be greater than that found with tetracycline and chloramphenicol. This is in marked contrast to the *in vitro* titres, where only small differences are found.

We thank Mr. F. P. Doyle and his colleagues for the preparation of penbritin.

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# ABSORPTION AND EXCRETION OF "PENBRITIN"

BY

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"Penbritin" (6[D(−)-α-aminophenylacetamido]penicillanic acid; B.R.L. 1341) is a new penicillin derived from the penicillin nucleus, 6-aminopenicillanic acid, with a broad spectrum of activity against both Gram-positive and Gram-negative organisms (for full microbiological details, see Rolinson and Stevens, 1961).

This paper describes the investigations undertaken to determine a suitable dosage of penbritin for preliminary clinical trials.

Since penbritin is highly stable in acid medium it could be well absorbed orally. Experiments were therefore designed to investigate the serum concentrations and urinary excretions obtained with various oral doses of the new penicillin.

## Methods

Seventeen subjects (10 male and 7 female) aged 19–29 years (mean 25 years), weight 50–91 kg. (mean 65 kg.), took part in this study. Each subject was designated a letter of the alphabet and each retained the same letter throughout the investigations. Except where otherwise stated, all doses were given in the fasting state, the subjects having refrained from taking breakfast until after the two-hour specimen of blood had been withdrawn from the antecubital vein. All specimens were taken with Bayer "O" "venules," and assays were carried out on the freshly separated serum on the same day as the test was performed.

### Assay Procedure

Penbritin concentrations were determined by the cup-plate biological assay method using *Sarcina lutea* ATCC 9341 as test organism. Nutrient agar ("oxoid" No. 2) was seeded with 5 ml. of an overnight broth culture per 500 ml. of agar and poured into large assay plates to give an agar depth of approximately 4 mm. Plugs of agar were removed to give holes of 7 mm. diameter, which were filled with the solutions to be assayed. The plates were incubated at 30° C. overnight. The ring diameters were then measured and the concentrations read off in the usual way from the standard line.

Standard solutions of penbritin of 1, 0.5, 0.2, 0.1, 0.05, and 0.02 μg./ml. were used on each assay plate. Standards and unknowns were so arranged on plates as to compensate both for any variations in agar thickness and for the time factor in filling the plate.

The samples to be assayed were diluted to give a concentration of approximately 0.2 μg./ml. The diluent for the unknown samples and for the standard solutions was a 4% solution of bovine plasma albumin fraction V (Armour Pharmaceutical Co.) in M/20 phosphate buffer, *p*H 7. This solution had been found by experiment to have the same effect as human serum on the assays of this penicillin by this method.

Tetracycline concentrations were determined by the cup-plate biological assay method using a strain of *Bacillus mycoides* as test organism. Standard solutions of tetracycline of 20, 10, 5, 2, 1, and 0.5 μg./ml. were used on each assay plate.

The samples to be assayed were plated neat and the diluent for the standard solutions was human serum.

*Experiment 1.*—A 250-mg. capsule of penbritin was given to 10 subjects; blood samples were taken at ½, 1, 2, 4, and 6 hours after dosing, and the serum was assayed for the concentration of penbritin in terms of μg./ml. The 0–6-hour urine fractions were also collected and assayed for penbritin. Table I sets out the individual and mean results obtained in this experiment.

*Experiment 2.*—Two 250-mg. capsules were given to seven subjects and a similar procedure was adopted to that in experiment 1. Table II sets out the results obtained in this experiment.

*Experiment 3.*—Three 250-mg. capsules were given to seven subjects, and the investigation was carried out as in experiments 1 and 2. The results are set out in Table III.

*Experiment 4.*—Finally, four 250-mg. capsules were given to 10 subjects and a similar procedure was adopted as in the previous experiments. Table IV sets out the results obtained.

Fig. 1 shows graphically the means obtained in the four experiments, and Fig. 2 shows a dose-response curve for the two-hour levels.

TABLE I.—*Serum Concentrations and Urinary Excretion of Penbritin After a Single 250-mg. Dose*

| Subject | Dose mg. | Serum Concentrations (μg./ml.) | | | | | Urine mg. in 6 hr. |
|---|---|---|---|---|---|---|---|
| | | ½ hr. | 1 hr. | 2 hr. | 4 hr. | 6 hr. | |
| A | 250 | 0·3 | 2·6 | 2·7 | 0·5 | 0·1 | 83 |
| B | 250 | 1·4 | 3·5 | 2·1 | 0·3 | 0·08 | 92 |
| C | 250 | 0·2 | 2·6 | 2·3 | 0·3 | 0·1 | 111 |
| D | 250 | 0·7 | 2·2 | 2·4 | 0·5 | 0·2 | 51 |
| G | 250 | 1·4 | 1·8 | 1·7 | 0·6 | 0·3 | 59 |
| H | 250 | 1·4 | 2·7 | 2·0 | 0·5 | 0·2 | 125 |
| K | 250 | 1·0 | 1·3 | 1·2 | 0·7 | 0·3 | 108 |
| M | 250 | 1·1 | 2·8 | 2·4 | 0·4 | 0·2 | 57 |
| N | 250 | 0·2 | 0·8 | 1·3 | 0·5 | 0·1 | 64 |
| P | 250 | 0·4 | 1·6 | 1·3 | 0·3 | 0·1 | 71 |
| Mean | .. | 0·81 | 2·19 | 1·94 | 0·46 | 0·17 | 82 (33%) |

TABLE II.—*Serum Concentrations and Urinary Excretion of Penbritin After a Single 500-mg. Dose*

| Subject | Dose mg. | Serum Concentrations (μg./ml.) | | | | | Urine mg. in 6 hr. |
|---|---|---|---|---|---|---|---|
| | | ½ hr. | 1 hr. | 2 hr. | 4 hr. | 6 hr. | |
| A | 500 | 1·0 | 3·1 | 3·6 | 0·9 | 0·2 | 169 |
| G | 500 | 0·5 | 2·4 | 2·8 | 1·0 | 0·4 | 105 |
| L | 500 | 0·02 | 0·5 | 3·9 | 1·0 | 0·3 | 63 |
| N | 500 | 0·5 | 0·9 | 3·6 | 1·0 | 0·2 | 119 |
| P | 500 | 2·5 | 4·9 | 3·2 | 0·45 | 0·1 | 135 |
| R | 500 | 0·18 | 2·8 | 4·7 | 1·0 | 0·2 | 135 |
| Z | 500 | 0·5 | 4·6 | 4·7 | 0·8 | 0·2 | 158 |
| Mean | .. | 0·74 | 2·7 | 3·8 | 0·88 | 0·2 | 126 (25%) |

TABLE III.—*Serum Concentrations and Urinary Excretion of Penbritin After a Single 750-mg. Dose*

| Subject | Dose mg. | Serum Concentrations (μg./ml.) | | | | | Urine mg. in 6 hr. |
|---|---|---|---|---|---|---|---|
| | | ½ hr. | 1 hr. | 2 hr. | 4 hr. | 6 hr. | |
| B | 750 | 0·04 | 4·8 | 4·8 | 0·9 | 0·2 | 186 |
| C | 750 | 1·3 | 3·2 | 4·2 | 1·1 | 0·2 | 162 |
| D | 750 | 0·4 | 5·3 | 4·8 | 1·4 | 0·3 | 216 |
| H | 750 | 1·2 | 4·0 | 5·0 | 1·5 | 0·3 | 216 |
| I | 750 | 0·3 | 4·4 | 6·1 | 1·7 | 0·4 | 188 |
| K | 750 | 0·2 | 5·0 | 5·3 | 2·3 | 0·6 | 344 |
| M | 750 | 0·2 | 2·8 | 5·2 | 1·3 | 0·4 | 182 |
| Mean | .. | 0·52 | 4·2 | 5·1 | 1·5 | 0·3 | 213 (28%) |

TABLE IV.—*Serum Concentrations and Urinary Excretion of Penbritin After a Single 1,000-mg. Dose*

| Subject | Dose mg. | Serum Concentrations (μg. ml.) | | | | | Urine mg. in 6 hr. |
|---|---|---|---|---|---|---|---|
| | | ½ hr. | 1 hr. | 2 hr. | 4 hr. | 6 hr. | |
| A | 1,000 | 1·8 | 5·1 | 5·5 | 1·3 | 0·4 | 322 |
| B | 1,000 | 2·5 | 7·2 | 7·5 | 1·7 | 0·4 | 400 |
| D | 1,000 | 0·1 | 1·4 | 8·5 | 3·7 | 0·9 | 450 |
| F | 1,000 | 0·2 | 3·4 | 7·6 | 2·8 | 0·9 | 371 |
| G | 1,000 | 0·5 | 4·5 | 8·4 | 2·0 | 0·7 | 214 |
| K | 1,000 | 1·9 | 3·9 | 4·5 | 1·8 | 0·9 | 293 |
| L | 1,000 | 0·4 | 2·3 | 6·6 | 2·4 | 0·9 | 323 |
| M | 1,000 | 0·3 | 3·7 | 8·4 | 1·8 | 0·6 | 300 |
| N | 1,000 | 0·5 | 2·0 | 5·3 | 1·7 | 0·4 | — |
| P | 1,000 | 1·2 | 4·2 | 5·6 | 1·0 | 0·2 | — |
| Mean | .. | 0·94 | 3·77 | 6·79 | 2·02 | 0·63 | 334 (33%) |

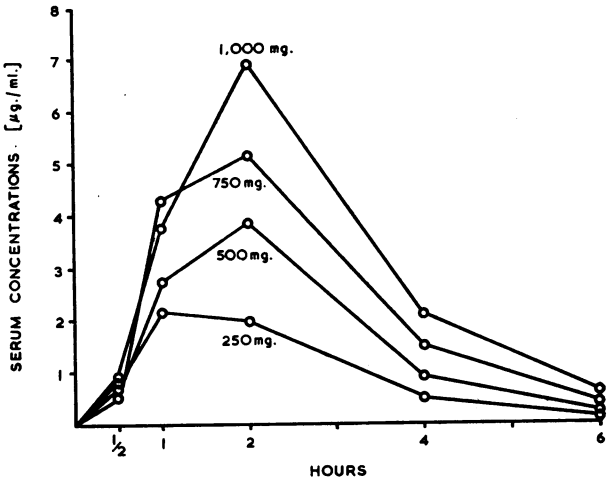


FIG. 1.—Mean serum concentrations of penbritin.

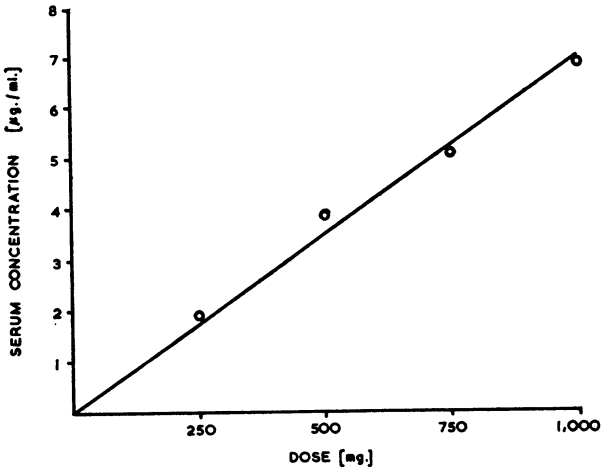


FIG. 2.—Dose-response curve for penbritin (two-hour levels for 250-, 500-, 750-, and 1,000-mg. doses).

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**Concentration of Penbritin in Urine**

Tables I–IV show the total excretion of penbritin in milligrams in the 0–6-hour fraction of urine; this is also expressed as a mean percentage of the administered dose. It will be seen that some 30% of the given dose is excreted in the urine within six hours. Data relating to the *concentration* of an antibiotic in the urine in terms of μg./ml., however, are of greater interest from the therapeutic aspect, but this depends upon the volume of urine excreted over the given period of time.

Table V shows the range of urine concentrations of penbritin obtained with the various doses.

*Experiment 5.*—It has been shown that approximately 30% of a given dose of penbritin is excreted in the urine within six hours. The total amount of a given dose of penbritin excreted in the urine may not, however, appear within six hours, although for most acid-stable penicillins some 95% of the total amount excreted appears in that time. A 24-hour urine study in four subjects was carried out. Each subject received two 250-mg. capsules on the morning of the study, having breakfasted as usual. Urine was collected over the 0–4 hour, 4–8 hour, 8–12 hour, and 12–24 hour period. The results are shown in Table VI. It will be seen that some 97% of the amount excreted in the urine appears within eight hours of dosing.

The following conclusions can be drawn from the results of the above investigations: (1) peak serum concentrations are generally obtained at about two hours after dosing (compared with half to one hour for other acid-stable penicillins); (2) significant serum concentrations are still present at six hours after dosing (0.2 μg./ml. compared with 0.02 μg./ml. for other acid-stable penicillins); (3) doubling the dose virtually doubles the peak serum concentrations (Fig. 2) (this is not seen with the tetracyclines); (4) some 30% of a given dose is excreted in the urine over a six-hour period; (5) a six-hourly or eight-hourly dosage might be considered for therapeutic trials; (6) 250 mg. 6–8 hourly should be adequate for the treatment of common Gram-positive infections (minimum inhibitory concentration=0.01–0.05 μg./ml.) and those due to *H. influenzae* (minimum inhibitory concentration=0.1–0.5 μg./ml.); (7) 750 mg. or more six-hourly would be required for the majority of infections due to Gram-negative organisms (minimum inhibitory concentration=0.5–5 μg./ml.); (8) 250–500 mg. eight-hourly might be adequate for the treatment of most infections of the urinary tract in view of the high concentrations obtained in the urine (150–1,000 μg./ml.).

TABLE V.—*Urine Concentrations μg./ml. (0–6 Hours)*

| Dose (mg.) | Range of Concentration (10 Subjects) |
|---|---|
| 250 | 150–1,000 μg./ml. |
| 500 | 250–1,000 ,, |
| 750 | 500–2,000 ,, |
| 1,000 | 550–2,500 ,, |

TABLE VI.—*24-Hour Urinary Excretion Study of Penbritin After a Single 500-mg. Dose*

| Subject | Dose mg. | Penbritin (mg.) | | | | Total 0–24 hr. |
|---|---|---|---|---|---|---|
| | | 0–4 hr. | 4–8 hr. | 8–12 hr. | 12–24 hr. | |
| B | 500 | 121 (24%) | 79 (15·8%) | 3·3 | 1·9 | 205 (41%) |
| D | 500 | 99 (19·8%) | 85 (17%) | 3·5 | 0·32 | 188 (37%) |
| T | 500 | 69 (13·8%) | 39 (7·8%) | 1·45 | 0·92 | 110 (22%) |
| V | 500 | 93 (18·5%) | 64 (12·8%) | 1·24 | 2·7 | 161 (32%) |

*Experiment 6.*—For comparative purposes, 10 subjects received two 250-mg. capsules of tetracycline and a similar procedure was adopted as in the previous experiments except that no half-hour specimen was taken. The results are set out in Table VII. Serum

TABLE VII.—*Serum Concentrations and Urinary Excretion of Tetracycline After a Single 500-mg. Dose*

| Subject | Dose mg. | Serum Concentration (μg./ml.) | | | | Urine mg. in 6 hr. |
|---|---|---|---|---|---|---|
| | | 1 hr. | 2 hr. | 4 hr. | 6 hr. | |
| A | 500 | 2·0 | 2·8 | 2·5 | 1·8 | 101 |
| C | 500 | 1·7 | 1·9 | 2·4 | 0·8 | 66 |
| D | 500 | 0·9 | 0·8 | 0·9 | 0·3 | 17 |
| G | 500 | 1·5 | 2·0 | 2·0 | 1·1 | 31 |
| H | 500 | 0·7 | 0·9 | 0·9 | 0·3 | 30 |
| K | 500 | 0·8 | 1·9 | 1·9 | 1·3 | 72 |
| L | 500 | 1·0 | 1·9 | 2·4 | 1·3 | 27 |
| M | 500 | 1·3 | 1·8 | 2·1 | 1·2 | 85 |
| N | 500 | 0·8 | 1·6 | 2·0 | 1·1 | 72 |
| P | 500 | 2·1 | 2·8 | 2·4 | 1·5 | 82 |
| Mean .. | | 1·3 | 1·8 | 2·0 | 1·1 | 58 (12%) |

TABLE VIII.—*Serum Concentration on Day 1 and Day 4 After the Administration of 500 mg. of Penbritin Every Eight Hours for Four Days*

| Subject | Dose mg. | Day | Serum Concentrations (μg./ml.) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | ½ hr. | 1 hr. | 2 hr. | 4 hr. | 6 hr. | 8 hr. |
| B | 500 t.d.s. | 1 | 0·1 | 0·6 | 4·8 | 1·4 | 0·22 | 0·08 |
| | | 4 | 0·12 | 1·0 | 3·2 | 0·7 | 0·23 | 0·06 |
| D | 500 t.d.s. | 1 | 0·1 | 0·16 | 5·9 | 1·5 | 0·17 | 0·08 |
| | | 4 | 0·27 | 1·7 | 3·3 | 0·8 | 0·19 | 0·06 |
| K | 500 t.d.s. | 1 | 0·24 | 0·75 | 4·6 | 1·3 | 0·22 | 0·14 |
| | | 4 | 0·17 | 2·6 | 2·0 | 0·5 | 0·21 | 0·06 |
| M | 500 t.d.s. | 1 | 0·07 | 0·17 | 4·3 | 1·2 | 0·48 | 0·12 |
| | | 4 | 1·05 | 3·7 | 2·3 | 0·8 | 0·21 | 0·05 |

concentrations obtained with 500 mg. of tetracycline are well below those of penbritin for the first two hours after dosing. The urine excretion of tetracycline over the six-hour period was only 12% of the administered dose; consequently the concentration of tetracycline in the urine (50–200 μg./ml.) was well below that obtained with an equivalent dose of penbritin (250–1,000 μg./ml.).

*Experiment 7.*—The data obtained so far relates only to single-dose studies, and in view of the rather slow absorption (peak serum concentrations at approximately two hours and significant levels at six hours) it was decided to investigate whether there was any accumulation of penbritin in the serum on a possible clinical regimen. Four subjects were given two 250-mg. capsules every eight hours for four days—that is, 12 doses. Serum concentrations were obtained on Day 1 and Day 4. No side-effects were reported in this small group. Results are shown in Table VIII. It will be seen that in this small number of subjects there was certainly no accumulation of penicillin on an eight-hourly regimen. In fact, the overall levels obtained on the fourth day were, on the whole, lower than those obtained on the first day.

### Summary and Conclusions

Investigations were carried out on a new penicillin (penbritin; B.R.L. 1341) to determine a suitable scheme of dosage for therapeutic trials.

Consideration of serum concentrations in terms of μg./ml., together with the minimum inhibitory concentrations of the antibiotic for various organisms, serves as a rough guide to the possible value of the penicillin *in vivo*, assuming that the presence of human serum does not markedly depress its antibiotic activity. Penbritin is little affected by serum (Rolinson and Stevens, 1961).

Penbritin is well absorbed orally, and some 30% of a given dose is excreted in the urine within six to eight hours of dosing.

The single- and multiple-dose studies described suggest that for therapeutic trials the following dosage regimen might be adopted:

250 mg. six-hourly for the treatment of infections due to Gram-positive organisms or those due to *H. influenzae*. 250–500 mg. six- or eight-hourly is suggested for infections of the urinary tract in view of the high concentrations of penbritin obtained in the urine. 750 mg. or more eight-hourly might well be required for the treatment of infections due to Gram-negative organisms other than those occurring in the urinary tract.

Our thanks are due to Miss J. Willacy and Miss E. Smith for the biological assays, to the volunteers who so willingly and regularly attended for their morning dose of penbritin, and to Miss J. E. Crawford for her help in the preparation of this paper.

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# "PENBRITIN": AN ORAL PENICILLIN WITH BROAD-SPECTRUM ACTIVITY

BY

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The isolation of 6-aminopenicillanic acid (Batchelor *et al.*, 1959) made possible the biosynthesis of many modifications of penicillin. Such modifications have already led to a compound with intensified anti-staphylococcal activity (methicillin) and to others with improved properties of absorption from the gut (phenethicillin, phenoxypropylpenicillin). To date, however, such compounds have shown a narrowing of antibacterial activity compared with penicillin G. An exception to this trend is represented by the derivative 6[D(−)-α-aminophenylacetamido]penicillanic acid ("penbritin"; B.R.L. 1341). This derivative was found to have a bactericidal effect at low concentrations against a variety of Gram-negative as well as Gram-positive organisms, and we are reporting here our microbiological and preliminary therapeutic results with it.

### Methods

The laboratory and clinical methods followed closely those already described in our reports on methicillin (Stewart, 1960, 1961; Stewart *et al.*, 1960). The following modifications were introduced in this study.

*Tests for Penicillinase.*—In addition to conventional methods we now use a membrane filter technique for identifying organisms as producers of extracellular penicillinase. In this method (Holt and Stewart, 1961)