A COMPARISON OF FOUR PHENOXYPENICILLINS

BY

JILLIAN M. BOND, Ph.D. J. W. LIGHTBOWN, M.Sc., Dip.Bact.
National Institute for Medical Research, Mill Hill, London

MARY BARBER, M.D. PAMELA M. WATERWORTH,* F.I.M.L.T.
Postgraduate Medical School, Hammersmith Hospital, London

The introduction of the acid-stable phenoxymethylpenicillin (penicillin V) (Spitzy, 1953 ; Schindelmaisser, 1954) made oral penicillin therapy possible for many infections. Although not so active on an equal weight basis as benzylpenicillin, the difference was not very great, and phenoxymethylpenicillin has proved its value. Subsequent to the discovery of 6-aminopenicillanic acid a number of semi-synthetic penicillins analogous to phenoxymethylpenicillin have been produced and made available with various claims regarding their superiority over phenoxymethylpenicillin. Phenoxyethylpenicillin was described in 1959 (Perron *et al.*, 1959–60 ; Gourevitch *et al.*, 1959–60 ; Pindell *et al.*, 1959–60 ; Morigi *et al.*, 1959–60 ; Cronk *et al.*, 1959–60), phenoxypropylpenicillin in 1961 (Williamson *et al.*, 1961), and phenoxybenzylpenicillin in 1962 (Rollo *et al.*, 1962).

As each phenoxypenicillin has been introduced it has been compared with one or more of those already available. Phenoxyethylpenicillin was claimed to be superior to phenoxymethylpenicillin, the phenoxypropyl derivative was claimed to be superior to both. Phenoxybenzylpenicillin was claimed to have advantages over phenoxymethylpenicillin and phenoxyethylpenicillin.

In each instance the evidence made available to support the different claims has not been convincing, and the need for a comparison of all four derivatives was pointed out by the *British Medical Journal* (1962). In the absence of a clinical comparison recourse must be made to the measurement of blood levels produced in healthy human volunteers and the relative antibacterial activities, on a weight basis. Such comparisons have formed the basis of the relative evaluations already published, but the results which can be obtained are dependent on the experimental technique used, and this has not always been clearly described.

Determination of Blood Level

Penicillins in blood serum are usually partially bound to protein, the complex being dissociable to differing degrees. The relative importance of bound and dissociated penicillin is arguable, but any comparison of a series of homologous penicillins must take careful account of the two states. In previous studies the aim has been to determine total penicillin in the blood (bound and dissociated); this is accomplished if the standards of comparison used in the biological assay are dissolved in a medium with the same affinity for the penicillin being assayed as the serum protein. If the diluent for the standard has a greater affinity for the penicillin than serum protein, the estimates of penicillin in the blood will be falsely high ; if a lesser affinity then the blood levels measured will be low. Since the four phenoxypenicillins have markedly different affinities for serum protein, the use of a common diluent may produce different degrees of discrepancy. Bunn and Knight (1961) demonstrated that errors of the order of 100% could be introduced if 7% bovine albumin was used as diluent in the assay of various penicillins in serum, a concentration close to that used by Williamson *et al.* (1961).

The further complication that the sera of different individuals bind penicillin to varying extents (Lightbown and Sulitzeanu, 1957) means that a true measure of the total penicillin in a serum sample can be obtained only if the standard samples are dissolved in homologous serum, a procedure which is tedious but possible in a controlled study. With such a technique using relatively pure penicillins a measure of the total weight of antibiotic per millilitre of serum can be made. The concentration of free antibiotic must be measured separately.

One laboratory has reported comparative blood levels of different phenoxypenicillins by a method ascribed to Kunin *et al.* (1959). This technique, first used to compare blood levels produced by a given dose of a series of different tetracycline derivatives, assays the different antibiotics in serum samples against standard dilutions of one arbitrarily chosen member of the series. Williamson *et al.* (1961) in this way reported the activities of serum samples containing phenoxypenicillins in terms of a benzylpenicillin standard. The method contravenes all the accepted principles of biological standardization (Jerne and Wood, 1949 ; Miles, 1952), and yields results which are valueless and may be misleading. The values obtained will be a property of the test organism chosen, usually *Sarcina lutea*, and will depend on the many biological variables of the assay ; they give no indication at all of the relative values of the different antibiotics *in vivo* except perhaps for an animal with the specific nutrient agar of the assay circulating through its blood system, infected with *S. lutea*.

If the standard of comparison used in the assay is prepared from material identical with the antibiotic present in the serum samples then the assay will measure the weight of antibiotic present per millilitre of serum. The comparison of the antibacterial activities of the different antibiotics must be measured independently.

Determination of Antibacterial Activity

In previous comparative examinations of the phenoxypenicillins the effect of serum protein on antibacterial activity has been largely ignored. Blood levels approximating only to the total have been reported together with comparative values for minimum inhibitory concentrations against various micro-organisms measured in nutrient broth, the implicit suggestion being made that the relative activities which are found in broth will obtain in serum or tissue fluid. Gourevitch *et al.* (1959–60, 1961) showed that in the presence of serum the relative activities of the phenoxypenicillins against *Staph. aureus* were altered. It is therefore necessary to compare minimum inhibitory concentrations in serum as well as in broth.

Methods

Design of Trial

Four oral penicillins of the phenoxy group were selected for comparison. These were: (1) phenoxymethylpenicillin (penicillin V, supplied by Eli Lilly as K salt) ; (2) phenethicillin (phenoxyethylpenicillin ; " broxil," supplied by Beecham Research Laboratories Ltd. as K salt) ; (3) propicillin (phenoxypropylpenicillin ; " brocillin," supplied by Beecham Research Laboratories Ltd. as K salt); and

*Working with a full-time grant from the Medical Research Council.

(4) phenbenicillin (phenoxybenzylpenicillin ; " penspek," supplied by Distillers Co. (Biochemicals) Ltd. as K salt, purity 98.6%). The international approved names are used throughout this paper.

Each antibiotic was made up in capsules of 250 mg. at the Hammersmith Hospital Dispensary. Healthy volunteers, of both sexes, ranging from 17 to 50 years in age, and 7 to 15 st. (45 to 95 kg.) in weight, were given a single oral dose of 250 mg. after a light breakfast. Serum levels were estimated after one, two, four, and six hours.

The trial was divided into two experiments. In July, 1962, 10 subjects (Nos. 1–10) were given each of the four antibiotics at weekly intervals, in the order phenoxymethylpenicillin, phenethicillin, phenbenicillin, and propicillin. In October, 1962, this procedure was repeated with 10 different subjects (Nos. 11–20) ; again each one received all four antibiotics at weekly intervals.

A sample of blood was taken before the dose was given, to use for the preparation and dilution of standards as described in the next section ; further blood samples were taken after one, two, four, and six hours. The serum was separated by centrifugation, stored at 4° C., and assayed the next day. Urine samples were collected from some of the patients in each group and examined both for antibiotic content by direct assay and for chemical change of the antibiotic by chromatography.

Assay of Serum Samples

The assay technique used was that described by Lightbown and Sulitzeanu (1957) using 10 by 10-in. (25 by 25-cm.) glass plates and fish-spine beads. The test organism was *S. lutea* NCTC 8340. Samples were assayed for both total and free, or unbound, antibiotic content.

Estimation of Total Antibiotic.—Two concentrations of a standard solution of the appropriate antibiotic were made up in the serum of each subject, taken before the dose was given, and used as standard for the assay of the four undiluted serum samples from the same subject. In this way the antibiotic in the standards was bound to serum proteins to exactly the same extent as in the serum samples, and the assay would thus estimate the total antibiotic in the sample. The material used to prepare the standard solutions was a portion of that used to prepare the capsules. Thus variation of content of optical isomers and contaminating penicillins between standard and test was avoided.

Estimation of "Free" Unbound Antibiotic.—Serum samples were filtered through dialysis tubing under negative pressure and the resulting protein-free filtrate was assayed against the appropriate antibiotic standards in phosphate buffer ($\mu=0.1$, pH $=7.0$): 3 ml. of serum in 1-cm. dialysis tubing (" visking cellophane ") under 600 mm. pressure for two hours (at 4° C.) gave about 1 ml. of clear colourless filtrate. Controls, consisting of antibiotic in phosphate buffer instead of serum, were set up and run concurrently with the serum samples. Both filtrates and sac contents from these controls were assayed against standards in buffer.

Estimation of Urine Samples

In the first experiment the urine passed by five male subjects in the first six hours was collected and the volume measured. A 150-ml. sample from each was acidified to pH 2 with phosphoric acid, and then extracted immediately with three 20-ml. amounts of ether. The resulting emulsion was broken, the ether extract dried with anhydrous magnesium sulphate, 1 ml. removed, and diluted at least 1/100 in buffer and assayed against standard in phosphate buffer. The remaining ether was further concentrated and examined chromatographically using a butanol, ethanol, H_2O, 4:1:5 solvent system and biological development, the test organism being *Bacillus subtilis* NCTC 8236. A modification of the method of Goodall and Levi (1947) was also used.

In the second experiment the volume of urine passed by five male patients in the first eight hours was measured and a sample from each diluted in buffer and assayed directly for antibiotic content.

Measurement of Minimum Inhibitory Concentrations of Phenoxypenicillins in Presence and Absence of Serum

Experiments were carried out with fresh pooled human serum or serum supplied by the Lister Institute. In both cases the serum was Seitz-filtered. Appropriate serial dilutions of the penicillins were added in 0.5-ml. quantities

TABLE I.—*Phenoxymethylpenicillin. Total Levels of Antibiotic in Serum (μg./ml.) After a Single Oral Dose of 250 mg.*

Subject No.	Time after Administration			
	1 Hour	2 Hours	4 Hours	6 Hours
1	4·60	0·99	0·106	0·052
2	1·21	0·61	0·117	0·055
3	2·78	0·71	0·134	Trace
4	1·31	0·57	0·149	0·057
5	5·30	0·64	0·129	0·064
6	2·46	0·60	0·074	0·053
7	1·38	0·51	0·073	Trace
8	1·41	0·76	0·242	0
9	1·38	0·43	0·109	0·058
10	0·34	1·27	0·446	0·065
Mean 1–10	2·22	0·71	0·158	0·046
11	0·88	0·47	0·504	0·077
12	1·16	1·67	0·238	0·061
13	3·01	1·00	0·136	0·064
14	1·45	1·30	0·363	0·056
15	1·57	1·76	0·471	0·060
16	4·26	1·60	0·144	0·064
17	0·18	0·35	0·785	0·685
18	0·70	0·40	0·074	0·063
19	2·02	0·51	0·264	0·053
20	5·87	0·65	0·074	0·044
Mean 11–20	2·11	0·97	0·305	0·123
Mean 11–20 (No. 17 omitted)	2·32	1·04	0·252	0·060
Grand mean 1–20 (No. 17 omitted)	2·27	0·87	0·205	0·053

Trace = Approximately 0·050 μg./ml.

TABLE II.—*Phenethicillin. Total Levels of Antibiotic in Serum (μg./ml.) After a Single Oral Dose of 250 mg.*

Subject No.	Time after Administration			
	1 Hour	2 Hours	4 Hours	6 Hours
1	1·28	2·66	0·73	0·122
2	4·21	1·31	0·27	0·088
3	4·32	6·55	0·55	0·094
4	2·72	1·98	0·29	0·090
5	3·39	2·01	0·34	0·096
6	4·59	1·87	0·15	Trace
7	4·95	2·05	0·25	0·092
8	0·99	2·36	0·35	0·076
9	1·72	0·81	0·20	0·081
10	8·10	1·05	0·14	Trace
Mean 1–10	3·63	2·27	0·33	0·084
11	1·54	1·05	0·25	0·097
12	3·32	2·41	0·29	0·068
13	5·21	3·01	0·32	0·070
14	3·90	2·03	0·30	0·089
15	2·38	2·02	0·29	0·083
16	3·18	2·34	0·25	0·068
17	3·67	3·03	0·46	0·088
18	0·64	0·51	0·57	0·075
19	0·98	2·15	0·26	0·079
20	6·61	1·21	0·18	0·068
Mean 11–20	3·14	1·98	0·32	0·079
Mean 11–20 (No. 17 omitted)	3·08	1·86	0·30	0·077
Grand mean 1–20 (No. 17 omitted)	3·37	1·97	0·32	0·081

Trace = Approximately 0·050 μg./ml.

to 4.5 ml. of neat serum or broth. The tubes were inoculated with 0.02 ml. of an overnight broth culture diluted 1 in 500 (small inoculum) or undiluted (large inoculum).

Results

Total Antibiotic

The individual results (in μg/ml.) are given in Tables I to IV, the two groups being distinguished for each antibiotic. The results of Subject 17 are given, but were not included in the final grand mean, since this person gave very abnormal serum levels with three of the four antibiotics.

There was considerable variation between subjects with each antibiotic, especially at one and two hours. The differences between the four antibiotics can best be seen in Fig. 1, where mean total serum levels (means of 19 patients, No. 17 omitted) have been plotted against time.

TABLE III.—*Propicillin. Total Levels of Antibiotic in Serum (μg./ml.) After a Single Oral Dose of 250 mg.*

Subject No.	Time after Administration			
	1 Hour	2 Hours	4 Hours	6 Hours
1	5·25	2·75	0·54	0·112
2	3·50	1·88	0·44	0·113
3	5·78	4·30	0·51	0·102
4	2·88	2·27	0·35	Trace
5	5·39	2·03	0·28	,,
6	4·13	2·59	0·27	,,
7	5·52	2·30	0·38	0·160
8	2·47	1·04	0·39	0
9	1·01	0·87	1·15	0·231
10	5·95	1·54	0·18	Trace
Mean 1–10	4·19	2·16	0·45	0·104
11	1·21	1·02	0·54	0·112
12	3·53	2·55	0·84	0·190
13	1·31	2·98	1·31	0·173
14	4·86	2·36	0·39	0·119
15	2·44	3·11	0·66	0·151
16	3·27	2·21	0·26	0·086
17	0·89	1·09	2·34	0·990
18	1·05	0·87	0·24	Trace
19	5·21	3·17	0·34	0·095
20	3·17	1·04	0·18	Trace
Mean 11–20	2·70	2·04	0·71	0·208
Mean 11–20 (No. 17 omitted)	2·90	2·15	0·53	0·121
Grand mean 1–20 (No. 17 omitted)	3·58	2·15	0·49	0·113

Trace = Approximately 0·080 μg./ml.

TABLE IV.—*Phenbenicillin. Total Levels of Antibiotic in Serum (μg./ml.) After a Single Oral Dose of 250 mg.*

Subject No.	Time after Administration			
	1 Hour	2 Hours	4 Hours	6 Hours
1	10·57	6·55	1·41	0·52
2	1·71	5·43	2·12	0·58
3	10·42	5·54	0·95	Trace
4	4·61	5·35	1·11	0·38
5	8·06	8·14	1·43	0·47
6	9·75	5·92	0·93	0·37
7	9·22	5·15	0·75	0·32
8	1·33	4·46	2·21	0·58
9	1·29	1·84	3·64	0·89
10	1·68	4·24	2·26	0·40
Mean 1–10	5·86	5·26	1·68	0·48
11	5·89	3·34	1·05	0·41
12	5·27	6·90	1·70	0·46
13	1·18	3·03	4·11	0·71
14	1·89	1·85	1·65	0·70
15	2·89	4·11	2·09	0·71
16	7·52	6·94	1·73	0·51
17	1·92	3·98	2·82	1·20
18	2·32	4·86	1·28	0·43
19	12·76	6·26	1·20	0·52
20	8·10	2·01	0·47	0·28
Mean 11–20	4·97	4·33	1·81	0·59
Mean 11–20 (No. 17 omitted)	5·31	4·37	1·70	0·53
Grand mean 1–20 (No. 17 omitted)	5·60	4·84	1·69	0·50

Trace = Approximately 0·250 μg./ml.

These results were examined statistically to see if the differences between blood levels were significant. At each time interval the difference between the serum levels of two preparations to be compared was found for each individual. These differences were then tested for the significance of their deviation from zero by a t test. In this way the between-subject variation was removed. Initially phenoxymethylpenicillin was compared with each of the other three penicillins, then propicillin and phenbenicillin were each compared with phenethicillin. The results are summarized in Table V.

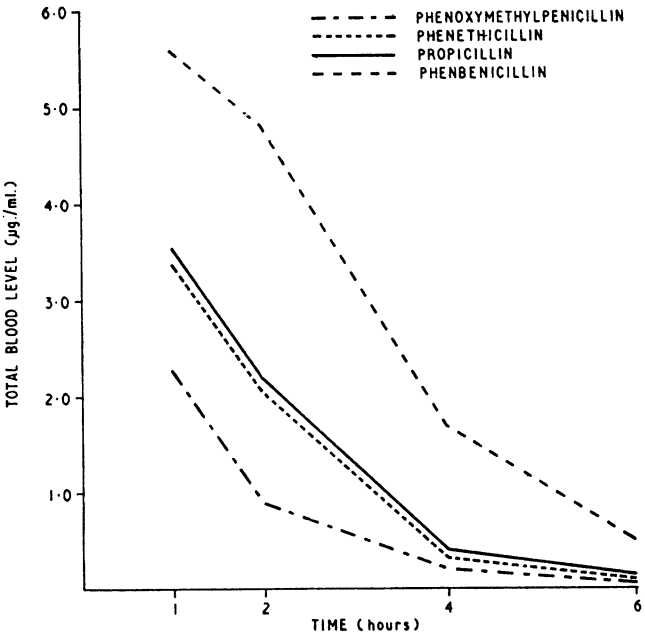

FIG. 1.—Mean blood levels of total antibiotic after a single oral dose of 250 mg. in 19 subjects.

TABLE V.—*Evaluation of Differences Between Levels of Total Antibiotic of Four Phenoxypenicillins in 19 Subjects After a Single Oral Dose of 250 mg. (by Means of t Test). Values of t*

		Time after Administration			
		1 Hour	2 Hours	4 Hours	6 Hours
Comparison of phenoxymethylpenicillin with:	Phenethicillin	2·01 B	4·12 HS	2·80 S	4·8 HS
	Propicillin	2·75 S	6·24 HS	3·6 HS	5·5 HS
	Phenbenicillin	4·38 HS	9·48 HS	11·8 HS	12·3 HS
Comparison of phenethicillin with:	Propicillin	0·39 NS	0·42 NS	2·44 B	2·6 S
	Phenbenicillin	2·09 B	6·30 HS	6·26 HS	18·4 HS

HS = High significant. Probability of obtaining value of t by chance < 0·001.
S = Significant. Probability of obtaining value of t by chance 0·001–0·02.
B = Borderline. Probability of obtaining value of t by chance 0·02–0·10.
NS = Not significant. Probability of obtaining value of t by chance > 0·10.

The ages and weights of the 20 subjects ranged from 17 to 50 years and 7 to 15 st. (45 to 95 kg.). The total serum levels at one hour and four hours were examined for possible variation with the age or the weight of the subjects, but no correlation with either of these factors was found.

Free Antibiotic

With phenethicillin, propicillin, and phenbenicillin all one- and two-hour serum samples from Subjects 11–20 in the second experiment were filtered as described under Methods, and the filtrates assayed. With phenoxymethylpenicillin only 13 of the possible 20 samples were tested in this way. The individual free levels are given in Table VI, and the mean free serum levels are shown in Fig. 2.

The amount of antibiotic bound to the serum proteins was assumed to be the difference between the total and free

levels. The individual and mean values obtained in this way with each of the antibiotics, and expressed as a percentage of the total antibiotic present, are given in Table VII.

In the first experiment of this comparison a different technique of filtration was used. This was a modification of the method described by Coolidge (1940). Serum samples were filtered through dialysis membrane by centrifuging the dialysis sacs containing the serum at 30,000 *g* in a refrigerated centrifuge. However, it was found with this method that the antibiotics did not filter freely. Thus in control experiments with antibiotic in buffer instead of serum the antibiotic was concentrated in the sac. The results obtained by this method have not been included, and it is stressed that any method of filtration of serum should be carefully controlled to avoid differential filtration of the antibiotic itself.

TABLE VI.—*Levels of Free Antibiotic (μg./ml.) After a Single Oral Dose of 250 mg.*

Subject No.	Phenoxymethyl-penicillin		Phenethicillin		Propicillin		Phenbenicillin	
	1 hr.	2 hrs.	1 hr.	2 hrs.	1 hr.	2 hrs.	1 hr.	2 hrs.
11	0·21		0·41	0·30	0·14	0·13	0·40	0 13
12		0·30	0·69	0·58	0·35	0·28	0·12	0·14
13	0·49		0·98	0·72	0·14	0·30	(Trace)	0·09
14	0·15		0·69	0·40	0·40	0 21	0·06	0·05
15	0·30	0·32	0·57	0·56	0·31	0·25	0·06	0·11
16	1·00		0·82	0·65	0·42	0·26	0·13	0·15
17	0·05	0·11	0·81	0·66	0·09	0·11	0·06	0·08
18	0·15		0·25	0·19	0·11	0·10	0·07	0·16
19	0·32		0·28	0·45	0·54	0·38	0 21	0·11
20	0·57	0·14	0·89	0·34	0·31	0·14	0·18	0·09
Mean	0·36	0·22	0·64	0·49	0·28	0·22	0·14	0·11

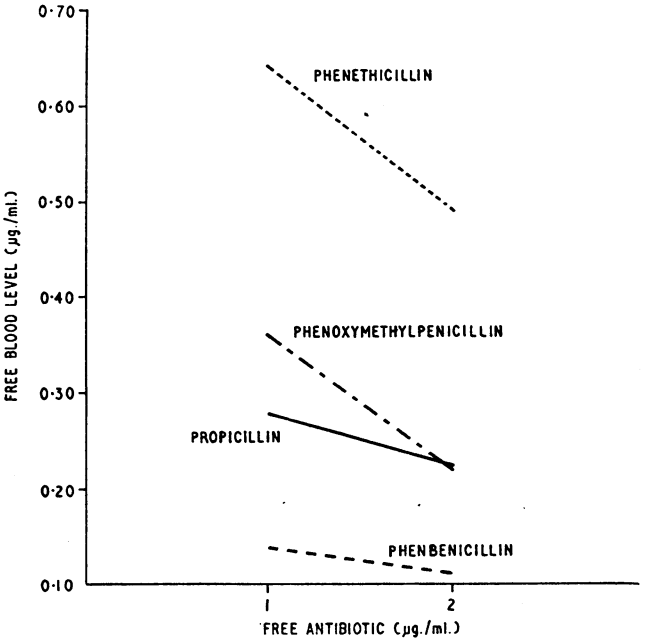

FIG. 2.—Blood levels of free antibiotic after a single dose of 250 mg.

TABLE VII.—*Percentage of Antibiotic Bound to Serum Protein with Four Phenoxypenicillins*

Subject No.	Phenoxymethyl-penicillin		Phenethicillin		Propicillin		Phenbenicillin	
	1 hr.	2 hrs.	1 hr.	2 hrs.	1 hr.	2 hrs.	1 hr.	2 hrs.
11	76·1		73·7	71·9	88·4	87·6	93·3	96·1
12		81·9	79·2	76·0	90·0	88·9	97·8	98·0
13	83·9		81·2	76·1	89·6	89·9	*	97·0
14	89·9		82·3	80·1	91·9	91·2	97·1	97·2
15	80·8	81·9	76·0	72·2	87·5	92·0	98·0	97·9
16	76·5		74·2	72·3	87·1	88·1	98·3	98·1
17	70·6	68·8	77·9	78·2	90·1	89·6	97·1	96·8
18	77·9		61·8	62·7	89·8	88·0	96·9	98·3
19	84·2		71·2	79·3	89·7	88·0	98·4	98·3
20	90·2	79·2	86·5	71·6	90·4	86·7	97·8	95·6
Mean	81·1	77·9	76·4	74·0	89·5	89·0	97·2	97·2
Grand mean	80·1		75·2		89·3		97·2	

* Free antibiotic below the level detectable by assay.

Inactivation of Phenoxypenicillins in Serum at 37° C.

Samples of serum (*p*H 7) containing the four phenoxypenicillins were incubated at 37° C. and the total penicillin content was assayed at intervals. Inactivation occurred at approximately the same rate for all four penicillins—that is, 10–20% of activity being lost in 5 hours.

Recovery of Antibiotic in the Urine

The amounts of antibiotic excreted into the urine of the subjects examined in each group are given in Table VIII.

TABLE VIII.—*Recovery of Phenoxypenicillins in Urine After a Single Oral Dose of 250 mg.*

Subject No.	Antibiotic Activity Expressed as mg. of Dosage Material			
	Phenoxymethyl-penicillin	Phenethicillin	Propicillin	Phenbenicillin
	Experiment 1: Urine up to 6 Hours			
5	86·5	55·0	77·3	49·4
6	49·4	65·5	61·2	59·3
7		71·0	75·9	81·9
8		41·9	47·0	60·8
9		39·5		18·0
10	57·7	75·1	62·3	45·1
Mean	64·5	58·0	64·8	52·4
	Experiment 2: Urine up to 8 Hours			
11	45·4	55·7	44·3	55·7
12	81·2	73·5	63·6	66·3
13	57·6	106·9	76·0	56·3
14	85·5	95·6	101·3	34·2
16	56·1	75·3	74·4	35·4
Mean	65·2	81·4	71·9	49·6

The results of these assays are difficult to evaluate, since it was shown chromatographically that all four antibiotics had been modified, with the production, in each case, of at least two biologically active components. These were either not present in the original material or present in different proportions. The differences between standard and sample invalidate the assays, and if, as is likely, the two or three components present had different activities against the test organism the estimated weights give little guide to the weight of antibiotic material excreted. The dose-response lines obtained with urine samples and the standard solutions in buffer did not show marked departure from parallelism, as was found by Jensen *et al.* (1962) with benzylpenicillin. Thus the invalidity of the results would not be obvious in the absence of chromatographic examination.

Minimum Inhibitory Concentrations of Phenoxypenicillins

Results with Staph. aureus.—A typical experiment showing the sensitivity of a strain of *Staph. aureus* to the four phenoxypenicillins in serum and broth with a small and large inoculum is given in Table IX; a number of other penicillins are included for comparison. It will be seen that under all conditions of test benzylpenicillin gave the lowest minimum inhibitory concentration. Phenoxymethylpenicillin and phenethicillin were the next most effective, and in both cases there was a fourfold increase in the inhibitory concentration in the presence of serum. Propicillin and phenbenicillin both showed a high degree of serum interference. The activity of ampicillin was unaffected by serum. It will also be seen that in tests with methicillin and cloxacillin against a penicillinase-producing

staphylococcus the former was unaffected by serum whereas the latter showed a sixteenfold decrease in activity.

Table X summarizes the fold increase in minimum concentration in a number of experiments with different strains of *Staph. aureus* and different batches of serum. It will be seen that phenbenicillin shows the highest degree of serum inactivation, after which come propicillin, phenethicillin, and phenoxymethylpenicillin in that order.

Results with Streptococcus pyogenes.—In experiments with a single strain of *Str. pyogenes*, using neat culture as the inoculum, the results in relation to bacteriostasis were as shown in Table XI. The order of serum-binding was similar to that seen in experiments with *Staph. aureus*. With *Str. pyogenes*, serum caused a much greater inactivation of the bactericidal activity of all the penicillins, so that if the cultures were plated out after 20 hours survivors were found in concentrations of 0.12 μg./ml. of all the penicillins, even benzylpenicillin. This was not seen with *Staph. aureus*.

TABLE IX.—*Sensitivity of a Strain of Staph. aureus to Penicillins in Tests with a Small and Large Inoculum in Broth or Serum. Figures Represent Minimum Inhibitory Concentrations in μg./ml.*

	Small Inoculum		Large Inoculum	
	Broth	Serum	Broth	Serum
Benzylpenicillin	0·03	0·03	0·06	0·12
Phenoxymethylpenicillin	0·06	0·25	0·06	0 25
Phenethicillin	0·06	0·25	0 06	0 25
Propicillin	0·12	0·50	0·12	1·00
Phenbenicillin	0·25	2·00	0·25	2·00
Ampicillin	0·25	0·25	0·25	0 25
Methicillin*			4·00	4·00
Cloxacillin*			0·50	8·00

* Tested with a penicillinase-producing strain.

TABLE X.—*Summary of Experiments Comparing Sensitivity of Staph. aureus to Penicillins in Serum and Broth. Fold Decrease in Sensitivity in Serum—i.e., Ratio* $\frac{\text{M.I.C. in Serum}}{\text{M.I.C. in Broth}}$. *Each Figure Represents a Different Test*

	Small Inoculum							Large Inoculum			
Benzylpenicillin	0	0	0	0	0	0	0	2	0	2	4
Phenoxymethylpenicillin	4	4	0	0	0	0	0	4	4	4	2
Phenethicillin	4	4	4	2	2	4	4	4	8	8	4
Propicillin	4	4	4	4	4	8	4	8	2	8	4
Phenbenicillin	8	16	16	8	8	16	16	8	16	32	8
Ampicillin	0	0	0	0	0	0	0	0	0	0	0

TABLE XI

	M.I.C. in Serum	Fold Increase Compared with Broth
Benzylpenicillin	0·03	4
Phenoxymethylpenicillin	0·06	4
Phenethicillin	0·25	8
Propicillin	0·25	8
Phenbenicillin	1·0	32

Discussion

The results obtained with the four phenoxypenicillins may be compared in two ways. Firstly, on the basis of the total blood levels obtained and the minimum inhibitory concentrations measured in the presence of 95% serum, and, secondly, on the basis of the free blood levels and the minimum inhibitory concentrations obtained in broth. In Table XII these comparisons are made on the basis of the ratio of the blood level to the appropriate minimum inhibitory concentration. The higher the ratio the more effective one would expect the antibiotic to be.

Against *Staph. aureus*, phenoxymethylpenicillin and phenethicillin are clearly superior to propicillin and phenbenicillin by both methods of comparison. The differences between the first two homologues are marginal at one hour, but at two hours phenethicillin is probably the better. Since the dilution steps used in measuring the minimum inhibitory concentrations were twofold, this will limit the significance of the differences between the ratios. With *Str. pyogenes*, again by both methods, phenoxymethylpenicillin and phenethicillin are superior, with phenoxymethylpenicillin giving the best results.

The results suggest that, from the point of view of available activity at a given time after a given dose, phenoxymethylpenicillin and phenethicillin are to be preferred. These are the two penicillins showing the lowest degree of serum-binding. It might be argued, in the case of phenbenicillin, that the large proportion of antibiotic bound to serum protein is advantageous in that it will serve as a depot, prolonging the duration of the blood level. As has been suggested by Goldstein (1949), this could be beneficial if the level of free antibiotic was at the same time high enough to give an adequate therapeutic effect. In the case of phenbenicillin this would be expected only with a much increased dosage.

TABLE XII.—*Comparison of Four Phenoxypenicillins on the Basis of the Ratio of Total or Free Blood Level to the Appropriate Minimum Inhibitory Concentration*

Ratio	Total Blood Level M.I.C. in Serum				Free Blood Level M.I.C. in Broth			
Organism	*Staph. aureus*		*Str. pyogenes*		*Staph. aureus*		*Str. pyogenes*	
Time	1 hr.	2 hrs.	1 hr.	2 hrs.	1 hr.	2 hrs.	1 hr.	2 hrs.
Phenoxymethylpenicillin	9·0	3·48	37·5	14·5	6·0	3·7	24·0	14·7
Phenethicillin	13·4	8·0	13·4	8·0	10·7	7·3	21·3	14·6
Propicillin	4·7	2·87	14·2	6	2·3	1·8	9·3	7·3
Phenbenicillin	2·74	2·4	5·48	1	0·56	0·44	4·7	3·7

It should be noted that the values we have reported for the percentage binding by serum protein are much higher than those previously cited.—for example, Pindell *et al.* (1959–60). In the experiments of the latter authors the binding was measured at a very high concentration of penicillin (5 mg. in 5 ml. of serum). This was dialysed against 20 ml. of saline. Under these conditions one would expect to find a lower degree of binding than at the comparatively low therapeutic levels (see Goldstein, 1949).

It has been shown previously, and confirmed here, that phenbenicillin gives more prolonged total blood levels than the other phenoxypenicillins, and it might be thought that this is a reflection of the increased affinity to serum protein. However, a study of the data for individual blood levels shows that in a relatively larger proportion of cases the peak blood level was reached at a later time for phenbenicillin—for example, 10 out of 19 later than one hour for phenbenicillin compared with 3 out of 19 for phenoxymethylpenicillin. This would suggest that the prolonged blood level is the result of a slower absorption rather than a slower excretion. Preliminary comparisons of the relative rates of elimination of phenbenicillin and phenoxymethylpenicillin after intravenous injection in rabbits has indicated that phenbenicillin disappears from the blood at least as rapidly as phenoxymethylpenicillin.

As was found previously with benzylpenicillin, so also with certain of the phenoxypenicillins, the degree of serum-binding varied from subject to subject. Variation may be expected either because of differences between subjects in affinity of serum protein for the penicillins or because of differences in the levels of free antibiotic. In the absence

of differences of affinity of individual sera for a given penicillin, the degree of binding would be expected to be maximal and constant at very low concentrations of free antibiotic and to vary inversely with concentration of free antibiotic at higher concentrations. Both phenbenicillin and propicillin would seem to be maximally bound over the whole range of free antibiotic concentrations observed (see Fig. 3); the variation between samples is small and probably represents mainly experimental error.

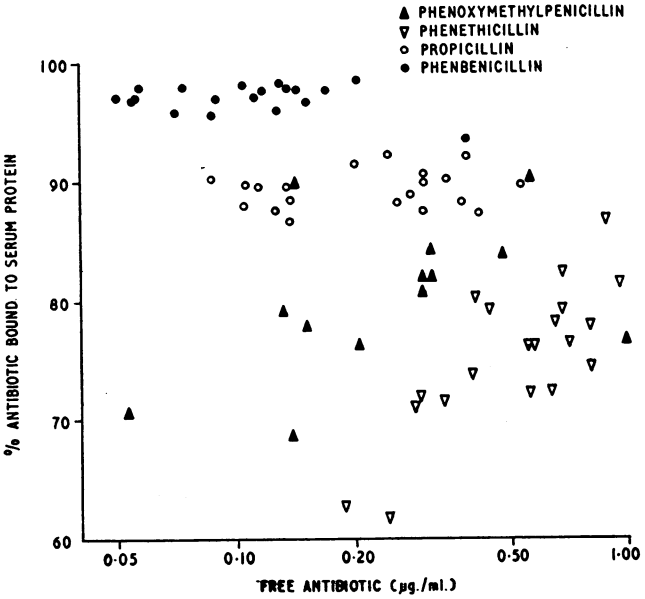

FIG. 3.—Relationship between free antibiotic and the percentage bound to serum protein. (Based on levels at one and two hours.)

With phenoxymethylpenicillin and phenethicillin the variation is much greater and there is a suggestion in Fig. 3 that the lowest degree of binding occurs at the lowest levels of free penicillin. This would be contrary to theoretical expectation unless the affinity of the serum proteins for phenoxymethylpenicillin and phenethicillin does in fact vary appreciably between subjects. If in those individuals with serum having a low affinity for these penicillins the excretion is more rapid than in those having a high affinity, then the relationship seen in Fig. 3 might be expected. In the case of phenethicillin the percentage binding in samples taken at two hours is less than at one hour but this is not significant.

Comment has been made on the relatively low recovery of phenbenicillin in urine in the light of the relatively high blood levels which it produces. In this study the recovery of antibiotically active material in urine expressed as weight of the particular penicillin administered was lowest for phenbenicillin. However, chromatographic analysis has shown that with each of the four penicillins considered the biologically active material excreted differs markedly in composition from the original drug, as was shown for phenbenicillin by Rollo *et al.* (1962) and for cloxacillin by Knudsen *et al.* (1962). Under these conditions it is impossible to make a quantitative comparison of the weights of the four phenoxypenicillins which are excreted. The fact that all four phenoxypenicillins are found in other biologically active forms in the urine does, of course, raise the possibility that these forms exist in the blood. So far this situation has not been described, but if it does occur the difficulty of comparing these four penicillins would be greatly increased.

Summary

Four phenoxypenicillins which are available for oral therapy have been compared. Blood-level determinations of free and total antibiotic have been studied in human volunteers, after a single oral dose, and these have been assessed in relation to the antibacterial activity of the compound *in vitro* in the presence and absence of serum.

The results predict that phenoxymethylpenicillin and phenethicillin would be equally effective against staphylococcal or streptococcal infections and that both would be more active than either propicillin or phenbenicillin.

We are indebted to Miss H. Leighton, of N.I.M.R., for technical assistance in carrying out the antibiotic assays, and to Miss J. Allen, of Hammersmith Hospital, for preparing the antibiotics in capsules. We also thank Miss M. Mussett for help and advice on the statistical evaluation of the results. Finally, we thank the 20 "volunteers" on the staff at Hammersmith Hospital who took part in these experiments.

REFERENCES

Brit. med. J., 1962, **1**, 99.
Bunn, P., and Knight, R. (1961). *Antibiot. and Chemother.*, **11**, 190.
Coolidge, T. B. (1940). *J. biol. Chem.*, **135**, 541.
Cronk, G. A., Naumann, D. E., Albright, H., and Wheatley, W. B. (1959–60). *Antibiot. Ann.*, **7**, 133.
Goldstein, A. (1949). *Pharmacol. Rev.*, **1**, 102.
Goodall, R. R., and Levi, A. A. (1947). *Analyst*, **72**, 277.
Gourevitch, A., Hunt, G. A., and Lein, J. (1959–60). *Antibiot. Ann.*, **7**, 111.
—— —— Luttinger, J. R., Carmack, C. C., and Lein, J. (1961). *Proc. Soc. exp. Biol. (N.Y.)*, **107**, 455.
Jensen, S. F., Lund, E., and Marner, I. L. (1962). *Acta path. microbiol. scand.*, **55**, 79.
Jerne, N. K., and Wood, E. C. (1949). *Biometrics*, **5**, 273.
Knudsen, E. T., Brown, D. M., and Rolinson, G. N. (1962). *Lancet*, **2**, 632.
Kunin, C. M., Dornbush, A. C., and Finland, M. (1959). *J. clin. Invest.*, **38**, 1950.
Lightbown, J. W., and Sulitzeanu, D. (1957). *Bull. Wld Hlth Org.*, **17**, 553.
Miles, A. A. (1952). *Bull. Wld Hlth Org.*, **6**, 131.
Morigi, E. M. E., Wheatley, W. B., and Albright, H. (1959–60). *Antibiot. Ann.*, **7**, 127.
Perron, Y. G., Crast, L. B., Gottstein, W. J., Minor, W. F., and Cheney, L. C. (1959–60). Ibid., **7**, 107.
Pindell, M. H., Tisch, D. E., Hoekstra, J. B., and Reiffenstein, J. C. (1959–60). Ibid., **7**, 119.
Rollo, I. M., Somers, G. F., and Burley, D. M. (1962). *Brit. med. J.*, **1**, 76.
Schindelmaisser, F. (1954). *Wein. med. Wschr.*, **104**, 369.
Spitzy, K. H. (1953). *Wein. klin. Wschr.*, **65**, 583.
Williamson, G. M., Morrison, J. K., and Stevens, K. J. (1961). *Lancet*, **1**, 847.

In his presidential address at the opening session of the annual conference of the National Society for Clean Air Dr. A. Parker, D.Sc., commended industry generally for an effort to improve the efficiency of combustion fuels, with a resulting reduction of smoke. He estimated that for the country as a whole the quantity of smoke emitted into the air by industry was now less than one-half of the amount emitted in 1956. This was a tribute partly to industry and partly to the efforts of the medical officers of health and the public health inspectors of the local authorities. Dr. Parker said that the domestic grates in this country emitted about three times as much smoke as was discharged from all the other uses of coal, and that the domestic chimney was by far the greatest cause of pollution at ground level. Yet the progress in dealing with domestic smoke was disappointingly slow. The so-called black areas of Great Britain covered about 4% of the area of the country, and by the beginning of April, 1963, smoke control was in operation over a total area of only 280 square miles, or less than one-tenth of the area that should eventually be covered. "It has on occasion been asserted that smoke control orders are an unwarranted interference with personal liberty. It seems to me that those who insist on polluting the air unnecessarily with smoke are interfering with the liberty of their neighbours who wish to breathe clean air, enjoy good health, and keep down damage to their property."