Hippuric acid excretion after benzylamine ingestion in man

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ABSTRACT The fate of ^{14}C -benzylamine after oral administration as the hydrochloride has been investigated in two male volunteers. Over 98% of the administered radiolabel was excreted in the urine as ^{14}C -hippuric acid within 24 hours. The rate of urinary hippuric acid excretion was extremely rapid, with more than 90% of the dose excreted after three hours.

Benzylamine has a widespread use as a chemical intermediate for dyestuffs, pharmaceuticals, cosmetics and plastic polymers (Rose and Rose, 1966). It has also been employed in the manufacture of corrosion inhibitors (Yasnitskii *et al.*, 1976; Makwana *et al.*, 1975) and in photography (Starukhina *et al.*, 1976). In spite of this, however, there appears to be a lack of knowledge of the biological fate in man of this ubiquitous chemical, although it has been over a century since interest was first shown in benzylamine metabolism. In 1877 Schmiedeberg fed benzylamine to dogs, and isolated hippuric acid from the urine, this finding being confirmed by Mosso (1890). Later, Imai (1924) showed that benzylamine was excreted by hens as benzoic acid and ornithuric acid, and by dogs as benzoic acid and hippuric acid.

In an attempt to fill this fundamental gap in our knowledge of benzylamine, the recently available ^{14}C -benzylamine was used as the hydrochloride to follow the metabolism of this compound in two male volunteers.

Materials and methods

^{14}C -BENZYLAMINE

Radiolabelled benzylamine was purchased from the Radiochemical Centre, Amersham, England, in the form of (methylene- ^{14}C) benzylamine hydrochloride (specific activity 56 mCi/mmol). The total radioactivity was diluted with distilled water to give a final solution containing 1.00 $\mu\text{Ci/ml}$.

Unlabelled benzylamine hydrochloride was pre-

pared by the dropwise addition of concentrated hydrochloric acid to a stirred solution of benzylamine in ether.

LIQUID SCINTILLATION COUNTING

A Packard Tricarb 3390 liquid scintillation counter was used for all radioactivity determinations. A dioxan-based scintillation cocktail was used, and efficiency of counting was determined by automatic external standardisation.

DOSE ADMINISTRATION AND SAMPLE COLLECTION

Each ^{14}C -benzylamine hydrochloride dose was prepared by dissolving unlabelled benzylamine hydrochloride (100 mg) in 20 ml (20 μCi) aqueous ^{14}C -benzylamine. After the two male volunteers had fasted overnight, they swallowed the benzylamine dose and the water that had been used to wash out the container several times. They ate a light breakfast one hour later, their urine was collected at timed intervals, the total volume measured and aliquots prepared for liquid scintillation counting.

SAMPLE PREPARATION

Samples of urine (100 μl) were added directly to the scintillation cocktail (10 ml) for radioactivity determinations. Aliquots of urine (20 μl) were run on TLC plates pre-coated with silica gel 60 F524 (Merck) and followed by development with benzene/acetic acid (90/30, v/v). The areas corresponding to benzylamine, hippuric acid and benzoic acid (as indicated by quenching of fluorescence at 254 nm by these concurrently run reference compounds) were scraped from the plate separately and the finely ground silica prepared for counting. The remainder of the silica was scraped from the plate in 1 cm sections and also prepared for counting as above.

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Results and discussion

The percentage of an oral dose of ^{14}C -benzylamine hydrochloride solution excreted in the urine at timed intervals is shown in the Table. The radioactivity in the urine was found, by TLC and subsequent liquid scintillation counting, to be associated with hippuric acid. No other radioactive metabolites were detected.

Table Urinary excretion of radioactivity following an oral dose* of ^{14}C -benzylamine hydrochloride

Subject	Time (hr)	Total urine volume (ml)	Radioactivity excreted per time interval (% dose)	Cumulative radioactivity excreted (% dose)
A	0.5	20	66.55	66.55
	1	16	17.62	84.17
	2	64	9.55	93.72
	3	45	2.42	96.14
	4	34	1.14	97.28
	5	25	0.49	97.77
	6	25	0.37	98.14
	7	25	0.23	98.37
	8	24	0.18	98.55
	10	50	0.20	98.75
	12	105	0.16	98.91
	24	630	0.29	99.20
B	0.25	50	3.02	3.02
	0.50	83	25.26	28.28
	1	92	37.84	66.12
	2	122	20.07	86.19
	3	130	4.56	90.75
	4	112	2.37	93.12
	5	90	1.29	94.42
	6	57	0.86	95.28
	7	39	0.57	95.85
	8	28	0.38	96.23
	9	36	0.33	96.56
	10	47	0.29	96.85
	12	120	0.35	97.20
	24	940	0.93	98.13

*20 μCi benzylamine hydrochloride (100 mg).

After 24 hours, over 98% of the administered radioactivity had been recovered for both subjects. The excretion of the radiolabel was extremely rapid, with over 90% of the dose excreted in the first three hours following the administration of the benzylamine.

It is apparent from our results that benzylamine is rapidly metabolised, probably via benzaldehyde, to benzoic acid, and finally excreted entirely as hippuric acid. This finding is in agreement with that of Bridges *et al.* (1970) who showed that, in man, benzoic acid was excreted in the urine exclusively as hippuric acid, 97% of the dose being eliminated within four hours of dosing. As there is so little difference in the excretion rate of hippuric acid derived from either

benzylamine or benzoic acid, the metabolism of benzylamine to benzoic acid must be an extremely rapid process. Thus, although benzylamine is known to be a mucosal irritant, its rapid conversion to the harmless benzoic acid is of some relevance in assessing its overall toxicity.

In view of the importance of urinary hippuric acid excretion as an index of industrial exposure of workers to toluene (Pagnotto and Lieberman, 1967; Ikeda and Ohtsuji, 1969) it may be feasible for benzylamine exposure to be monitored similarly. However, interpretation of urinary hippuric acid determinations would be difficult where workers were exposed to these two chemicals simultaneously.

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