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MICROBIOLOGICAL STUDIES ON A NEW BROAD-SPECTRUM PENICILLIN, "PENBRITIN"*

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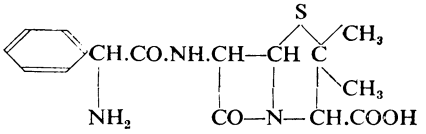
"Penbritin" (B.R.L. 1341) is a new penicillin prepared from 6-aminopenicillanic acid. It is of special interest because it is acid-stable and is active against a wide range of Gram-positive and Gram-negative bacteria. The compound is 6[D(−)α-aminophenylacetamido]penicillanic acid, with the structural formula



As the free acid it is sparingly soluble in water, and at neutral *p*H the solubility is only about 10% at room temperature.

Methods

Minimum inhibitory concentrations (M.I.C.) required to prevent growth of bacteria for 24 hours at 37° C. were determined by serial dilution in nutrient broth ("oxoid" No. 2) *p*H 7.2 and also in nutrient agar ("oxoid" No. 2). A concentration of 5% whole blood was incorporated in the media for the nutritionally exacting organisms. Serial dilution tests were normally inoculated with one drop of an overnight broth culture. Serial dilutions in agar were poured into Petri dishes and the surface was inoculated with one drop from an overnight broth culture.

Viable counts in broth cultures were made by preparing tenfold dilutions in agar and colony-counting in the usual way after incubation overnight.

Assays of penbritin in solution were carried out with the cup-plate method, using *Sarcina lutea* A.T.C.C. 9341 as test organism.

Sensitivity disks containing 10 μg. of penbritin were prepared using 6-mm.-diameter disks of Whatman 3 MM paper. By means of an Agla micrometer syringe, 0.01 ml. of a 0.1% solution of penbritin was applied to the disks, which were then air-dried at 37° C. before use.

Minimum Inhibitory Concentrations

The minimum inhibitory concentrations (M.I.C.) of penbritin required to prevent growth for 24 hours are shown in Table I. Results are also shown for penicillin G, tetracycline, and chloramphenicol for comparison.

*Penbritin is the registered trade mark of Beecham Research Laboratories Ltd.

Against the pyogenic cocci penbritin was only slightly less active than penicillin G, the M.I.C. values being generally one tube lower in the dilution tests. Compared with tetracycline and chloramphenicol, penbritin was substantially more active. Penbritin is not stable to penicillinase and therefore not effective against penicillinase-producing penicillin-resistant staphylococci. Against *Haemophilus influenzae*, penbritin showed a high level of activity and was slightly more active in this respect than was penicillin G, tetracycline, or chloramphenicol. Penbritin was also highly active against *Neisseria catarrhalis*.

Among the Gram-negative bacilli, penbritin showed a range of activity generally similar to that of tetracycline. For strains of *Escherichia coli, Klebsiella pneumoniae*, and species of *Shigella*, the M.I.C. values were close. Against *Aerobacter aerogenes*, penbritin showed little activity, partly because a high level of penicillinase production is typical of these organisms. Certain strains of *Proteus* were resistant to penbritin, although sensitive to tetracycline, again due in part to penicillinase production. On the other hand, certain strains of *Proteus* resistant to tetracycline were sensitive to penbritin.

Penbritin showed consistently high activity against species of *Salmonella*. Results are given in Table II for the M.I.C. values for representatives of the *Salmonella* groups. Parallel tests were also carried out with penicillin G, tetracycline, and chloramphenicol alongside penbritin. Of these four antibiotics, penbritin was the most active. Strains of *Salm. typhi* were generally inhibited by 0.25–0.5 μg./ml. of penbritin and *Salm. typhi-murium, Salm. paratyphi-A*, and *Salm. paratyphi-B* by 1.25–2.5 μg./ml. No strains were encountered which were resistant. Compared with tetracycline, the M.I.C. values for penbritin were generally one tube better, and, compared with chloramphenicol and penicillin G, penbritin was much more active.

In the experiments reported in Tables I and II, many of the test organisms were recently isolated clinical cultures. This is not sufficient, however, to indicate the proportions of sensitive and resistant strains among the cultures routinely encountered clinically. In order to establish this, larger numbers of organisms were examined, and in Table III results are given for the M.I.C. values of all the isolations of coliform organisms

and *Proteus* species made in the Middlesex Hospital, the West Middlesex Hospital, and Redhill County Hospital during a period of two months. The total number of coliforms isolated, including *E. coli* types, *Klebsiella*, and *Aerobacter*, was 341. Of these, 277 (81%) showed an M.I.C. of 5 μg./ml. or less. The largest single group consisted of those with an M.I.C. of 2.5 μg./ml., and 146 (43%) were in this category. A total of 64 (19%) showed an M.I.C. of 12.5 μg./ml. or over. Each of these cultures with an M.I.C. of 12.5 μg./ml. or over was examined (Table IV) and identified according to the classification of Wilson (1935). Of these 64 cultures only 14 proved to be typical *E. coli* type I or II—that is, 4% of all coliforms. The majority of the resistant strains were those showing citrate utilization comprising the intermediate type and *A. aerogenes*.

Of the total number of 121 *Proteus* cultures examined, 99 (82%) showed an M.I.C. of 5 μg./ml. or less and

TABLE I.—*Sensitivity of Bacteria to Penbritin*

Species	Strain	Sensitivity* to 10-μg. disks Penbritin	M.I.C. (μg./ml.) Serial Dilution in Agar: Penbritin	Pen. G	Tetracycline	Chloramphenicol
Staph. pyogenes (Oxford)	1227	37	0·05	0·02	0·25	2·5
Staph. pyogenes (Smith)	1474	31	0·05	0·02	0·25	2·5
Staph. pyogenes var. *aureus*	1232	—	0·05	0·01	0·25	2·5
,, ,,	1233†	—	0·05	0·02	0·25	2·5
,, ,,	N.C.I.B. 8588	—	0·06	0·02	0·12	2·5
,, ,,	1399†	—	0·05	0·02	0·25	2·5
,, ,,	1402†	—	0·05	0·02	0·25	5·0
,, ,,‡	1083†	—	125·0	125·0	125·0	50·0
Str. pyogenes β type	1489†	25	0·01	0·005	0·25	2·5
,, ,,	1490†	23	0·02	0·01	0·25	2·5
,, ,,	1488†	—	0·01	0·06	0·25	2·5
Str. pyogenes α type	1479†	29·5	0·02	0·005	0·25	5·0
,, ,,	1482†	—	0·01	0·005	0·25	1·25
Diplococcus pneumoniae	1222†	—	0·02	0·05	0·25	2·5
,, ,,	1224†	33	0·05	0·02	0·25	2·5
,, ,,	1251†	—	0·02	0·1	0·25	1·25
Str. faecalis	1591	12·5	1·25	2·5	1·25	2·5
,, ,,	N.C.I.B. 8191	11·0	5·0	12·5	1·25	5·0
,, ,,	N.C.I.B. 8192	13·0	5·0	12·5	1·25	2·5
,, ,,	1274	17·0	1·25	1·25	12·5	5·0
N. catarrhalis	N.C.T.C. 3622	30	0·12	0·005	0·6	0·25
H. influenzae	1584†	—	0·5	0·5	1·25	0·5
,, ,,	1585†	—	0·25	0·5	0·5	0·5
,, ,,	1583†	30	0·25	0·5	1·25	0·5
H. parainfluenzae	1586†	22·5	0·12	0·25	1·25	0·5
Alcaligenes faecalis	N.C.I.B. 8156	7·0	5·0	5·0	2·5	5·0
E. coli	1014†	6·0	5·0	50·0	2·5	2·5
,, ,,	1015†	8·0	2·5	25·0	2·5	2·5
,, ,,	1590†	4·0	5·0	50·0	5·0	5·0
,, ,,	1589†	7·0	2·5	25·0	5·0	5·0
,, ,,	1013†	5·0	5·0	25·0	5·0	5·0
A. aerogenes	N.C.I.B 418	3·0	250·0	250·0	1·25	1·25
Kleb. pneumoniae	1052	—	1·25	6·25	1·25	2·5
Shigella shigae	N.C.T.C. 4837	16·5	0·5	5·0	0·6	2·5
,, *schmitzi*	1079	—	0·6	5·0	0·6	2·5
,, *flexneri*	1078	14·5	1·25	12·5	1·25	2·5
,, *sonnei*	N.C.T.C. 8220	11·0	5·0	50·0	5·0	2·5
Salm. paratyphi-A	1070	16·5	0·6	6·25	1·25	2·5
Salm. paratyphi-B	1385†	11·5	1·25	5·0	2·5	5·0
Salm. typhi	N.C.T.C 8393	21	0·25	2·5	2·5	2·5
,, *typhi-murium*	1416†	16·5	1·25	6·25	—	5·0
Pr. vulgaris	1055†	13·0	5·0	5·0	62·5	12·5
,, ,,	N.C.T.C. 401	6·5	5·0	25·0	0·6	1·25
,, *mirabilis*	1431†	13·0	1·25	5·0	62·5	12·5
,, *morganii*	N.C.T.C. 2815	0	62·5	>500·0	0·5	6·25
Ps. pyocyanea	1058	0	>250·0	>500·0	50·0	125·0

* Diameter of zone of inhibition *minus* diameter of disk.
† Recently isolated clinical cultures.
‡ Penicillinase-producing penicillin-resistant strain also resistant to tetracycline and chloramphenicol.

TABLE II.—*Minimum Inhibitory Concentrations of Penbritin for Species of Salmonella*

Species	Strain	M.I.C. (μg./ml.) Serial Dilution in Agar: Penbritin	Pen. G	Tetracycline	Chloramphenicol
Salm. paratyphi-A	1070	1·25	6·25	1·25	2·5
,, *paratyphi-B*	1385*	0·6	5·0	1·25	5·0
,, ,,	1386*	5·0	5·0	5·0	6·25
,, ,,	1387*	2·5	12·5	2·5	5·0
,, ,,	1071	1·25	6·25	2·5	5·0
,, *typhi-murium*	1076	0·25	2·5	1·25	1·25
,, ,,	1077	1·25	5·0	2·5	5·0
,, ,,	1416*	1·25	6·25	—	5·0
,, ,,	1417*	1·25	5·0	2·5	5·0
,, ,,	1434*	1·25	5·0	2·5	5·0
,, ,,	1435*	1·25	5·0	2·5	5·0
,, ,,	1462	1·25	5·0	2·5	5·0
,, *cholerae-suis*	1068	1·25	2·5	2·5	5·0
,, *thompson*	1418*	0·6	6·25	1·25	2·5
,, ,,	1464	1·25	5·0	2·5	6·25
,, *typhi*	1072	0·5	1·25	1·25	2·5
,, ,,	N.C.T.C.8393	0·25	2·5	0·5	2·5
,, ,,	1074	0·5	1·25	1·25	2·5
,, ,,	1075*	1·2	5·0	5·0	5·0
,, ,,	N.C.T.C. 786	0·25	1·25	0·5	0·6
,, ,,	1388*	0·5	2·5	1·25	2·5
,, ,,	1389*	0·5	2·5	1·25	2·5
,, ,,	1390	0·25	2·5	0·6	2·5
,, ,,	1391*	0·25	1·25	0·6	2·5
,, ,,	1392*	0·25	1·25	0·6	2·5
,, ,,	1393*	1·25	5·0	1·25	5·0
,, *enteritidis*	1463	1·25	5·0	2·5	5·0
,, *gallinarum*	1302	0·5	5·0	1·25	2·5
,, *london*	1069	1·25	6·25	2·5	5·0
,, *binza*	1419*	1·25	6·25	1·25	5·0

* Recently isolated clinical cultures.

TABLE III.—*Sensitivities to Penbritin of the Clinical Isolates of Coliforms, Proteus Species, and Strains of Str. faecalis Obtained in Three Hospitals Over a Period of Eight Weeks*

Organisms	Total No. of Isolates	Sensitivity of Each Isolate to Penbritin (Serial Dilution in Agar) M.I.C. μg./ml.											
		>250	250	125	50	25	12·5	5	2·5	1·25	0·5	0·25	0·12
Coliforms	341	22	5	8	8	7	14	72	146	52	7		
		64						277					
Proteus	121	11	1		2	6	2	15	5	48	29	2	
		22						99					
Str. faecalis	11								2	4	1	1	3
								11					

TABLE IV.—*Identification of the 64 Penbritin-resistant Coliforms (M.I.C. 12.5 μg./ml. or Over) Shown in Table III*

	Resistant Coliforms
E. coli Type I	7
,, ,, ,, II	7
Intermediate, Type I	17
Klebsiella	4
A. aerogenes, Type I	18
,, ,, ,, II	2
Irregular Type I	2
,, ,, VIII	1
Unidentified	6

79 (65%) were inhibited by 1.25 μg./ml. Results are also shown in Table III for the sensitivities of the isolations of *Str. faecalis* from the West Middlesex Hospital over the same period. All were sensitive to 2.5 μg./ml.

Sensitivity Disks

A loop from an overnight broth culture was spread on a plate in the usual way and paper disks containing 10 μg. of penbritin were placed on the surface. Zones of inhibition were measured after incubation overnight. Results are shown in Table I alongside the M.I.C. values by serial dilution. It will be seen that a reasonable correlation exists between the results for sensitivity as judged from the use of the disks and the M.I.C. values. The results with *A. aerogenes* might appear an exception, but this culture is known to produce penicillinase and the M.I.C. is greatly affected by inoculum size. In the experiments with the disks a loop spread on a plate constitutes a relatively small inoculum.

Effect of Inoculum Size

Results are given in Table V for the effect of inoculum size on the M.I.C. values for penbritin, tetracycline, and chloramphenicol using *Salm. typhi* N.C.T.C. 8393 as test organism. It will be seen that cell numbers had little effect on the M.I.C. for penbritin, even when the inoculum was as large as 0.5 ml. of an overnight broth culture. In contrast, the M.I.C. for tetracycline was increased tenfold by the increase in inoculum size. In Table V the results are also shown for certain other cultures. With a strain of *Shigella sonnei* the inoculum size again had little effect on the activity of penbritin, but with *A. aerogenes* and a strain of *Pr. vulgaris* a large inoculum resulted in a very high M.I.C. value typical of the effect obtained with penicillin G and penicillinase-producing staphylococci. Evidence of penicillinase production by these cultures is given below.

TABLE V.—*Effect of Inoculum Size on the M.I.C. Values for Penbritin*

Culture	Inoculum (18-hour Broth Cultures) per 5 ml.	M.I.C. μg./ml. (Serial Dilution in Broth)		
		Penbritin	Tetracycline	Chloramphenicol
Salm. typhi N.C.T.C. 8393	0·5 ml.	0·6	2·5	5·0
	0·2 ,,	0·5	1·25	2·5
	0·03 ,,	0·5	0·6	2·5
	0·03 ,, of 1/100 dilution	0·25	0·5	2·5
	0·03 ,, of 1/10,000 dilution	0·25	0·25	1·25
Pr. vulgaris 1292	0·03 ml.	50·0		
	0·03 ,, of 1/1,000 dilution	5·0		
A. aerogenes 1003	0·03 ml.	125·0		
	0·03 ,, of 1/1,000 dilution	5·0		
Sh. sonnei 1081	0·03 ml.	1·25		
	0·03 ,, of 1/1,000 dilution	1·25		

Penicillinase Production as a Factor in the Resistance to Penbritin

Penicillinase production is not confined to the staphylococci and Gram-positive bacilli. Many strains among the Gram-negative bacilli also produce penicillinase. In Table VI results are given with strains of *E. coli*, *Pr. vulgaris*, and *A. aerogenes* which indicate that, in certain cases at least, resistance to penbritin is associated with destruction of the antibiotic presumably due to penicillinase. The experiments involved serial dilution tests in broth and assay of the penbritin content of each tube after the incubation period overnight. Loss of activity overnight at 37° C. in the absence of any test organism was only about 10%; consequently in such instances where loss of activity was considerable this could be attributed almost entirely to destruction by the test organism.

The results for *E. coli* 1009 with an M.I.C. of 5 μg./ml. show that at the highest concentration just permitting growth there was slight inactivation, the penbritin content being approximately half that present at the time of inoculation. In other words, the degree of inactivation by the test organism could be sufficient to result in an M.I.C. value perhaps one tube higher than would be the case if there had been no destruction, but this would be the limit to which penicillinase could affect the M.I.C. in this particular case. On the other hand, with the strain of *E. coli* 1140 showing an M.I.C. of 500 μg./ml. inactivation was considerable. In the tubes containing up to 25 μg./ml. the penbritin was completely destroyed, and in the tube initially containing 250 μg./ml. more than 50% was destroyed by the test organism. This degree of destruction alone would be responsible for a high M.I.C. value, but it is of interest to note that in this particular case the culture was also inherently resistant to penbritin and grew in tubes in which up to 100 μg. of penbritin per ml. remained even after the incubation period overnight. With *Pr. vulgaris* and *A. aerogenes* (Table VI) both caused extensive inactivation of penbritin, and in these cases the penicillinase factor might well be entirely responsible for the resistance. This is supported by the results with a small inoculum of the culture of *A. aerogenes*. In this instance the extent of penbritin inactivation was much diminished and the M.I.C. was also correspondingly lower.

Activity of Penbritin

Effect of pH

The *p*H of sterile nutrient broth was adjusted aseptically to give a range of *p*H values and the M.I.C. values determined by serial dilution using various test organisms. Results are shown in Table VII. With the Oxford *Staphylococcus* and a strain of *Pr. mirabilis* a range of *p*H from 5.5 to 8 had no significant effect on the activity of the compound. On the other hand, with both strains of *E. coli* used and with a strain of *Str. faecalis*, penbritin was approximately 10 times more active at *p*H 5.5 than at *p*H 8. This effect did not

TABLE VI.—*Penicillinase Production as a Factor Affecting M.I.C. Values for Penbritin. Broth Serial Dilution Tests Assayed for Penbritin after Incubation Overnight at 37° C.*

E. coli 1009:										
Penbritin before inoculation (μg./ml.)				50	25	12·5	5·0	2·5	1·25	0·5
,, after incubation overnight (μg./ml.)				41	21	9·6	2·3	0·9	0·4	0·1
Visible growth after incubation overnight				—	—	—	—	+	+	+
E. coli 1140:										
Penbritin before inoculation (μg./ml.)	500	250	125	50	25	12·5	5·0			
,, after incubation overnight (μg./ml.)	500	100	10	0·5	0	0	0			
Visible growth after incubation overnight	—	+	+	+	+	+	+			
Pr. vulgaris 1292:										
Penbritin before inoculation (μg./ml.)	500	250	125	50	25	12·5	5·0			
,, after incubation overnight (μg./ml.)	400	210	60	0	0	0	0			
Visible growth after incubation overnight	—	—	—	+	+	—	+			
A. aerogenes 1003:										
Large inoculum (0·03 ml. of overnight broth):										
Penbritin before inoculation (μg./ml.)	500	250	125	50	25	12·5	5·0			
,, after incubation overnight (μg./ml.)	440	0	0	0	0	0	0			
Visible growth after incubation overnight	—	+	+	+	+	+	+			
Small inoculum (0·03 ml. of 1/1,000 dilution of overnight broth):										
Penbritin before inoculation (μg./ml.)	500	250	125	50	25	12·5	5·0	2·5		
,, after incubation overnight (μg./ml.)	420	270	110	44	22	0	0	0		
Visible growth after incubation overnight	—	—	—	—	—	—	+	+		

appear to be correlated with the extent to which the *p*H of the medium was influenced by the growth of the test organism, nor was there any correlation with the effect of *p*H on growth itself. With the strain of

TABLE VII.—*Effect of pH on Activity of Penbritin (Serial Dilution in Broth)*

Culture	*p*H of Broth before Inoculation	*p*H of Inoculated Broths after 24 Hours' Incubation (No Penbritin Present)	M.I.C. (μg./ml.)
Oxford *Staph.*	5·2	—	0·05
	7·2	—	0·12
	8·5	—	0·05
E. coli 1009	5·2	—	0·5
	7·2	—	2·5
	8·5	—	25·0
E. coli 1012	5·5	6·9	1·25
	7·2	7·6	2·5
	8·0	8·1	12·5
Str. faecalis 1207	5·5	5·4	0·5
	7·2	7·2	1·25
	8·0	7·7	5·0
Pr. mirabilis 1431	5·5	5·6	0·5
	7·2	7·4	0·5
	8·0	7·9	1·25

Pr. mirabilis and *Str. faecalis* the growth in control tubes at *p*H 5.5 appeared to be somewhat lighter than at the higher *p*H values, but with the other organisms growth was heavy at all the *p*H values.

Effect of Serum

Results are shown in Table VIII for the effect of 40% human serum on the M.I.C. values for penbritin using a strain of *E. coli* and the Oxford *Staphylococcus* as test organisms. It will be seen that serum had little effect on activity, and the M.I.C. values were only one tube lower than in the controls.

TABLE VIII.—*Effect of Human Serum on M.I.C. Values of Penbritin (Serial Dilution in Broth)*

	M.I.C. (μg./ml.)	
	Control	+40% Human Serum
Oxford *Staph.*	0·02	0·05
E. coli	1·0	2·5

Bactericidal Activity

Overnight broth cultures were used to inoculate fresh sterile nutrient broth to give a viable count of about 10^7 cells/ml. Incubation was carried out at 37° C. with different concentrations of penbritin, and viable counts were made at intervals of time. Results are given in Figs. 1 and 2 for the Oxford *Staphylococcus* and for a strain of *E. coli*. With the Oxford *Staphylococcus* a concentration of 0.5 μg./ml. almost completely sterilized the culture in 24 hours, and a concentration of 0.1 μg./ml. greatly reduced the viable count.

With the strain of *E. coli*, penbritin also showed a high degree of bactericidal activity at concentrations only slightly higher than the M.I.C. value. For the strain of *E. coli* used in Fig. 2 the M.I.C. was 2.5 μg./ml. and a concentration of 5 μg./ml. almost completely sterilized the culture in seven hours.

In Figs. 3, 4, and 5 comparative results are given for the bactericidal activity of penbritin, tetracycline, and chloramphenicol, using a strain of *Salm. typhimurium* as test organism. Tetracycline, at concentrations as high as 5 μg./ml., showed little more than a bacteriostatic effect. With chloramphenicol, some bactericidal activity was apparent with 5 μg./ml., but with lower concentrations the effect was largely bacteriostatic. In contrast, penbritin at a concentration of

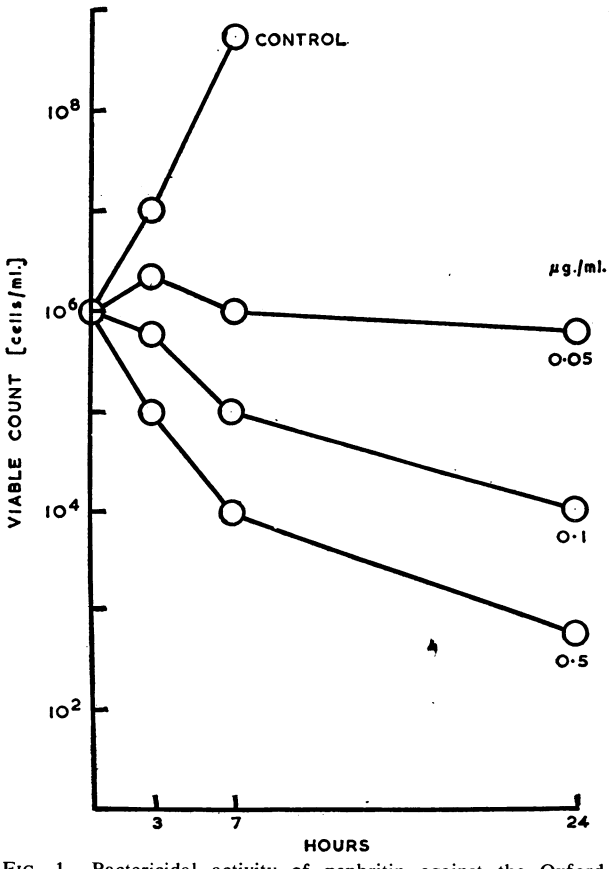


FIG. 1.—Bactericidal activity of penbritin against the Oxford *Staphylococcus*.

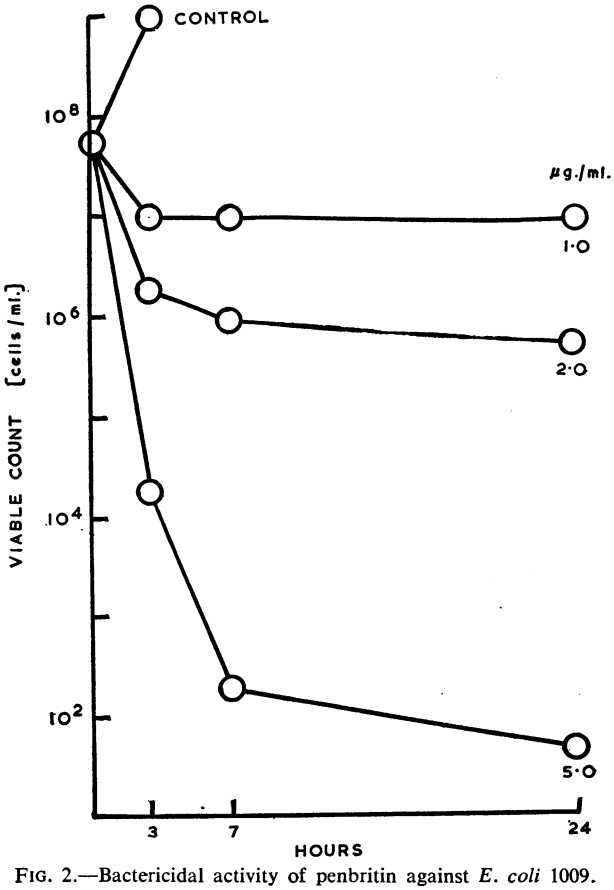


FIG. 2.—Bactericidal activity of penbritin against *E. coli* 1009.

5 μg./ml. completely sterilized the culture in 24 hours, and in three hours a kill of greater than 99.9% was obtained. Lower concentrations were also markedly bactericidal.

Stability of Penbritin in Acid Solution

Penbritin shows a high degree of stability in solution at low *p*H. Results are given in Table IX for the stability at 37° C. at *p*H 2. After two hours the loss in activity was only some 5%, and even after storage overnight under these conditions nearly 50% of the activity was retained. Comparative tests showed that penbritin was, in fact, rather more acid-stable than penicillin V.

TABLE IX.—*Acid Stability of Penbritin. Stability at pH 2 and 37° C.*

Time (hours)	0	1	2	4	6	24
Activity remaining (%)	100	100	95	88	80	42

At neutral *p*H, and at room temperature, sterile solutions of penbritin retained approximately 80% of the activity after one week. Non-sterile solutions exposed to the atmosphere and risk of contamination usually showed a more rapid loss of activity, due very probably to penicillinase-producing contaminants.

Penbritin is not stable to penicillinase and is destroyed at approximately the same rate as penicillin G.

Emergence of Resistance to Penbritin

Broth cultures of the Oxford *Staphylococcus, Salm. typhi* 1388, and *E. coli* 1009 were transferred every two or three days into serial dilution tubes of penbritin using as inoculum the tube with the highest concentration of penbritin which still permitted growth. Results are shown in Table X. It will be seen that the results obtained with penbritin are typical of a penicillin, the emergence of resistant strains being stepwise, each increase in resistance being only some twofold to fourfold. Consequently, the emergence of resistant strains during treatment with penbritin is not likely to be a serious factor, at least so far as the more sensitive organisms are concerned, nor is it likely to be important in urinary infections where the concentrations of penbritin achieved in the urine are high and therefore likely to be adequate even against strains which are substantially more resistant than normal.

TABLE X.—*Emergence of Penbritin-resistant Strains. Cultures Were Transferred Every Two or Three Days from Serial Dilution Tests in Broth, the Last Tube Showing Growth Being Used as Inoculum for the Next Transfer*

No. of Transfers	M.I.C. (μg./ml.)		
	E. coli 1009	*Salm. typhi* 1388	Oxford *Staph.*
0	1·25	0·5	0·05
1	1·25	1·25	0·05
2	12·5	5·0	0·25
3	25·0	5·0	0·5
4	50·0	12·5	1·25
5	125·0	25·0	1·25
6	125·0	25·0	1·25
7	125·0	50·0	5·0

Discussion

High activity against the Gram-positive cocci together with very little activity against the Gram-negative bacilli is almost characteristic of a penicillin, at least so far as those penicillins in clinical use are concerned. Some degree of increased Gram-negative activity to give a broader spectrum is shown by *p*-aminobenzylpenicillin (Tosoni, Glass, and Goldsmith, 1958) and also by the

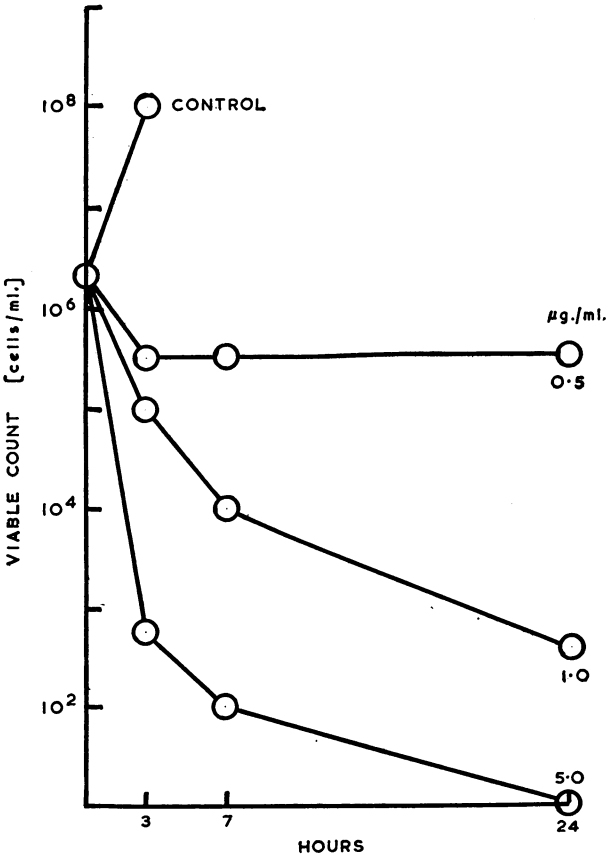


FIG. 3.—Bactericidal activity of penbritin against *Salm. typhi-murium* 1076.

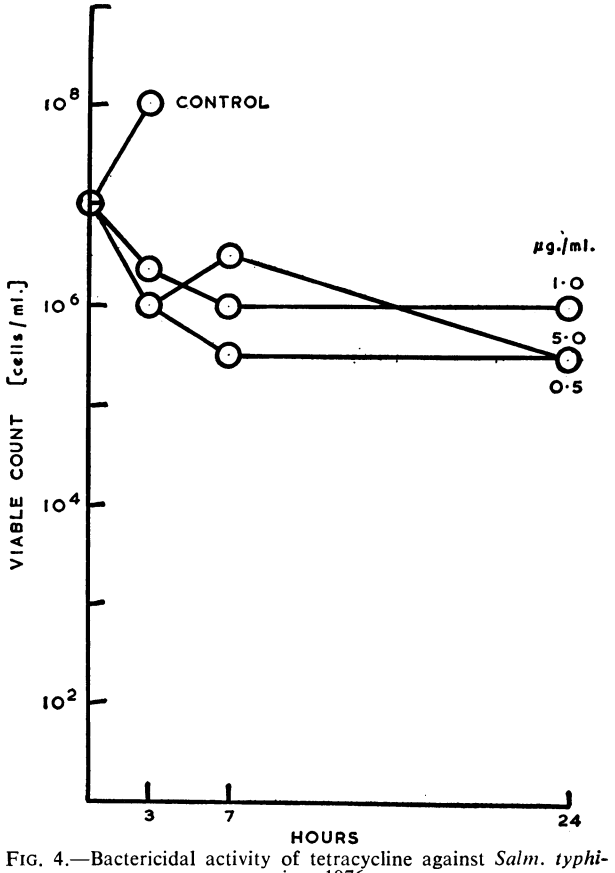


FIG. 4.—Bactericidal activity of tetracycline against *Salm. typhi-murium* 1076.

penicillin cephalosporin N (Heatley and Florey, 1953; Abraham, Newton, and Hale, 1954), but neither shows Gram-negative activity comparable with that of penbritin, nor are they acid-stable.

Compared with penicillin G, penbritin is about 10 times more active against the Gram-negative bacilli generally, and is only slightly less active against the Gram-positive cocci. This results in a spectrum in which activity is comparable with that of tetracycline and chloramphenicol in the Gram-negative field and much higher against the Gram-positive cocci. Penbritin can therefore be regarded as a broad-spectrum penicillin. In comparative tests between penbritin, tetracycline,

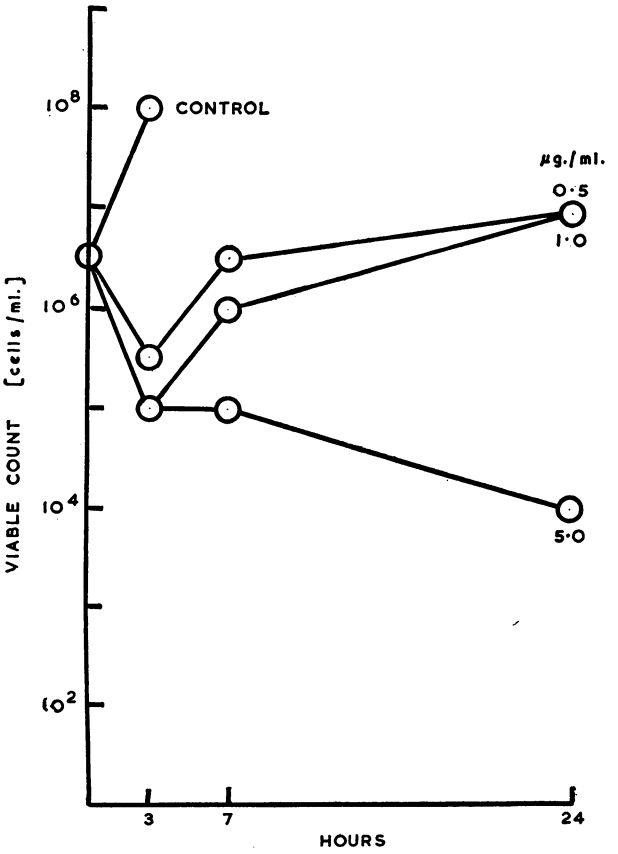


FIG. 5.—Bactericidal activity of chloramphenicol against *Salm. typhi-murium* 1076.

chloramphenicol, and penicillin G for certain organisms penbritin was the most active. This was true for *Salmonella* species and *H. influenzae*. Among the coliforms and species of *Proteus*, a certain number of penbritin-resistant strains occur, these strains being insensitive to penbritin in certain cases because of penicillinase production resulting in destruction of penbritin, although in some strains there is also evidence of inherent resistance. Among the coliforms and *Proteus* strains encountered clinically, the majority (about 80%) were sensitive to 5 μg./ml. or less, but about 20% showed an M.I.C. of 12.5 μg./ml. or greater.

Penbritin retains certain other characteristics of a penicillin—namely, that activity is little affected by the presence of serum and also that a high degree of bactericidal activity is shown. Cultures of *E. coli* and *Salm. typhi-murium* were virtually sterilized by 5 μg. of penbritin per ml. overnight. This is in marked contrast to tetracycline and chloramphenicol, where the effect is largely bacteriostatic. Penbritin also resembles other penicillins with respect to emergence of resistant strains. This is stepwise in the manner of resistance to penicillin G, and problems associated with resistance to penbritin are not likely to be very different from those associated with other penicillins in current use.

Against some organisms, activity of penbritin was found to be greatly affected by *p*H. This is of significance in connexion with urinary infections, and because of this penbritin might be expected to be more effective in certain cases with an acid urine. The effect of *p*H, however, was not general for all the bacteria tested, and further work is in progress to establish the extent and significance of the effect of *p*H on penbritin activity.

Summary

Penbritin (B.R.L. 1341) is a new penicillin which is acid-stable and active against a wide range of Gram-positive and Gram-negative bacteria.

Activity against Gram-negative bacilli is similar to that of tetracycline and chloramphenicol.

Against *Salmonella* species and *Haemophilus influenzae*, penbritin is slightly more active than penicillin G, tetracycline, or chloramphenicol.

Penbritin is more active than tetracycline against the pyogenic cocci and only slightly less active than penicillin G.

Penbritin is not stable to penicillinase and therefore not active against penicillin-resistant staphylococci or other penicillinase-producing organisms.

Activity of penbritin is not greatly affected by the presence of serum. Against certain organisms penbritin is more active at a slightly acid *p*H than at higher *p*H values.

Penbritin is highly bactericidal.

Emergence of resistant strains develops stepwise in the typical penicillin manner.

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A new British Standard (B.S. 3354:1961) specifies requirements for sinus forceps with box joints made of stainless steel for a range of sizes of 5, 6, 7, and 8 in. nominal overall length. The particular uses of sinus forceps make them vulnerable at the joint, and consequently box-jointed instruments only have been specified. Similar instruments with screw joints are available, however, for those applications for which the screw joint may give a satisfactory performance. This standard may be obtained from the British Standards Institution, Sales Branch, 2 Park Street, London W.1 (price 3s. each; postage extra to non-subscribers).