Excretion of ^{35}S -Tobias acid (2-naphthylamino, 1-sulphonic acid) by the rat after oral and intravenous administration

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Marchisio, M. A., Dubini, M., Serra, G., Mennini, T., and Manara, L. (1976). *British Journal of Industrial Medicine*, 33, 269-271. Excretion of ^{35}S -Tobias acid (2-naphthylamino, 1-sulphonic acid) by the rat after oral and intravenous administration. Urinary and faecal excretion of radioactivity after either an intravenous or oral (1 mg/kg) dose of ^{35}S -labelled Tobias acid (2-naphthylamino, 1-sulphonic acid), a dyestuff intermediate structurally similar to the powerful carcinogen 2-naphthylamine, was studied in rats. The Tobias acid was eliminated from the body within 24 hours of administration, almost exclusively through the urine. TLC-chromatography of faecal extracts and urine did not disclose the presence of excreted products other than unchanged Tobias acid and the search for inorganic $^{35}\text{SO}_4$ in the urine by BaCl_2 precipitation was negative. There was significant absorption from the gastrointestinal tract, but neither cleavage of the sulphonic group nor other biotransformation by the intestinal flora was apparent under the test condition. There was no evidence that the sulphonic group of Tobias acid is cleaved in the body to a significant extent to give 2-naphthylamine. This information should help in the evaluation of the occupational hazard potential of Tobias acid.

Tobias acid (2-naphthylamino, 1-sulphonic acid) (Figure) an important dyestuff intermediate, may be considered as typical of sulphonated amino naphthalene compounds; it is also largely used as intermediate in the dye industry. The only structural difference between Tobias acid and the powerful carcinogenic compound 2-naphthylamine is the presence in Tobias acid of a sulphonic group in position 1, ortho position to the amino group.

Our survey of the literature did not reveal whether sulphonic groups bound to aromatic rings are stable in a biological environment; this is particularly relevant for sulphonic derivatives of naphthylamine.

The following questions need to be resolved:

1. Is Tobias acid retained or excreted?
2. What is the principal excretion route?
3. In what chemical form is it eliminated?

FIGURE Tobias acid (2-naphthylamino, 1-sulphonic acid)

4. Does cleavage of its sulphonic group occur in the body and is it converted to 2-naphthylamine?

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To help answer these questions, we have studied the kinetics of elimination of orally and intravenously administered Tobias acid in rats by means of the ^{35}S -labelled compound.

Materials and methods

Labelled Tobias acid (synthesized by G. Vicario and P. G. Ramella, Istituto Donegani, Montedison, Novara, Italy) was obtained at a specific activity of 4.5 mCi/mmol by sulphonation of 2-naphthylamine with $^{35}\text{SO}_3$ in tetrachloroethane and purified by crystallization with acids from an aqueous solution. The radiochemical purity estimated by TLC chromatography was more than 98%.

Experiments were performed on male Sprague-Dawley rats (Charles River, Italy); their average weight was 220 g. The animals were kept in individual cages and maintained at a standard temperature (22°C, 60% relative humidity) with free access to food and water (Altromin®, diet). Animals were treated either intravenously (tail vein) or orally (gavage) with 1 mg (equivalent to 16.4 μCi at the time of administration) ^{35}S -Tobias acid per kg body weight, dissolved in 2 ml saline. Urine and faeces were collected separately for each 24-hour period, up to 72 hours. Radioactivity measurements were performed by liquid scintillation, using a Packard TriCarb 2425 automatic counter and a dioxane based scintillation fluid (Manara, Mennini, and Carminati, 1972). Counting efficiency determined on random samples by internal standardization and monitored on all the samples by the external standardization device of the counting instrument was constant at about 95%. Sufficient counts were collected so as not to exceed a maximum counting error of $\pm 5\%$ for the samples containing the lowest activities. The counting rate determined for the entire stock of counting vials used in the experiments before adding the radioactive aliquots averaged 20 counts/min. Appropriate correction by the ^{35}S decay factor was provided for the counting data obtained from the assays performed at different times after administration of the labelled compound to the animals; all of the activity values (disintegration per minute) were normalized at the time of administration.

Fifty μl aliquots of urine or $17\,000 \times \text{g}$ supernatant of faecal homogenate (in 30 ml distilled water, omnimixer homogenizer) were counted dissolved in 15 ml scintillation fluid as an estimate of total ^{35}S -radioactivity. For the detection of inorganic $^{35}\text{SO}_4$, 0.2 ml 1 mol/l acetic acid and 0.4 ml 5% BaCl_2 were added to 1 ml urine and the activity found in the clear supernatant was compared to that of non-processed urine. TLC chromatography was carried out on standard 20×20 cm chromatoplates and on glass rods (Manara *et al.*, 1972; Manara, Ghinzone, and Mennini, in preparation) both were coated with silica gel G Merck and developed in a solvent system consisting of n-Butanol, n-Propanol, H_2O , and 25% ammonia (10:5:4:1). Chromatoplates were scanned instrumentally (Packard model 7201) and in order to achieve a high degree of accuracy the silica gel on the rods at the R_f of Tobias acid (0.5) was scraped into liquid scintillation vials and counted; under such conditions, the silica gel settles on the bottom of the vial and does not affect the counting; more than 90% of authentic ^{35}S -Tobias acid was eluted consistently and counted in solution. Urine aliquots were chromatographed before and after incubation with betaglucuronidase (overnight at 37°C, 4 ml urine plus 2 ml 1 mol/l acetate buffer pH 4.5 containing 0.52 enzymatic units from *Helix pomatia*). Appropriate blanks and recoveries were carried out; an average of 95% and 75% for ^{35}S -Tobias acid added to urine or faecal homogenates respectively were recovered and counted after chromatography.

Results

The Table gives the data on the urinary and faecal excretion of ^{35}S after intravenous (animals 1, 2, and 3) and oral (animals 4, 5, and 6) administration of 1 mg/kg Tobias acid. Intravenous injection resulted in prompt elimination in the urine of most of the administered label (up to 84% recovered from rat 1, within the first 24-hour period) with only a minor fraction appearing in the faeces (a maximum of 2.6% after 72 hours, rat 1). After oral administration of ^{35}S -Tobias acid, most of the activity was

TABLE
EXCRETION OF ^{35}S ACTIVITY AFTER ADMINISTRATION OF 1 mg/kg ^{35}S -LABELLED
TOBIAS ACID TO RATS

Rat no.	Activity administered, DPM	Activity excreted, DPM								
		0-24 hours		24-48 hours		48-72 hours		Total % excretion 0-72 hours		
		Urine	Faeces	Urine	Faeces	Urine	Faeces	Urine	Faeces	Urine + faeces
1	7828	6620	174.4	74.6	28.3	26.0	NM	86	2.6	88
2	7464	5210	91.2	39.6	7.9	22.4	NM	71	1.3	72
3	7828	5860	35.4	49.3	12.4	13.8	NM	76	0.6	76
4	8556	4560	1926.0	102.0	106.2	13.4	NM	55	24.0	78
5	8010	2300	3155.0	46.3	44.2	13.6	NM	29	40.0	69
6	7828	2990	3231.0	181.8	254.1	13.7	NM	41	44.0	85

Figures, if not otherwise stated, are DPM (disintegration per minute) in thousands; rats nos. 1, 2, and 3 were dosed intravenously and rats nos. 4, 5, and 6 orally.

NM = Not measurable

again promptly cleared from the body; the total (urine plus faeces) excretion was comparable in size and time of occurrence to that observed after intravenous injection, but, in contrast to the latter, urinary and faecal elimination were of a similar order.

Chromatography of faecal extracts and of urine aliquots revealed a single peak of activity at the same R_f of authentic Tobias acid on instrumental scanning. Removal and counting of silica gel at the same R_f of authentic Tobias acid after chromatography on glass rods of faecal extracts and urine aliquots, accounted for over 75% and 90% respectively of the applied activity (not corrected for the appropriate recovery). Equivalent results were observed after chromatography of urine, whether incubated with beta-glucuronidase or non-processed. Addition of $BaCl_2$ did not produce any loss in urine activity (such loss would have indicated the presence of significant amounts of ³⁵SO₄).

Discussion

Rats exposed to a single administration of Tobias acid showed that:

1. The compound was rapidly and extensively eliminated from the body after intravenous injections, almost exclusively in urine.
2. Elimination took place in the form of unchanged Tobias acid and, within the limits of the experimental error, it could be concluded that conjugation or biotransformation processes did not occur to any significant extent.
3. There was no evidence that the sulphonic group of Tobias acid was cleaved in the body to give 2-naphthylamine.
4. Oral administration resulted in significant absorption from the gastrointestinal tract, but neither cleavage of the sulphonic group nor other biotransformation by the intestinal flora was apparent under experimental conditions.

Some of these conclusions may be further supported: in spite of the extensive elimination of Tobias acid, no attempts were made to wash the cages and to account for the fraction of the eliminated activity which presumably was trapped therein, which leads us to believe that the dose administered was virtually all excreted; and the relatively small

administered dose (1 mg/kg) would not be expected to give rise to saturation of drug metabolizing enzymes in the body. Therefore our finding that there was no excreted product other than unchanged Tobias acid is of significance.

Conversely, although it can safely be concluded that a significant fraction of the administered Tobias acid was not converted in the body to 2-naphthylamine, the possibility that we were dealing with non-detectable traces of 2-naphthylamine should not be rejected. Further investigation based on the use of high specific activity ring-labelled Tobias acid could set more accurately lower limits of detection for 2-naphthylamine, thus providing a more definite answer.

Our findings are consistent with those previously reported by McMahon and O'Reilly (1969) who studied the elimination of a group of arylamino-sulphonic acids using a method based on the procedure of Bratton *et al.* (1939); their investigation of several naphthylaminosulphonic acid isomers indicated that, if the amino group is hindered by an adjacent group or ring, no metabolite is obtained.

It is believed that the results made available by our study, despite the limitations, will help in the evaluation of the occupational hazard potential of Tobias acid.

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