total activity in individuals with the normal homozygous state.

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Identification of *p*-Hydroxyphenoxymethyl Penicillin as a Metabolite of Phenoxymethyl Penicillin

WHEN urine of normal adults to whom phenoxymethyl penicillin (penicillin V (200 mg)) had been administered was chromatographed[1], a second inhibition zone in addition to the penicillin V zone was observed (Fig. 1). The R_F value of this product was identical with that of *p*-hydroxyphenoxymethyl penicillin, obtained from a sample of crude penicillin V[2]. This metabolite represents about 10 per cent of the activity found in the urine.

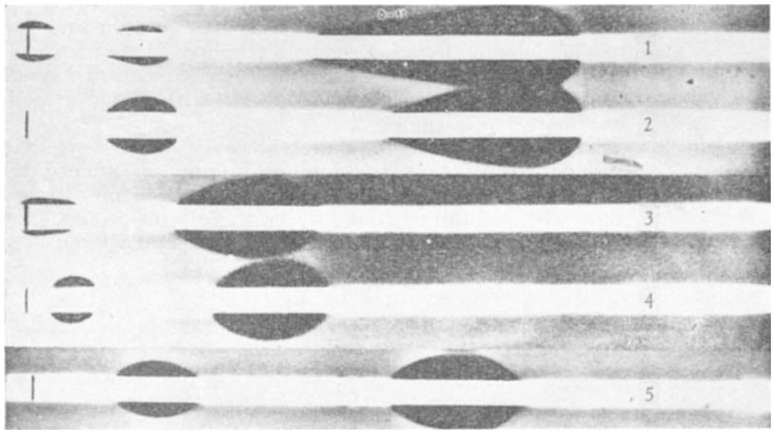

Fig. 1. Bioautography of paper chromatograms of urine after oral administration of penicillin V and phenethicillin. Whatman No. 1 paper, buffered with 10 per cent sodium citrate at *p*H 5·5 (Nos. 1 and 2) or *p*H 6·0 (Nos. 3, 4, 5). Developing solvent: ether saturated with 28 per cent ammonium sulphate. R_F values of penicillin V: 0·60 ± 0·06 at *p*H 5·5 (Nos. 1 and 2), 0·32 ± 0·04 at *p*H 6·0 (Nos. 3 and 4); *p*-hydroxypenicillin V: 0·13 ± 0·02 at *p*H 5·5 (Nos. 1 and 2), 0·05 ± 0·01 at *p*H 6·0 (Nos. 3 and 4); phenethicillin: 0·49 ± 0·05 and metabolite: 0·17 ± 0·02 at *p*H 6·0 (No. 5)

To confirm the structure of this substance, pooled urine samples were extracted with butyl acetate, after acidification to *p*H 2·0 with phosphoric acid. The penicillin was extracted from the organic solvent into bicarbonate solution and re-extracted into ether after acidification. The ether solution was applied on a column of silica gel, equilibrated with 2 M phosphate buffer of *p*H 6·2. The column was developed with ether containing increasing amounts of methanol (0·5–3 per cent), as described by Behrens *et al.*[3]. The column was cut into 1-in. sections, which were eluted with phosphate buffer of *p*H 7·0. The top section contained the metabolite and some penicillin V. The product eluted from the top of the column was hydrolysed by refluxing in 3 N hydrochloric acid for 24 h, and the side-chain acid was extracted with ether. By circular chromatography using system *E* of Högström[4], phenoxyacetic and *p*-hydroxyphenoxyacetic acid could be identified. There was also an indication of the presence of the *ortho*-acid on the paper chromatogram (Table 1).

Table 1. PAPER CHROMATOGRAPHY OF PHENOXYACETIC ACIDS

Acid*	R_F†	Colour‡
o-$HOC_6H_4OCH_2COOH$	0·41	Violet
m-$HOC_6H_4OCH_2COOH$	0·23	Red-violet
p-$HOC_6H_4OCH_2COOH$	0·15	Blue
$C_6H_5OCH_2COOH$	0·37	—

* The hydroxyphenoxyacetic acids were prepared by reaction of the appropriate dihydric phenol with chloroacetic acid[5,6].
† Butanone-2 : water : diethylamine, 921 : 77 : 2 (ref. 4). The acids were detected with bromophenol blue indicator or by diazo reagent.
‡ Colour obtained by reaction with diazotized *p*-nitroaniline[7].

Further confirmation of the structure of the side-chain acid was obtained by gas chromatography of the methyl esters. The acid was treated with methyliodide (7 m.mole) and 0·5 M sodium methylate (2 m.mole) for 5 h at 65° in a sealed tube. In this way phenoxyacetic acid was transformed into the methyl ester, and the hydroxyphenoxyacetic acid into the methyl ester of the methoxy acid. These esters could be separated by gas chromatography on a column of 'Reoplex 400' (20 per cent) at 180° (ref. 8). By this method the presence of *p*-hydroxyphenoxyacetic acid was confirmed, and a small peak of *o*-hydroxyphenoxyacetic acid was detected.

The predominant active metabolite of penicillin V in human beings is *p*-hydroxy isomer. In the urine of some persons who had received penicillin V, we found a second metabolite (probably the dihydroxy derivative), which remained at the start on the paper chromatogram (Fig. 1, lanes 1 and 3).

A more polar biologically active metabolite was also observed when the urine of persons who had received α-phenoxyethylpenicillin (phenethicillin) was examined (Fig. 1). Although the structure of this metabolite was not established, it seems very likely that hydroxylation of the benzene ring had also occurred.

The presence of active metabolites in the urine after administration of the following penicillins has been described: benzylpenicillin (penicillin *G*)[9,10], penicillin V, phenethicillin and propicillin[10], phenoxybenzyl penicillin (phenbenicillin)[10,11], 3,4-dichloro-α-methoxybenzyl penicillin[10,12], oxacillin and cloxacillin[10,13].

The hydroxylation of aromatic compounds in the organism is well known, and metabolites of this type have been described for such drugs as phenobarbital, diphenylhydantoin, and phenylbutazone[14]. These transformation products are often conjugated with glucuronic acid, and possibly penicillin V is transformed into a substance of this type that is inactive. Another inactive degradation product could be penicilloic acid, as was demonstrated with benzylpenicillin[15].

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