**The Effect of Oral Inorganic Sulphate on the Metabolism of
4-Hydroxyphenethylamine (Tyramine) in Man: Tyramine *O*-Sulphate Measurement
in Human Urine**

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1. Urinary excretion of tyramine *O*-sulphate in six normal human subjects after an oral dose of 125 mg of tyramine hydrochloride alone, and with a supplement of Na_2SO_4 , was determined by using chromatographic and electrophoretic separations followed by spectrophotofluorimetry. 2. In every case the excretion of tyramine *O*-sulphate was increased significantly after sulphate supplementation.

Baumann (1876) first showed that the formation of sulphate esters takes place in dogs fed on a diet containing phenol, and advanced the view that exogenous sulphate could augment the excretion of phenol as phenyl sulphate. Laidlaw & Young (1948, 1953) confirmed this finding by administering $\text{Na}_2^{35}\text{SO}_4$ subcutaneously to male rats simultaneously with intraperitoneal or oral dosing of 2-naphthylamine, or 1- or 2-naphthol. They showed marked increases in the ester sulphate fraction of the urine in each case, namely 2-amino-1-naphthyl [^{35}S]sulphate and the corresponding naphthyl [^{35}S]sulphates. Dziewiatkowski (1949) also confirmed Baumann's (1876) original observation by dosing male rats orally with phenol and $\text{Na}_2^{35}\text{SO}_4$. Tacker *et al.* (1970) investigated tyramine metabolism in rats and found no sulphate conjugation. Nevertheless, because of known differences in metabolism in different species and the differences in compounds used, these studies can only give a broad indication of the possible metabolism in man.

In man, a metabolite of orally ingested tyramine in the urine is tyramine *O*-sulphate (Mullen & Smith, 1971). The aim of the present study was to determine whether orally ingested inorganic sulphate augments the production of tyramine *O*-sulphate in man. To do this, an accurate method for the measurement of urinary tyramine *O*-sulphate was developed.

Materials and methods

Materials. (a) Chemicals. Tyramine hydrochloride (puriss grade), anhydrous Na_2SO_4 (AnalaR grade), 1M- HNO_3 (AnalaR grade) and 1-nitroso-2-naphthol were obtained from British Drug Houses Ltd., Poole, Dorset, U.K. [$1\text{-}^{14}\text{C}$]Tyramine hydrochloride (250 $\mu\text{Ci}/\text{mg}$) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K., and was used for the synthesis of [$1\text{-}^{14}\text{C}$]tyramine *O*-sulphate, by the method of Mattock & Jones (1970). Both samples of

tyramine were pure by chromatography and electrophoresis. Other chemicals were of AnalaR grade.

(b) Standards. Aqueous solutions of tyramine *O*-sulphate containing 1–10 μg of tyramine/ml were prepared. Standards run through the complete separation procedure and compared with identical standards assayed directly showed the recovery to be 95–100%.

Mode of administration of tyramine. Tyramine hydrochloride (125 mg) was administered to six adult subjects (three males and three females) in gelatin capsules, and anhydrous Na_2SO_4 (300 mg) was administered in similar capsules. The subjects received no medication for at least 48 h before and during the study, and ate no foods known to contain tyramine (Sen, 1969; Marley & Blackwell, 1970). Fluid intake was voluntarily limited but adequate. After the subjects had voided the early-morning urine specimen on day 1, a tyramine capsule was taken with breakfast. On day 2 no tyramine was given, and on day 3 a tyramine capsule and an Na_2SO_4 capsule were taken together with breakfast. Urine was collected and stored at 4°C over a 9 h period without added preservative, as it has been shown previously by using [$1\text{-}^{14}\text{C}$]tyramine hydrochloride that 95% of the total administered radioactivity was excreted by man in this period (Smith *et al.*, 1972). Urine was adjusted to pH 3 with conc. HCl, the volume was measured and 100 ml portions were stored at –20°C until assayed within 5 days of collection.

Assay of tyramine *O*-sulphate in urine. Four discrete stages made up the procedure, namely: paper chromatography of urine followed by elution of tyramine *O*-sulphate band; hydrolysis of the eluate; paper electrophoresis of liberated tyramine and elution of the tyramine band; spectrophotofluorimetry.

(1) Chromatography of urine. Whatman no. 1 paper (25 cm × 25 cm) was used. Each sheet was cut to contain two 10 cm-wide lanes, separated by 2 mm vertical slits. Five sheets were placed on a frame

(Smith, 1969) and developed overnight by using ascending chromatography at 4°C in butan-1-ol-acetic acid-water (12:3:5, by vol.) as solvent. Standard tyramine *O*-sulphate or urine (0.5 ml) was applied as a streak to the origin and standard [^{14}C]-tyramine *O*-sulphate (20 $\mu\text{Ci/ml}$; 10 μl) was applied to the same origin. The separated tyramine *O*-sulphate band (R_F approx. 35) was located by using a spark-chamber scanner (Smith *et al.*, 1973), cut out and eluted with 9 ml of 0.1 M-HCl into a glass-stoppered test tube.

(2) Hydrolysis of tyramine *O*-sulphate. The eluate was adjusted to approx. 1 M-HCl and heated for 2 h at 105°C in a sealed tube. The hydrolysate was dried on a rotary evaporator at 45°C in the original tube. The residue was dissolved in 0.2 ml of 0.1 M-HCl with vigorous agitation on a vortex mixer (Fisons Scientific Apparatus, Loughborough, Leics., U.K.).

(3) Paper electrophoresis of liberated tyramine. Whatman no. 1 paper (25 cm $\times$ 25 cm) was used in a Shandon model Q11 high-voltage electrophoresis apparatus. Each sheet was cut to contain three 6 cm-wide lanes, separated by 2 mm slits. A portion (0.15 ml) of the redissolved hydrolysate or standard tyramine was applied to the origin and electrophoresis was carried out for 15 min at 2 kV and 40 mA in a pyridine-acetic acid-water (20:6.3:1973, by vol.) buffer adjusted to pH 5.3. The tyramine band was located in the spark chamber, excised and eluted with 3 ml of 0.1 M-HNO₃ and made up to 4 ml with the same HNO₃.

(4) Spectrophotofluorimetry. An Aminco-Bowman instrument with an attached Du Mont type 304-A cathode-ray oscilloscope was used. A sample (2 ml) of the eluate after electrophoresis was pipetted into a ground-glass-stoppered tube. 1-Nitroso-2-naphthol (1 ml; 0.1%, w/v) in 95% (v/v) ethanol (Gerngross *et al.*, 1933) was added with rapid mixing. Then 1 ml of 1 M-HNO₃, containing 2% (v/v) NaNO₂ (2.5%, w/v), freshly prepared (Udenfriend & Cooper, 1952), was added with further vigorous mixing. The mixture was incubated in the dark at 60°C for 1 h, cooled rapidly to room temperature in a fume-cupboard draught (10 min) and extracted with 1,2-dichloroethane (10 ml) for 1 min. The tubes were spun for 10 min at 3000 rev./min in a centrifuge to separate the two phases completely. The fluorescence of the upper aqueous layer was measured by using an activation wavelength of 465 nm and an emission wavelength of 565 nm as determined by the use of a cathode-ray oscilloscope.

Results and discussion

Temperature and time of incubation. Incubation of the 1-nitroso-2-naphthol reagent with tyramine, in the dark in a shaking water bath (Gallenkamp Ltd.,

London E.C.2, U.K.), at various temperatures and times showed an optimum of 60°C for 60 min.

Interfering substances and specificity. Although many *o*-unsubstituted phenols react with 1-nitroso-2-naphthol similarly to tyramine, all are eliminated either during the chromatographic and electrophoretic purification stages, or by their specific fluorescence characteristics. Further, it is likely that the concentration of their conjugates would be significantly less than that of tyramine *O*-sulphate after a 125 mg dose of tyramine.

Sensitivity and reproducibility. The method described here is capable of measuring 0.5 μg of tyramine/ml of urine, equivalent to a minimum excretion of 0.5–1.0 mg of tyramine as tyramine *O*-sulphate per 24 h sample. Reproducibility of standards and of urine duplicates was within $\pm 4\%$. Recoveries were monitored by measuring radioactivity at various stages. The results are shown in Table 1. It was possible to measure radioactivity on collets of paper prepared from excised areas after chromatography and electrophoresis. The dry collets were placed in a scintillation fluid [7.5 g of 2,5-diphenyloxazole and 0.75 g of 1,4-bis-(5-phenyloxazol-2-yl)benzene in 2 litres of toluene], and counted for radioactivity in a Packard

Table 1. Recovery of the purification procedure monitored by radioactivity measurements at various stages

Values for d.p.m. are means $\pm$ S.D. ($n = 4$).

Stage of purification procedure	Radioactivity (d.p.m.)
Added to origin for chromatography	1040 $\pm$ 19
Excised at R_F 0.30–0.38	1026 $\pm$ 14
Added to origin for electrophoresis	988 $\pm$ 26
Excised band after electrophoresis	960 $\pm$ 35
Final eluate for fluorescence assay	950 $\pm$ 40

Table 2. Urinary excretion of tyramine *O*-sulphate after oral doses of 125 mg of tyramine hydrochloride alone or with a 300 mg supplement of Na₂SO₄ in six adult humans

A paired *t* test gave a value of $t = 3.82$ corresponding to $P < 0.02$ with 5 degrees of freedom.

Sub- ject	Sex	Age (years)	Tyramine <i>O</i> -sulphate excreted (mg)		Increase (mg) (%)	
			After tyramine	After tyramine + sulphate		
1	Male	27	18.7	24.1	5.4	35
2	Male	47	12.4	17.2	4.8	38
3	Male	50	9.0	12.7	3.7	41
4	Female	23	20.2	26.9	6.7	34
5	Female	29	10.8	11.6	0.8	7
6	Female	60	13.2	24.3	11.1	85

Tri-Carb model 3375 scintillation counter. Retrieval of the collet from the scintillation fluid and drying in air had no effect on the subsequent nitrosonaphthol assay. Urine assayed after standing for 9 h at room temperature, or after standing overnight at 4°C with 300 mg of Na₂SO₄ added, showed no change in tyramine *O*-sulphate content.

Originally the extraction procedure of Oates (1961) as modified by Smith & Kellow (1972) was used, but by using a tracer of [1-¹⁴C]tyramine it was found that losses were both high and variable. An entirely new extraction procedure has been devised, although the original nitrosonaphthol fluorimetric method has been retained.

The quantity of both substances administered was within physiological range, and in the molar ratio of 1:3 for tyramine:inorganic sulphate. The dose of tyramine was equivalent to that found in approx. 150 g of Cheddar cheese (Sen, 1969; Marley & Blackwell, 1970). Anhydrous Na₂SO₄ was administered at one-twentieth of its usual cathartic dose of 15 g of Na₂SO₄·10H₂O (Fingl, 1970). The results in Table 2 show that the amount of tyramine *O*-sulphate excreted after a fixed dose of tyramine varies considerably between individuals, and supplementing the dose of tyramine with inorganic sulphate always causes an increase in urinary tyramine *O*-sulphate. This increase was found to be highly significant by a paired *t* test in the group studied. It follows that dietary cysteine and methionine are not necessarily the sole sources of urinary ester sulphate. Further, the view that inorganic sulphate does not function as a precursor of organic sulphate esters (Binkley, 1949) can no longer be sustained. Indeed, our results are what would be expected from the results obtained by Laidlaw & Young (1948, 1953) and Dziewiatkowski (1949) using Na₂³⁵SO₄ with other phenols, all of which formed phenolic [³⁵S]sulphate esters.

Inorganic sulphate is known to occur in a normal diet (Davidson & Passmore, 1966), especially in green vegetables, but exact contents do not seem to have been determined. Tyramine occurs in many foods included in the normal Western diet (Sen, 1969; Marley & Blackwell, 1970). It has been shown to be completely absorbed from the human small intestine, as after oral dosing of [1-¹⁴C]tyramine hydrochloride radioactivity can be totally accounted for in the urine (Smith *et al.*, 1972).

The data of Michels & Smith (1965) from studies *in vivo* in rats indicate that the availability of sulphate is related to the ratio of 'neutral' to inorganic sulphur in the diet, and that the intestinal absorption of

methionine may be related to the inorganic-sulphur content of the diet. These workers also conclude that with a normal diet inorganic sulphate contributes as much to the metabolic sulphate pool as does 'neutral' sulphur.

The finding that inorganic sulphate affects phenolic-amine metabolism may have important consequences. For example, it would seem worth while to investigate the protecting ability of inorganic sulphate against the inadvertent ingestion of foods containing phenolic amines in patients receiving anti-depressant drugs of the type that inhibit monoamine oxidase (EC 1.4.3.4).

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- Baumann, E. (1876) *Pflügers Arch. Gesamte Physiol.* **12**, 285–291
- Binkley, F. (1949) *J. Biol. Chem.* **178**, 821–826
- Davidson, S. & Passmore, R. (1966) *Human Nutrition and Dietetics*, 3rd edn., p. 161, E. and S. Livingstone, Edinburgh and London
- Dziewiatkowski, D. D. (1949) *J. Biol. Chem.* **178**, 389–393
- Fingl, E. (1970) in *The Pharmacological Basis of Therapeutics*, 4th edn. (Goodman, L. S. & Gilman, A. eds.), Macmillan, London and Toronto
- Gerngross, O., Voss, K. & Herfield, H. (1933) *Chem. Ber.* **66**, 435–442
- Laidlaw, J. C. & Young, L. (1948) *Biochem. J.* **42**, 1p
- Laidlaw, J. C. & Young, L. (1953) *Biochem. J.* **54**, 142–148
- Marley, E. & Blackwell, B. (1970) *Advan. Chemother. Pharmacol.* **8**, 185–239
- Mattock, P. & Jones, J. G. (1970) *Biochem. J.* **116**, 797–803
- Michels, F. G. & Smith, J. T. (1965) *J. Nutr.* **87**, 217–220
- Mullen, P. E. & Smith, I. (1971) *Brit. J. Pharmacol.* **41**, 413p
- Oates, J. A. (1961) in *Methods in Medical Research* (Quastel, J. H., ed.), pp. 169–182, Year Book Medical Publishers, Chicago
- Sen, N. P. (1969) *J. Food Sci.* **34**, 22–26
- Smith, I. (1969) in *Chromatographic and Electrophoretic Techniques* (Smith, I., ed.), chapter 27, Heinemann, London, and Interscience, New York
- Smith, I. & Kellow, A. H. (1972) *Clin. Chim. Acta* **40**, 353–358
- Smith, I., March, S. E. & Gordon, A. J. (1972) *Clin. Chim. Acta* **40**, 415–419
- Smith, I., March, S. E., Mullen, P. E. & Mitchell, P. D. (1973) *J. Chromatogr.* **82**, 75–83
- Tacker, M., McIsaac, W. M. & Creaven, P. J. (1970) *Biochem. Pharmacol.* **19**, 2763–2773
- Udenfriend, S. & Cooper, J. R. (1952) *J. Biol. Chem.* **196**, 227–233