BACTERIOLOGICAL AND PHARMACOLOGICAL PROPERTIES OF PHENOXYBENZYLPENICILLIN

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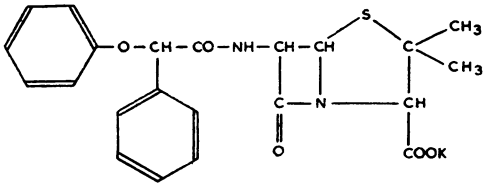
Phenoxybenzylpenicillin (" penspek " ; D.C.(B).L. 306) is 6(α-phenoxyphenylacetamido) penicillanic acid. The structural formula of the potassium salt is:



It may be prepared by the addition of a suitable precursor to a typical penicillin fermentation, or by the reaction of 6-aminopenicillanic acid with α-phenoxyphenylacetyl chloride or other suitable reagents.

The pure potassium salt is a white powder, readily soluble in water. In acid solution (*p*H 2), at 37° C., it has a half-life of 2 hours 3 minutes in comparison with 3 hours 34 minutes for penicillin V and 6 minutes for penicillin G.

We have shown that its antibacterial spectrum is similar to penicillin V and phenethicillin and that it produces particularly high and sustained blood levels in human volunteers.

Bacteriology

Methods

Minimum inhibitory concentrations (M.I.C.) required to prevent growth of bacteria for 18 hours at 37° C. were determined by serial dilution in nutrient broth (" oxoid " No. 2) with the following exceptions: for all streptococci and *Neisseria meningitidis* the broth was fortified with 5% horse serum; for *Corynebacterium diphtheriae* and *N. gonorrhoeae* 1% glucose was added to the broth and one drop of horse serum to each tube (7.5 ml.); for *Haemophilus influenzae* serial dilution tests were carried out in a specially prepared heated blood agar.

The inoculum for all organisms except *Staphylococcus aureus* and *H. influenzae* was 0.05 ml. of an 18-hour broth culture per 7.5 ml. For the staphylococcus a heavy inoculum of 1×10^7 organisms was used. For *H. influenzae* the agar in each tube was inoculated by spreading one loopful of an overnight broth culture over the surface.

The bactericidal activities of phenoxybenzylpenicillin, penicillin V, and phenethicillin were estimated by determining viable counts at ascending concentrations in nutrient broth at 0, 3, 6, and 24 hours. Eighteen-hour cultures of the sensitive strain of *Staph. aureus* 6718 were used to inoculate the nutrient broth to give a viable count of 10^8 cells/ml. Viable counts in the broth cultures were made by preparing tenfold dilutions in agar and counting the number of resulting colonies after overnight incubation at 37° C.

Sensitivity to *Bacillus cereus* penicillinase was determined by measuring the rate of CO_2 evolution from a sodium bicarbonate solution. This is expressed as the ratio of the rate of reaction for phenoxybenzylpenicillin to the rate of reaction for penicillin V.

Results

Streptococcus pyogenes and Streptococcus pneumoniae.—The minimum concentrations of phenoxybenzylpenicillin required to inhibit the growth of a number of strains of *Str. pyogenes* and *Str. pneumoniae* (Table I) show, in general, a close similarity to those of penicillin V and phenethicillin. Thus in six out of the seven strains of *Str. pyogenes* the M.I.C. for phenoxybenzylpenicillin was 0.025 μg./ml., compared with 0.012 μg./ml. for penicillin V and 0.025 μg./ml. for phenethicillin. With *Str. pneumoniae* the antibacterial activity of phenoxybenzylpenicillin was similar to that of penicillin V and phenethicillin (Table I).

Staphylococcus aureus.—Phenoxybenzylpenicillin was slightly less active than penicillin V against the seven sensitive strains tested, but all the seven strains tested were sensitive to less than 0.12 μg./ml. (Table II). Against penicillin-resistant strains phenoxybenzyl-

TABLE I.—*Sensitivity of Strains of Streptococcus pyogenes and Streptococcus pneumoniae to Phenoxybenzylpenicillin*

Organism		M.I.C. (μg./ml.)		
Species	Strain	Penicillin V	Phenoxybenzylpenicillin	Phenethicillin
Str. pyogenes	CN10	0·012	0·025	0·025
	GU1*	0·012	0·025	0·025
	GU2*	0·012	0·025	0·025
	GU24*	0·012	0·025	0·025
	GU48*	0·012	0·025	0·025
	GU64*	0·012	0·025	0·05
Str. pneumoniae	BO1	0·05	0·1	0·025
	Pn1	0·05	0·025	0·1
	GU3*	0·025	0·012	0·012
	GU8*	0·09	0·2	0·25
	GU60*	0·05	0·025	0·05

* Recently isolated clinical cultures.

TABLE II.—*Sensitivity of Strains of Staphylococcus aureus to Phenoxybenzylpenicillin*

Strain	M.I.C. (μg./ml.)		
	Penicillin V	Phenoxybenzylpenicillin	Phenethicillin
Sensitive: 7 strains*	0·03 (0·008–0·04)	0·07 (0·03–0·12)	0·03 (0·008–0·04)
Resistant:			
U134	600	200	200
U137	400	ca. 300	>400
U163	300	25	150
U95	300	200	200
U107	200	100	150
U146	200	38	100
U157	200	100	100
U125	150	40	100
U67	100	100	75
U74	100	200	200
U61	200	150	200
U155	100	60	50
U164	100	40	50
U165	75	100	75
U136	75	10	10
U161	75	40	38
U162	4·0	4·0	0·75
U130	2·5	5·0	1·9

* Mean and range.

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penicillin, like phenethicillin, was more active than penicillin V. This may be due to its lower sensitivity to penicillinase. Phenoxybenzylpenicillin is inactivated by penicillinase, but at a slower rate than penicillin V. A comparison of relative sensitivities to *B. cereus* penicillinase gave the following ratios: penicillin V 1.0, phenethicillin 0.66, and phenoxybenzylpenicillin 0.67.

In vitro Activity against Other Species of Bacteria.—Against the wider range of pathogenic bacteria the activity of phenoxybenzylpenicillin was, in general, similar to that of penicillin V and phenethicillin (Table III). Thus against *Str. faecalis* and *Str. agalactiae* the M.I.C.s were 5.0 and 0.075 μg./ml. respectively; for penicillin V the figures were 2.5 and 0.05, and for phenethicillin 5.0 and 0.1. Against *C. diphtheriae* phenoxybenzylpenicillin was less active than either penicillin V or phenethicillin, the respective M.I.C.s being 0.25, 0.02, and 0.075 μg./ml. The activity of all three penicillins was identical against one strain of *N. gonorrhoeae* with M.I.C.s of 0.05 μg./ml. Against a second strain (GU12) similar M.I.C.s were obtained for phenoxybenzylpenicillin and phenethicillin. However, this strain was appreciably more sensitive to penicillin V. Against the Gram-negative pathogens no exceptional activity was observed. Inhibition of growth, if observed, occurred with all three penicillins only at relatively high concentrations.

TABLE III.—*Sensitivity of Other Bacteria to Phenoxybenzylpenicillin*

Species	Strain	M.I.C. (μg./ml.)		
		Penicillin V	Phenoxybenzylpenicillin	Phenethicillin
Str. faecalis ..	8213	2·5	5·0	5·0
Str. agalactiae	7	0·05	0·075	0·1
C. diphtheriae	3984	0·02	0·25	0·075
N. gonorrhoeae	R17	0·05	0·05	0·05
" "	GU12	0·01	0·1	0·05
N. meningitidis	8339	0·025	0·05	0·025
H. influenzae	AHI	3·0	2·0	2·0
Pr. vulgaris ..	4175	320	640	>640
Ps. pyocyanea	—	>10,000	1,280	10,000
E. coli ..	—	>200	>640	>500
Salm. typhi ..	5762	66	200	666
Salm. paratyphi A	8386	>200	>200	1,000
Salm. paratyphi B ..	8390	200	1,000	>1,000
Salm. typhimurium ..	5710	200	>200	1,000
Sh. dysenteriae	8218	>80	80	400
Sh. flexneri ..	8525	100	40	400
Sh. sonnei ..	8220	80	160	>400
Salm. enteritidis	5694	200	>200	ca. 1,000

Bactericidal Activity of Phenoxybenzylpenicillin.—The bactericidal activity of phenoxybenzylpenicillin was tested against sensitive strains of *Staph. aureus*. The penicillin was found to sterilize the culture at a concentration approximately three times that at which it inhibited growth (M.I.C.). These results are similar to those obtained with penicillin V.

Absorption and Excretion

Methods

The subjects were healthy male and female volunteers from 18 to 50 years old who had been screened for absence of penicillin sensitivity.

The penicillins under investigation were assigned at random to the subjects, so that on each day an equal number in each group received the respective treatments. The groups were crossed over so that each subject received each of the preparations in turn, thus allowing for individual variation.

All subjects were instructed to fast overnight and no food was eaten until two hours after dosing. The penicillins were administered as capsules or tablets in the form of the potassium salts.

Blood samples (0.2 ml.) were taken by finger-prick at half, one, two, four, six, and in some cases at eight hours after dosing and immediately diluted with an equal volume of anticoagulant (disodium hydrogen citrate, 16.7 g.; glucose, 25 g.; distilled water to 1 litre).

Urine was collected over three periods according to the experiment, 0 to 3 hours, 3 to 6 hours, 6 to 12 hours. Dilutions were made in *p*H 7 buffer to give two final solutions containing approximately 1.0 and 0.25 μg./ml. The blood and urine samples were assayed by the *Sarcina lutea* disk-plate method using the different penicillins as their respective standards. For the blood estimations the standards were prepared in citrated human blood.

Results of First Trial

The first experiment was carried out on three groups of six subjects in a triple crossover design. The blood and urine levels in μg./ml. were estimated after administration of 150-mg. doses of phenoxybenzyl-

TABLE IV.—*Individual Blood Concentrations after Oral Administration of Phenoxybenzylpenicillin, Penicillin V, and Phenethicillin in 18 Subjects. Dose 150 mg.*

Subject	Blood Levels (μg./ml.) Hours After Dose														
	Phenoxybenzylpenicillin 150 mg.					Penicillin V 150 mg.					Phenethicillin 150 mg.				
	½	1	2	4	6	½	1	2	4	6	½	1	2	4	6
A 1	0·58	3·9	1·26	0·39	N	0·49	0·65	0·14	0·05	N	0·45	0·84	0·39	0·28	N
2	2·0	2·4	0·92	0·35	0·56	1·17	1·11	0·19	N	N	1·24	1·79	0·54	N	N
3	2·25	4·3	1·23	0·40	N	1·50	0·99	0·30	N	N	2·82	1·50	0·39	N	N
4	11·20	8·32	2·38	0·62	N	1·45	1·77	0·23	0·04	N	2·72	2·40	0·94	0·17	N
5	11·20	5·96	1·70	0·32	N	0·89	0·97	0·25	N	N	2·41	1·32	0·26	N	N
6	11·20	5·80	2·14	0·34	0·32	3·09	1·77	0·26	N	N	3·30	1·42	0·39	N	N
B 1	12·17	4·52	1·14	0·12	N	1·04	0·54	0·20	N	N	2·60	1·12	0·31	N	N
2	N	3·28	1·96	0·42	0·46	0·83	0·94	0·24	0·08	N	2·45	2·30	0·39	N	N
3	9·7	6·80	2·94	0·49	N	1·98	1·42	0·39	N	N	6·70	2·30	0·60	0·10	N
4	*	*	*	*	*	1·11	0·66	0·16	N	N	0·41	1·02	0·55	N	N
5	6·72	9·92	3·75	0·57	0·10	0·89	1·12	0·25	N	N	0·19	1·12	0·26	0·16	N
6	0·51	2·90	4·53	0·93	0·53	3·05	2·62	0·44	N	N	0·32	0·33	0·89	0·48	N
C 1	0·82	3·30	2·95	0·71	0·45	0·68	1·00	0·30	N	N	0·71	0·77	0·76	0·19	N
2	7·50	6·87	2·55	0·48	N	1·72	0·75	0·21	N	N	1·27	2·11	0·70	N	N
3	1·98	5·86	2·90	0·31	N	1·84	0·94	0·32	N	N	1·68	1·94	0·87	N	N
4	2·35	3·25	2·99	0·97	0·36	4·76	1·07	0·22	N	N	3·80	1·86	0·51	0·06	N
5	10·30	4·64	1·64	0·16	N	0·94	0·21	N	N	N	2·18	3·27	0·37	0·06	N
6	0·32	2·35	1·34	0·46	0·91	1·27	0·90	0·25	N	N	0·82	1·48	0·76	0·06	N
Mean	5·34	4·96	2·25	0·47	—	1·59	1·08	0·24	—	—	2·00	1·61	0·55	—	—

* This subject was unable to take part. N=Blood concentration below minimal detectable level; 0·05 μg./ml. for penicillin V and phenethicillin and 0·2 μg./ml. for phenoxybenzylpenicillin.

penicillin, penicillin V, and phenethicillin in capsules. The individual blood concentrations in μg./ml. for the respective penicillins are shown in Table IV and their means presented graphically in Fig. 1.

With all three penicillins the mean blood levels reached a peak within 30 minutes. With phenoxybenzylpenicillin the maximum mean blood concentration was

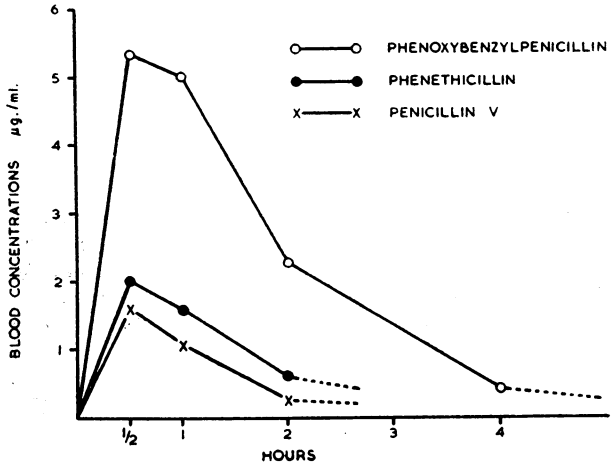

FIG. 1.—Mean blood concentrations after 150 mg. of phenoxybenzyl penicillin, penicillin V, and phenethicillin.

three times as high as that of penicillin V and two and a half times as high as that of phenethicillin.

In addition to the high peak, phenoxybenzylpenicillin gave more prolonged blood levels. After two hours the blood level of phenoxybenzylpenicillin was nearly ten times as high as that of penicillin V and four times as high as that of phenethicillin, and still exceeded the peaks for penicillin V and phenethicillin. At four hours most of the penicillin V and half of the phenethicillin blood levels were below the limit determinable by the assay method (Table IV), whereas substantial concentrations of phenoxybenzylpenicillin were shown to be present in all cases. After six hours there were no detectable penicillin V and phenethicillin blood levels in any of the subjects, whereas with phenoxybenzylpenicillin 40% still showed levels in excess of 0.3 μg./ml.

The urinary excretion of phenoxybenzylpenicillin followed a similar pattern to that of penicillin V and that of phenethicillin but was rather slower (Table V). As the total urinary excretion of phenoxybenzylpenicillin was lower than that of the other penicillins the urine was extracted and examined by paper chromatography and

TABLE V.—*Urinary Excretion of Phenoxybenzylpenicillin, Penicillin V, and Phenethicillin after Oral Administration*

Penicillin	Dose mg.	Weight Excreted (mg.) Hours After Dose				Percentage of Dose Excreted
		0–3	3–6	6–12	Total	
Phenoxybenzylpenicillin ..	150	28·24	6·07	1·37	35·68	23·8
Penicillin V ..	150	36·18	2·55	0·21	38·94	25·96
Phenethicillin ..	150	43·37	6·18	0·43	49·98	33·3

bioautography. It was found that, although the phenoxybenzylpenicillin administered was a single penicillin, the urine contained three active components. The major component was phenoxybenzylpenicillin, but two other components accounted for up to 20% of the activity. In the case of penicillin V and phenethicillin there were only traces of other active components in the urine.

Results of Second Trial

In the second trial the dose of phenoxybenzylpenicillin was increased to 250 mg. and comparison was made with the same weight of penicillin V. Two groups of normal subjects were used in a single crossover test.

Again phenoxybenzylpenicillin gave higher and more sustained blood levels than penicillin V (Table VI, Fig. 2). At one hour the mean blood concentration for phenoxybenzylpenicillin was nearly three times as high as that of penicillin V and at two hours nearly seven times as high. The blood levels had fallen below the

TABLE VI.—*Individual Blood Concentrations after Oral Administration of* 250 *mg. Penicillin V and Phenoxybenzylpenicillin in* 16 *Subjects*

Subject	Blood Levels (μg./ml.) Hours After Dose							
	Phenoxybenzylpenicillin 250 mg.				Penicillin V 250 mg.			
	1	2	4	8	1	2	4	8
1	9·60	4·46	2·66	0·84	3·23	0·76	0·08	0·74
2	8·86	4·70	0·78	0·38	1·74	0·54	N	N
3	1·25	6·20	4·60	0·21	3·66	0·84	0·44	N
4	5·09	8·64	0·91	N	1·20	0·80	0·37	N
5	4·70	3·40	0·35	N	1·19	0·38	0·06	N
6	4·20	5·29	0·32	N	2·22	0·42	0·15	N
7	4·86	2·70	0·76	0·52	2·22	0·44	0·10	N
8	7·70	3·10	1·26	0·32	3·25	*	2·80	0·36
9	21·60	5·06	2·16	0·86	2·49	1·36	0·32	0·66
10	4·2	6·99	1·51	0·38	0·94	0·80	N	N
11	6·09	4·19	2·56	N	8·90	1·20	*	N
12	19·20	6·16	*	N	1·98	0·26	1·06	0·14
13	5·02	2·08	1·08	0·34	1·52	0·50	N	N
14	2·76	1·60	1·26	N	2·62	0·58	0·18	N
15	3·50	7·05	1·28	0·28	2·32	0·87	0·25	N
16	10·50	5·90	2·26	N	1·92	1·10	0·08	N
Mean	7·45	4·85	1·58	—	2·59	0·72	0·39	—

N = Blood concentration below minimal detectable level: 0·05 μg./ml. for penicillin V and 0·2 μg./ml. for phenoxybenzylpenicillin.
* Assay invalidated.

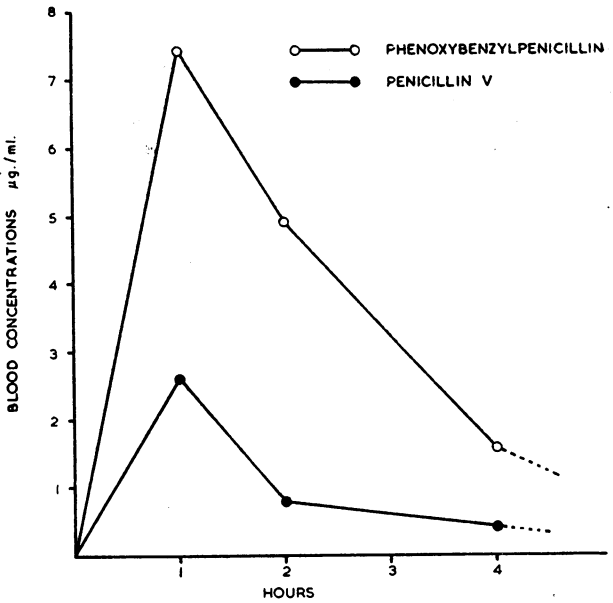

FIG. 2.—Mean blood concentrations after 250 mg. of phenoxybenzylpenicillin and penicillin V.

minimal detectable level at four hours in 3 out of the 16 subjects who had received penicillin V, whereas the mean blood concentration for those who had received phenoxybenzylpenicillin remained high. Only four of the subjects with penicillin V showed determinable levels at eight hours, whereas nine showed substantial blood concentrations of phenoxybenzylpenicillin.

Results of Third Trial

In this trial comparison was made of the blood levels obtained over four hours from a 125-mg. dose of phenoxybenzylpenicillin with those from 125 and 250 mg. of penicillin V administered in tablet form.

Three groups of normal subjects were used in a triple crossover design and blood estimations made at half, one, two, and four hours after administration. The peak blood level reached with 125 mg. of phenoxybenzylpenicillin was nearly five times as high as that reached with penicillin V at this dose level and twice as high as that of the 250 mg. dose of penicillin V (Table VII, Fig. 3). The blood concentrations with phenoxybenzylpenicillin were again better sustained than those with penicillin V though not so prolonged as in the previous trials.

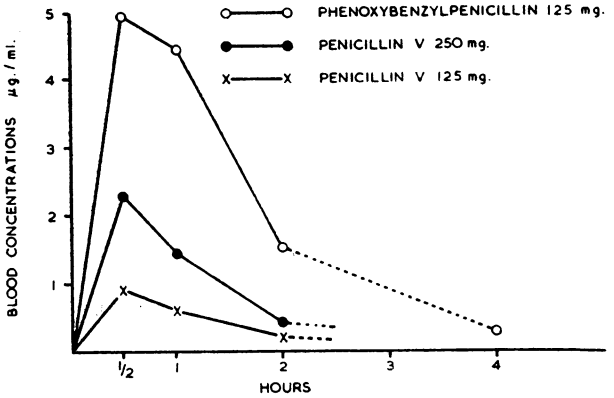

FIG. 3.—Mean blood concentrations after 125 mg. of phenoxybenzylpenicillin and 125 and 250 mg. of penicillin V.

Pharmacological Properties

The pharmacological properties of phenoxybenzylpenicillin have been studied in mice, rats, and cats. The acute LD50s of phenoxybenzylpenicillin in mice were 225 mg./kg. intravenously, 520 mg./kg. intraperitoneally, and 3,000 mg./kg. orally. Like penicillin V and phenethicillin, phenoxybenzylpenicillin has a low toxicity.

Chronic toxicity tests carried out in rats showed that daily doses as high as 0 5 g./kg. by mouth produced only a slight depression of growth and no toxic effects on the major organs over four weeks.

The effects of phenoxybenzylpenicillin on the blood-pressure and heart were studied in cats anaesthetized with chloralose. Single intravenous doses up to 50 mg./kg. were well tolerated with little change in the blood-pressure and respiration. Doses in excess of this (100 mg./kg.) caused a short-lasting fall in blood-pressure with a slight quickening in heart rate. No significant electrocardiographic changes were observed at these dose levels. Responses to the autonomic agents acetylcholine and adrenaline were unaffected, but there was a slight inhibition of the depressor response to histamine, becoming evident at 10 mg. of phenoxybenzylpenicillin per kg.

On the isolated heart of the guinea-pig (Langendorff preparation) single doses of 1 mg. injected into the perfusion cannula caused no gross changes in the heart rhythm and flow. Responses to adrenaline and acetylcholine remained unaffected. Similar results were obtained when 50 µg. of phenoxybenzylpenicillin per ml. was dissolved in the perfusion fluid.

Phenoxybenzylpenicillin did not inhibit urine flow in rats and cats and caused no depression of kidney efficiency.

Discussion

Phenoxybenzylpenicillin is a new penicillin chemically related to both penicillin G and penicillin V. After oral administration it is well absorbed, giving high and sustained blood levels superior to those of other related penicillins. Its bacterial spectrum *in vitro* is similar to that of penicillin V and phenethicillin.

Consideration of these properties indicates that phenoxybenzylpenicillin will show a therapeutic advantage over other penicillins tested (Garrod, 1960a, 1960b). Some authorities, however (McCarthy *et al.*, 1961), have indicated that this does not necessarily follow. Thus the final assessment of the penicillin can be made only after an extended period of use and observation in clinical practice, especially as laboratory methods cannot determine with certainty the influence of such factors in the individual as distribution in the body and inactivation due to serum binding or to breakdown.

TABLE VII.—*Individual Blood Concentrations after Oral Administration of 125 mg. Phenoxybenzylpenicillin and 125 mg. and 250 mg. Penicillin V*

Subject	Blood Levels (µg./ml.) Hours After Dose											
	Phenoxybenzylpenicillin 125 mg.				Penicillin V 250 mg.				Penicillin V 125 mg.			
	½	1	2	4	½	1	2	4	½	1	2	4
A 1	3·50	4·30	0·96	N	0·48	1·03	0·63	0·09	0·44	0·60	0·16	N
2	3·14	3·08	0·83	N	0·40	0·28	0·38	0·10	0·71	0·72	0·10	N
3	3·70	2·97	1·01	N	1·98	1·66	0·31	N	0·70	0·53	0·11	N
4	8·65	4·62	1·09	N	3·15	0·87	0·22	N	3·18	1·14	1·11	N
5	4·87	3·25	1·22	N	4·00	0·99	0·19	0·05	1·50	0·56	0·11	N
6	1·13	1·87	2·08	N	3·55	1·90	0·32	N	0·95	0·52	0·12	N
B 1	4·96	5·97	1·34	N	2·84	1·58	0·21	N	1·64	0·37	0·10	N
2	5·44	3·36	1·25	N	1·92	0·96	0·27	N	0·31	0·42	0·16	N
3	1·17	2·24	0·50	N	2·16	1·74	0·29	N	0·42	0·74	0·16	N
4	4·42	2·46	0·80	N	2·54	0·99	0·20	N	0·84	0·37	0·12	N
5	5·90	4·62	1·84	0·36	1·38	2·72	0·54	0·05	0·45	0·79	0·22	N
6	0·89	3·00	4·76	0·99	1·48	0·76	0·58	N	0·34	0·30	0·23	0·07
C 1	2·25	2·90	1·08	0·50	0·89	0·52	0·14	N	1·0	0·34	0·03	N
2	7·76	3·53	1·20	N	2·34	1·76	0·16	N	0·31	0·53	0·05	N
3	6·90	14·80	2·65	3·30	1·70	1·70	0·35	N	1·04	0·47	0·10	N
4	11·30	8·40	1·70	0·59	4·60	2·90	0·53	N	1·08	1·08	0·35	N
5	11·30	4·35	1·15	N	2·84	0·71	0·21	N	1·05	0·31	0·08	N
6	1·21	1·88	1·22	0·38	2·20	2·49	0·54	0·07	0·56	0·43	0·06	N
Mean	4·92	4·31	1·48	—	2·25	1·42	0·34	—	0·92	0·57	0·19	—

N = Blood concentration below minimal detectable level: 0·05 µg./ml. for penicillin V and 0·2 µg./ml. for phenoxybenzylpenicillin.

Initial clinical trials have been carried out which demonstrate the effectiveness of this penicillin against a variety of penicillin-sensitive organisms at a dosage lower than that normally recommended for other oral preparations. Results of these trials are reported by Carter and Brumfitt (1962).

Summary

Phenoxybenzylpenicillin is a new acid-stable, orally active penicillin chemically related to penicillins G and V and to phenethicillin.

In vitro its antibacterial spectrum and minimum inhibitory concentration against sensitive organisms has been shown to be similar to those of penicillin V and phenethicillin.

Studies in human volunteers at two dosage levels show that phenoxybenzylpenicillin gives higher and more prolonged therapeutic blood levels than penicillin V and phenethicillin. At the dose of 125 mg. phenoxybenzylpenicillin gave a peak blood level greater than that from 250 mg. of penicillin V.

Pharmacological tests have demonstrated a low oral toxicity. No allergic reactions were encountered, but phenoxybenzylpenicillin is likely to be similar to other penicillins in this respect.

The results suggest that phenoxybenzylpenicillin should have therapeutic advantages over existing oral penicillins and should be effective in lower dose levels at longer time intervals.

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BACTERIOLOGICAL AND CLINICAL STUDIES WITH PHENOXYBENZYLPENICILLIN

BY

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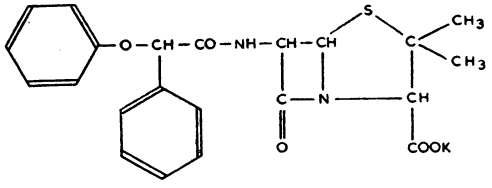
Since the discovery of 6-aminopenicillanic acid a number of new penicillins have been made available for laboratory and clinical assessment. Phenoxybenzylpenicillin (" penspek "; D.C.(B.)L. 306) is the potassium salt of 6(α-phenoxyphenylacetamido) penicillanic acid with the structural formula:



This paper describes absorption and excretion tests on human volunteers after oral administration of the compound. In addition microbiological assessment of phenoxybenzylpenicillin has been made, including a comparison with penicillins V and G. The results of these investigations justified a clinical trial in which the therapeutic effect of phenoxybenzylpenicillin was assessed in patients suffering from infections due to a variety of penicillin-sensitive organisms.

Absorption and Excretion

Assay Procedure.—Penicillin serum levels were determined by the cup-plate biological assay method using *Sarcina lutea* (ATCC 9341) as the test organism. Standard penicillin solutions of the particular penicillin being investigated were included on each assay plate and the standard and test solutions were arranged so as to compensate for errors due to such factors as variation in thickness of the agar. For assay of serum the diluent for unknown samples and for the standard penicillin solutions was a 3% solution of bovine plasma albumin Fraction V (Armour Pharmaceutical Co.) in M/20 phosphate buffer *p*H 7. For assay of urine the diluent for both unknown samples and standard penicillin solutions was M/20 phosphate buffer *p*H 7.

Serum Levels.—Five volunteers received 125 mg. of phenoxybenzylpenicillin by mouth two hours after a light breakfast, and venous blood was removed at timed intervals. One week later the same subjects received 125 mg. of penicillin V two hours after a light breakfast

TABLE I.—*Individual Serum Concentrations in Volunteers after a Single 125-mg. Dose of Phenoxybenzylpenicillin and Penicillin V*

Subject	Serum Concentrations (μg/ml.). Hours After Dose									
	Phenoxybenzylpenicillin					Penicillin V				
	½	1	2	4	6	½	1	2	4	6
A	6·0	3·0	0·6	0	0	1·4	1·5	0·3	0·04	0
B	0	1·6	1·7	0·09	0	0	1·8	0·6	0·15	0
C	1·0	2·0	1·4	0·1	0	0·1	0·5	0·6	0·04	0
D	0·2	2·8	1·0	0·09	0	0·1	0·4	0·7	0·05	0
E	1·1	2·2	1·2	0·1	0	0·05	0·8	0·5	0·04	0
Average	1·6	2·3	1·2	0·08	0	0·33	1·0	0·54	0·06	0

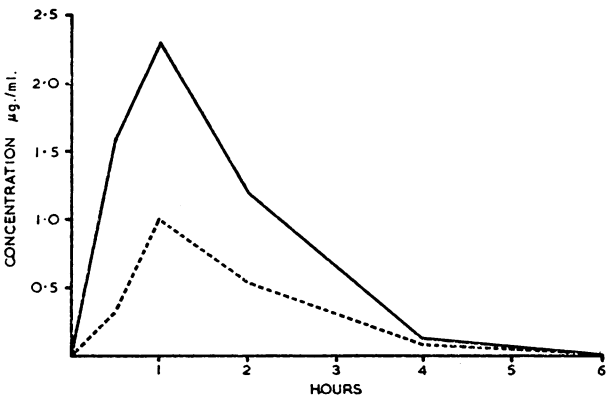


FIG. 1.—Comparison of serum levels of phenoxybenzylpenicillin and penicillin V in five healthy subjects after an oral dose of 125 mg. ----- penicillin V. ——— phenoxybenzylpenicillin.