The demethylation of methamphetamine by intestinal microflora

Methamphetamine (2-methylamino-1-phenylpropane) is a sympathomimetic drug which is liable to serious abuse (Kramer, Fischman & Littlefield, 1967). Its metabolism in the rat, guinea-pig and man has been described (Caldwell, Dring & Williams, 1972 a, b; Dring & Caldwell, 1973). These authors found that the route of elimination of [^{14}C] methamphetamine by guinea-pigs varied with the dose administered. With a dose of 10 mg kg^{-1}, 84% of ^{14}C appeared in the urine with 3% in the faeces, but at a dose of 45 mg kg^{-1}, 69% was in the urine and 18% in the faeces. The material in the faeces was composed of 3 metabolites, amphetamine (7%), norephedrine (4%) and an unknown (6%), in addition to the unchanged drug (1%).

The intestinal microflora have the capacity to metabolize a wide variety of drugs and foreign compounds in many ways (Scheline, 1968; Williams, 1972) and the detection of metabolites of this drug in the faeces raised the possibility of such metabolism in this case. Accordingly, the ability of the gut contents and individual strains of bacteria to biotransform this drug was examined to assess the significance of this site of metabolism.

[^{14}C]Methamphetamine hydrochloride ((±)-2-methylamino-1-phenyl-[1-^{14}C]-propane hydrochloride), m.p. 134–135°, specific activity 2·36 μCi mg^{-1}, was a sample prepared by Caldwell & others (1972a).

Methamphetamine hydrochloride, m.p. 132–134°, was purchased from K & K Labs., Plainview, N.Y., U.S.A. Amphetamine sulphate, m.p. 302° (decomp)., and 4′-hydroxyamphetamine hydrobromide, m.p. 190–192°, were the gifts of Smith, Kline and French Laboratories, Philadelphia, U.S.A. 4′-Hydroxymethamphetamine, m.p. 161–162°, was a sample prepared by Caldwell & others (1972a).

Table 1 gives R_F values of the compounds used and details of the chromatographic systems.

Scanning of paper and thin-layer chromatograms was performed with a Packard radiochromatogram scanner (model 7200), identification of ^{14}C peaks being made by comparison with R_F values of authentic compounds (see Table 1). ^{14}C content of peaks was estimated by cutting up chromatograms and using liquid scintillation counting as described by Caldwell & others (1972a).

Caecal and rectal contents (approx. 1g) of a freshly killed female Dunkin-Hartley guinea-pig were collected into sterile containers and homogenized in 9 volumes of

Table 1. *R_F values and colour reactions of methamphetamine and related compounds.* The solvents used were: A, methanol–chloroform (1:1, by vol) (Noirfailise, 1966); B, methanol–acetone (1:1, by vol) (Moerman, 1964). These were used for thin-layer chromatography with plates (20 × 20 cm) spread with 0·25 mm thick Alumina-G (Merck Ltd.) and silica gel G respectively which were heated at 120° for 1 h before use. The solvents were allowed to run from the origin to 12 cm.

Compound	System A	System B	Colour with Diazotized nitroaniline*	Colour with Dimethylamino-cinnamaldehyde**
Methamphetamine	0·81	0·24	none	pale pink
Amphetamine	0·45	0·59	pale pink	red
4′-Hydroxyamphetamine	0·39	0·51	purple	pale pink
4′-Hydroxymethamphetamine	0·64	0·18	purple	red

* Wickstrom & Salvesen (1952) ** Bridges (1963).

Todd-Hewitt broth. [^{14}C]Methamphetamine hydrochloride was added (1 mg; 166 000 dmin^{-1}) and the culture incubated for 72 h at 37° in an atmosphere of 90% H_2 + 10% CO_2. After incubation, the homogenate was centrifuged and the supernatant used for chromatography.

Radiochromatograms were prepared and in System A, three more ^{14}C peaks were found with R_F 0·79 (methamphetamine), 0·47 (amphetamine) and 0·00 (an unknown metabolite). Similarly, 3 peaks were seen in System B, with R_F 0·60 (amphetamine), 0·26 (methamphetamine) and 0·00 (unknown).

Quantitation of these peaks as described showed that with caecal contents, 29% of added ^{14}C was recovered as methamphetamine, 47% as amphetamine and 24% as unknown, while for rectal contents, the figures were 76, 13 and 11 respectively.

The unknown was not investigated further and may be an intermediate in *N*-demethylation or an alternative metabolic product. Paper chromatography on Whatman No. 1 paper in the butanol-ammonia buffer system of Fewster & Hall (1951) showed that the unknown was not benzoic acid.

Representative strains of the main bacterial genera found in mammalian gut were isolated from human faeces by the methods of Drasar (1967) and cultured in a peptone–yeast extract–basal medium (1% Evans peptone, 1% yeast extract, 0·4% mineral salts solution and 0·1% cysteine HCl) containing 0·2% of either methamphetamine HCl or 4′-hydroxymethamphetamine, and incubated for 3 days at 37°. The atmospheres used are shown in Table 2.

After incubation, the cultures (0·1 ml) were chromatographed using System A and B (Table 1) and the amines visualized with the sprays described. The sprays will detect a minimum of 3–5 μg of amine, so that the minimum conversion detected was about 2%. No substances interfering with the detection method were found in incubations without the substrate, and no activity observed in control incubations.

Table 2 shows the *N*-demethylation of methamphetamine by representative strains of the main bacterial genera found in mammalian intestine. Activity was noted as positive when two spots corresponding to the primary and secondary amine were seen with the visualizing agents used (see Table 1). Most activity was seen in strains of the Enterococci, incubated aerobically and with the Lactobacilli, incubated anaerobically. The Clostridia exhibited some *N*-demethylation activity, while the other genera showed little, if any, activity. 4′-Hydroxymethamphetamine was tested against a selection of the strains used and gave results identical to those for methamphetamine. It is, therefore, assumed that the activity for both substrates is very similar.

The data presented in this report show that the intestinal contents of guinea-pigs possess the ability to biotransform methamphetamine by *N*-demethylation and probably one other pathway. The differences seen in the extent of metabolism between the caecal and rectal contents are probably a reflection of the differences in the amount

Table 2. *N-Demethylation of methamphetamine by representative strains of intestinal microflora.*

Genus	Incubation atmosphere	No. of strains tested	% of strains giving a positive reaction
Enterobacteria	Air	15	7
Enterococci	Air	15	67
Lactobacilli	80%N_2+20%CO_2	8	75
Clostridia	90%H_2+10%CO_2	23	35
Bacteriodes	90%H_2+10%CO_2	14	7
Bifidobacteria	90%H_2+10%CO_2	6	0

of material from these two regions used in the incubations, rather than a fundamental difference in the activity of their flora.

Work using isolated strains of bacteria from mammalian gut has shown that the bulk of the *N*-demethylation activity is due to the Lactobacilli and the Enterococci, with a contribution from the Clostridia. The strains of Clostridia carrying out this *N*-demethylation are also able to *N*-demethylate choline and other amines (Hawksworth, 1973). The bacterial strains used were isolated from human intestinal contents and although there are differences between the intestinal flora of man and the guinea-pig, these are quantitative rather than qualitative (Hawksworth, Drasar & Hill, 1971). The experiments described herein do not permit quantitation of the extent of methamphetamine metabolism due to the gut flora.

The metabolism of methamphetamine, like most drugs, has been assumed to be carried out by the liver (Axelrod, 1955) but it is apparent that, like the psychotropic drug imipramine (Minder, Schnetzer & Bickel, 1971) the liver and the gut flora may both contribute to the *N*-demethylation of methamphetamine in animals. It is, however, doubtful whether methamphetamine metabolism by the gut flora is of importance in the human situation, as in man the flora is restricted to the lower gut which would not be reached by this and other readily absorbed compounds (Williams, 1972).

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