et al.*, 1967) and can be administered in sufficient dosage to obtain concentrations in the serum which are inhibitory to typical strains of *Ps. pyocyanea*, and the drug is also excreted in high concentration in the urine.

One characteristic of the activity of carbenicillin against *Ps. pyocyanea* is that in serial dilution tests to determine the minimum inhibitory concentration the drug does not show a sharp end-point when the inoculum is very heavy. This is illustrated in Fig. 1. Serial dilutions of carbenicillin in nutrient

* Pyopen is the registered trade mark of Beecham Research Laboratories for carbenicillin.

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agar were prepared in Petri dishes and an area of the agar surface of each plate was inoculated with an undiluted overnight broth culture of a strain of *Ps. pyocyanea* applied with a cotton-wool swab. In this way the inoculum on each plate was at least 10 million organisms. A similar series of plates was also inoculated in the same way, using the same culture of *Ps. pyocyanea* but diluted 1/100 to give an inoculum of approximately 100,000 organisms. The strain of *Ps. pyocyanea* used in this experiment was a recent clinical isolate and was typical with regard to its sensitivity to carbenicillin. With the use of the undiluted culture as inoculum growth was very markedly diminished at a concentration of 25 μg./ml. carbenicillin, but a number of colonies were present even at a concentration of 50 μg./ml. and growth could only be said to have been prevented on the plate containing 125 μg./ml. However, when the inoculum was diluted 1/100 inhibition of growth was very pronounced at a concentration of 12.5 μg./ml., and at a concentration of 25 μg./ml. only three or four colonies were seen.

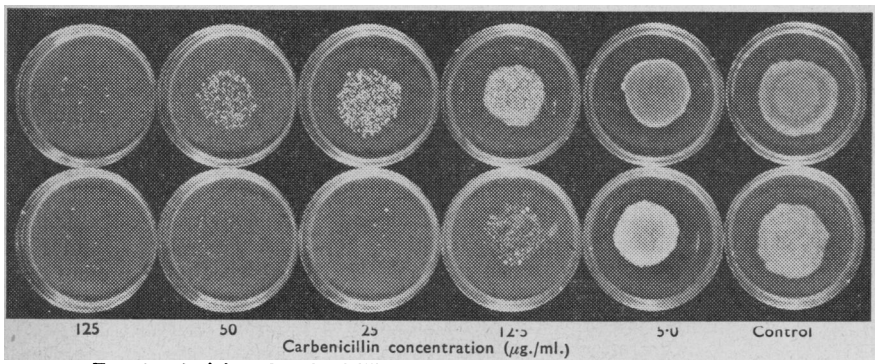

FIG. 1.—Activity of carbenicillin against a typical strain of *Ps. pyocyanea*. Serial dilutions of carbenicillin in nutrient agar were prepared in Petri dishes and an area of the agar surface of each plate was inoculated, using a cotton-wool swab. An undiluted overnight broth culture of *Ps. pyocyanea* was used to inoculate the upper row of plates and a 1/100 dilution of the same culture was used to inoculate the lower row.

Thus, in experiments of the type shown in Fig. 1, with the use of typical strains of *Ps. pyocyanea* and a very heavy inoculum level, some scanty growth may be seen with concentrations of carbenicillin as high as 125 μg./ml., and consequently if the criterion of the "minimum inhibitory concentration" is one of complete inhibition of all trace of growth the M.I.C. with a heavy inoculum may well be as high as 250 μg./ml. or more. However, the amount of growth at such concentrations is extremely meagre indeed, and with an inoculum only slightly smaller a sharp end-point is seen with complete inhibition of growth at a concentration of carbenicillin of 25–50 μg./ml.

Minimum inhibitory concentrations of carbenicillin for 111 clinical isolates of *Ps. pyocyanea* are shown in Table III. These inhibitory concentrations were determined by preparing serial dilutions of carbenicillin in agar as described above, and one drop of a 1/100 dilution of an overnight broth culture of each isolate was used as inoculum. It will be seen from Table III that the majority of the strains were inhibited by a concentration of 25–50 μg./ml. carbenicillin. In comparison, the inhibitory concentrations of ampicillin and benzylpenicillin against most strains of *Ps. pyocyanea* are of the order of 1,000–5,000 μg./ml. or greater.

TABLE III.—*Activity of Carbenicillin Against Pseudomonas pyocyanea*

No. of Strains	Minimum Inhibitory Concentration (μg./ml.)* and No. of Strains					
	250	125	50	25	12·5	5·0
111	5	8	61	31	6	

* Determined by serial dilution in agar. The inoculum was one drop of a 1/100 dilution of an overnight broth culture, and M.I.C. values were read after 18 hours at 37° C.

The effect of the inoculum size on the determination of the minimum inhibitory concentration is not due to destruction of the drug as in the case of penicillin G and penicillin-resistant staphylococci. Carbenicillin is itself stable to the penicillinase produced by typical strains of *Ps. pyocyanea*. Furthermore, the effect of inoculum size does not appear to be due to growth of resistant mutants present in a large inoculum. When tests are carried out using as inoculum the growth which occurs in the presence of high concentrations of carbenicillin the result obtained is the same as that seen with the original culture—that is, marked suppression of growth at concentrations of 25 μg./ml., but with scanty growth nevertheless persisting up to a concentration as high as 125 μg./ml.

As might be expected, this effect of inoculum size on the minimum inhibitory concentration of carbenicillin is also seen with tests carried out in liquid medium. With a heavy inoculum—for example, one drop of undiluted broth culture—growth appears normal with formation of pigment and pellicle up to a concentration of approximately 12.5 μg./ml. carbenicillin, but at 25 μg./ml. there is generally absence of pellicle and pigment and a markedly diminished amount of growth, although complete inhibition of growth may be obtained only with concentrations as high as 250 μg./ml. With a slightly smaller inoculum a sharp end-point is obtained, and with most strains of pseudomonas the minimum inhibitory concentration under these conditions is usually about 50 μg./ml.

Carbenicillin is bactericidal, and typical results with a strain of *Ps. pyocyanea* are shown in Fig. 2. Over the first seven hours a concentration of 50 μg./ml. usually results in a kill of at least 99% of the original inoculum, but thereafter some resumption of growth takes place which may even increase to a visible amount after 24 hours. When this growth is used as inoculum in a repeat test the same kill is again obtained over the first seven hours, followed by a certain amount of growth. The reason for this bactericidal effect followed by growth is not known, but it would not appear to be due to the selection of resistant mutants present in the original inoculum, nor is it due to inactivation of carbenicillin during the experiment.

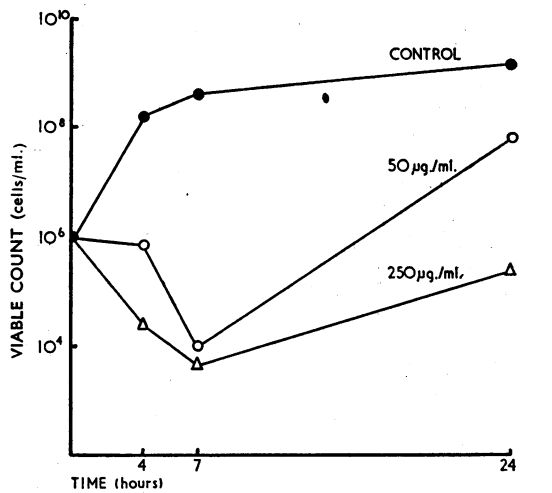

FIG. 2.—Bactericidal activity of carbenicillin against *Pseudomonas pyocyanea*.

Development of Resistance

Experiments involving repeated subculture of staphylococci and various Gram-negative bacilli, including *Ps. pyocyanea*, in the presence of carbenicillin, indicate that emergence of resistance takes place in a stepwise fashion which is characteristic of penicillins.

Effect of Serum on Activity

Carbenicillin is not highly bound to serum proteins. Sensitivity tests carried out in human serum show that the activity of carbenicillin is not significantly diminished in the presence of serum. Determinations of the extent of binding by ultrafiltration indicate that in human serum approximately 53% of the drug is unbound.

Disc Sensitivity Testing with Carbenicillin

With 6-mm. paper discs containing 100 μg. carbenicillin all strains of *Ps. pyocyanea* tested in this laboratory have given zones of inhibition of 12–20 mm. with a heavy inoculum and zones of 18–30 mm. with a dilute inoculum. With a heavy inoculum a number of colonies are usually visible within the zone of inhibition, although when these colonies are picked off and retested they show the same sensitivity as the original culture and again give rise to a number of colonies within the clear zone of inhibition.

In the case of Gram-negative bacilli other than pseudomonas —for example, *E. coli* and *Proteus* species—a 25-μg. disc would be suitable for sensitivity testing. With use of a heavy inoculum inhibition zones of 20 mm. or more are usually obtained with strains that are inhibited by 5 μg./ml. carbenicillin.

Absorption and Excretion in Man

Assay of Carbenicillin in Serum and Urine

The concentration of carbenicillin in serum and in urine was determined by the cup-plate biological assay method. Details of the technique were as previously described (Knudsen and Rolinson, 1960) except that *Ps. pyocyanea* NCTC 10490 (Ellsworth strain, 1973) was used as test organism. *Sarcina lutea* and *Staphylococcus aureus* are unsuitable as assay organisms because carbenicillin contains a small amount of benzylpenicillin and this may appear in the serum and in the urine together with the carbenicillin. Since *S. lutea* and *Staph. aureus* are many times more sensitive to benzylpenicillin than to carbenicillin, the presence of traces of benzylpenicillin may invalidate the assay. *Ps. pyocyanea* NCTC 10490, on the other hand, is sensitive to carbenicillin but relatively resistant to benzylpenicillin.. The culture is unusually sensitive to carbenicillin, showing a minimum inhibitory concentration of approximately 1.25 μg./ml., and using this strain it is possible to assay concentrations of carbenicillin as low as 2.5 μg./ml., while benzylpenicillin at concentrations of up to 500 μg./ml. fails to show any zone of inhibition. Standard solutions of carbenicillin from 1 to 100 μg./ml. were prepared in human serum, and, where necessary, serum specimens were suitably diluted in human serum to give a level within this range of concentration. For the assay of urine samples the standard solutions of carbenicillin were prepared in M/20 phosphate buffer *p*H 7, and urine samples were also diluted as required, using the same buffer.

Oral Administration

Carbenicillin in gelatin capsules was given at a dose of 500 mg. to healthy fasting volunteers. Serum samples were taken at ½, 1, 2, 4, and 6 hours after administration of the dose, and urine was collected over the period 0 to 6 hours. No carbenicillin (that is, less than 1 μg./ml.) was detected in the serum, and the quantity present in the urine amounted to less than 1% of the dose administered. It would appear, therefore, that carbenicillin is not absorbed to any appreciable extent when administered orally.

Intramuscular Administration

Carbenicillin was administered by intramuscular injection to groups of healthy adult volunteers as single doses of 250 mg., 500 mg., and 1,000 mg. The 250-mg. dose was given in a volume of 1 ml. of water for injection, and the 500-mg. and 1,000-mg. doses were dissolved in 2 ml. of water. Blood specimens were collected ½, 1, 2, 4, and 6 hours after administration, and the urine was collected during the six-hour period after dosing. Results of these studies are summarized in Table IV.

TABLE IV.—*Mean Serum Concentrations of Carbenicillin After Intramuscular Injection in Adult Volunteers*

No. of Subjects	Dose (mg.)	Serum Concentration (μg./ml.)						% of Dose Excreted in Urine over 0–6 hrs.
		½-hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.	6 hrs.	
10	250	5·9	7·9	5·7	2·6	<2·0	<2·0	71
9	500	13·7	17·7	13·2	—	2·0	<2·0	82
16	1,000	21·6	25·3	22·1	—	10·9	3·7	84

Mean serum peak levels of 7.9 μg./ml. carbenicillin were obtained one hour after administration of the 250-mg. dose, and these declined to <2 μg./ml. after four hours. Increasing the dose to 500 mg. resulted in serum concentrations twice as high as those obtained with the 250-mg. dose, but these declined to barely detectable concentrations after four hours. Impressive results were obtained with the 1,000-mg. dose, the mean serum concentrations being greater than 20 μg./ml. for the first two hours after injection, falling to 10.9 μg./ml. at four hours.

The amounts of carbenicillin excreted in the urine during the first six hours after injection were 71% of the dose with the 250-mg. dose, 82% of the 500-mg. dose, and 84% of the 1,000-mg. dose, producing average carbenicillin concentrations in the urine of 1,000 to 2,000 μg./ml. with the 500-mg. dose and 2,000 to 4,000 μg./ml. with the 1,000-mg. dose.

The effect of probenecid was investigated in a crossover study in which 10 subjects were given 1,000 mg. carbenicillin by the intramuscular route. One week later the same subjects took oral probenecid (1 g.) 10 hours and one hour before receiving 1,000 mg. intramuscular carbenicillin. The administration of probenecid resulted in a marked increase in the carbenicillin serum concentrations (Table V and Fig. 3) and in a significant reduction in the rate of elimination of carbenicillin from the serum.

Chromatographic examination of the urine after intramuscular administration of carbenicillin showed no evidence of the formation of biologically active metabolites.

Although carbenicillin is primarily of interest for its activity against *Ps. pyocyanea*, the compound is active against a wide range of bacteria, including *E. coli* and various ampicillin-resistant proteus species, and it seems probable that intramuscular doses of 500 to 1,000 mg. carbenicillin might be successfully used to attain serum, tissue, and urine concentrations adequate for the treatment of infections caused by these organisms. On the other hand, the activity of carbenicillin against *Ps. pyocyanea* is such that inhibitory concentrations of carbenicillin with respect to this particular pathogen are not likely to be achieved in serum and tissue by intramuscular

TABLE V.—*Effect of Probenecid on Serum Levels and Urinary Excretion of Carbenicillin in Healthy Adult Volunteers. (Carbenicillin Dose 1 g. by Intramuscular Injection)*

	No. of Subjects	Mean Serum Concentration (μg./ml.)					% of Dose Excreted in Urine over 0–6 hrs.
		½-hr.	1 hr.	2 hrs.	4 hrs.	6 hrs.	
With probenecid*	10	25·7	39·9	48·0	27·4	19·4	48
Without probenecid	10	26·8	29·4	22·2	10·8	3·3	84

* 1 g. 1 hour and 10 hours before administration of carbenicillin.

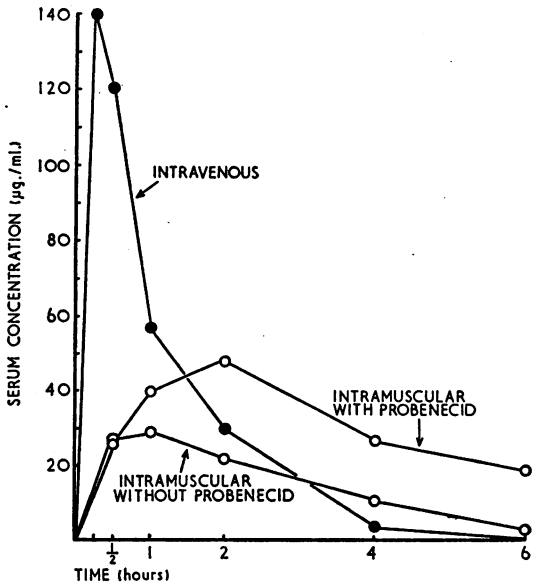

FIG. 3.—Serum concentrations in human volunteers after intramuscular and intravenous administration of 1 g. of carbenicillin.

dosing. Nevertheless, the urine concentrations attained after intramuscular administration of carbenicillin are greatly in excess of the minimum inhibitory concentrations of the drug against *Ps. pyocyanea*, and the results of clinical trials indicate that 1,000 mg. of carbenicillin given four times daily by the intramuscular route may be adequate for the treatment of acute urinary tract infections due to this organism.

Intravenous Administration

Two subjects were given 1,000 mg. of carbenicillin by slow intravenous injection. The penicillin was dissolved in 10 ml. of sterile water for injection and administered over a period of three minutes. Serum concentrations of greater than 100 μg./ml. carbenicillin were obtained during the first half-hour after administration of the compound, although at four hours only trace amounts of carbenicillin were detected (Table VI and Fig. 3).

From the bacteriological studies, concentrations of carbenicillin in the order of 100 μg./ml. would be expected to inhibit the growth of most strains of *Ps. pyocyanea*, but in view of the rapid elimination of the drug from the body frequent administration would probably be necessary. In practice the results of clinical studies have shown that, for the treatment of septicaemia, tissue infections, and chronic urinary tract infections due to *Ps. pyocyanea*, carbenicillin should be given at a dose of 1 g. hourly by the intravenous route, preferably together with probenecid, in order to maintain inhibitory concentrations in the body.

TABLE VI.—*Serum Concentration of Carbenicillin After Intravenous Administration of 1 g.*

Subject	Serum Concentration (μg./ml.)					
	¼-hr.	½-hr.	1 hr.	2 hrs.	4 hrs.	6 hrs.
A	140	127	60	28	3·7	<3·0
B	140	114	55	32	4·4	<3·0

Summary

Carbenicillin is a new semisynthetic penicillin active against *Pseudomonas pyocyanea*. The compound shows a broad spectrum of activity against Gram-positive and Gram-negative bacteria and is active against strains of *Proteus vulgaris*, *Pr. rettgeri*, and *Pr. morganii*, which are typically resistant to ampicillin. With a very heavy inoculum of *Ps. pyocyanea* marked inhibition of growth occurs with carbenicillin at 25 to 50 μg./ml., but some very scanty growth may persist even at concentrations of 125 to 250 μg./ml. With a slightly smaller inoculum complete inhibition of growth occurs at 25 to 50 μg./ml. Carbenicillin is bactericidal and is not highly bound to serum protein. Carbenicillin is not absorbed when given by mouth, but high concentrations of the drug are obtained in serum after intramuscular or intravenous administration, and these levels are increased and are more prolonged with concomitant use of probenecid. Carbenicillin is excreted in high concentration in the urine.

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