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THE COMPARATIVE METABOLISM OF MYLERAN-^{35}S IN THE RAT, MOUSE AND RABBIT

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Abstract—The fate of Myleran-^{35}S has been examined in three species. The rat and mouse excretes ^{35}S-labelled materials in similar proportions, whereas the rabbit excretes only methane sulphonic acid-^{35}S. The rabbit appears to be deficient in the urinary component which combines with methane sulphonic acid in the rat and mouse.

Chin-Tsu Peng[1] has shown that after injection into rats of Myleran-^{35}S (approx. 4 mg/kg in propylene glycol) the urinary metabolites (95–96 per cent of the dose after 32 hr) consisted mainly of methane sulphonic acid, some Myleran and other unidentified components. The amount of Myleran excreted unchanged within 24 hr was approximately 6 per cent of the dose. More recently, with the same species, Trams *et al.*[2] have shown that, following a smaller dose (2 mg/kg intraperitoneally, solvent assumed to be 10 per cent acetone), 60 per cent of the radioactive dose was excreted in 24 hr. Of this activity, 62–68 per cent was methane sulphonic acid, 25–30 per cent Myleran, 0·03–0·04 per cent sulphate and 5–8 per cent an unidentified component.

We have made some preliminary observations on the fate of Myleran-^{35}S in three species at the same dose level (10 mg/kg) used by us in earlier studies.[3] The compound, prepared according to the method of Chin-Tsu Peng,[1] with a specific activity of 8·6 mc/m-mole, was given intraperitoneally in arachis oil. The radioactivity present in urine and plasma was determined using 0·02 ml samples dried on aluminium trays with appropriate corrections for absorption.

In the rat, 50 per cent of the administered radioactive dose was excreted in the urine during the first 48 hr. The radioactive metabolites were separated by single-dimension paper chromatography (Fig. 1a) using two solvent systems, *n*-butanol:dioxan:2N ammonia (4:1:5) and *n*-butanol:2N acetic acid (1:1). Methane sulphonic acid and Myleran were identified whilst two components remained unidentified; one of these, (U1) lies very close to the methane sulphonic acid spot and the other (U2) is situated ahead of Myleran. Table 1 gives the R_f values of these metabolites in the two solvent systems. The radioactive material present in the 24 hr urine sample was mainly methane sulphonic acid and U1; these are not completely separated but autoradiographs suggest that approximately equal amounts were present. Only small amounts of injected Myleran (5 per cent) and U2 (2 per cent) were found. The plasma radioactivity reached a maximum level (6 per cent of the dose) approximately 3 hr after administration (Table 2) and consisted chiefly of unchanged Myleran (90 per cent) and methane sulphonic acid (8–10 per cent). No U2 was detected.

In the mouse, about 60 per cent of the radioactive dose appeared in the 24 hr urine sample. The relative amount of U1 was higher than in the rat, but the other three components found in the rat were also present in this species (Fig. 1b). The blood radioactivity reached a level of 1·6 per cent of the dose 1 hr after administration, 50–60 per cent being Myleran and the remainder methane sulphonic acid.

TABLE 1. RADIOACTIVE COMPONENTS OF URINE 0–24 HR AFTER ADMINISTRATION OF MYLERAN-^{35}S

	R_f values		Percentage distribution of radio-activity in urine		
Metabolite	BDN*	BA†	Rat	Mouse	Rabbit
Sulphate	origin	origin	—	—	—
$MeSO_2OH$	0·16	0·12	90	91	100
U1	0·11	0·18			—
Myleran	0·85	0·86	5	4	—
U2	0·98	0·93	2	3	—

* *n*-Butanol, dioxan, ammonia.
† *n*-Butanol, acetic acid.

The level of radioactivity in the plasma 2½ hr after injection into a rabbit was 1·4 per cent of the dose; of this 20–30 per cent was unchanged Myleran, whereas methane sulphonic acid accounted for most of the remainder (Table 2). In 24 hr, 30 per cent of the radioactive dose was excreted, entirely as methane sulphonic acid (Fig. 1c). *In vitro* tests were carried out to determine the stability of Myleran in plasma, urine and buffered solution. Labelled Myleran was dissolved in urine and plasma from each species (approx. 100 μg/ml) as well as in phosphate buffer at pH 8·0. In each case Myleran hydrolysed with a half-life of 11–12 hr at 37 °C. In urine and plasma from the

TABLE 2. RADIOACTIVE COMPONENTS OF PLASMA 2–4 HR AFTER INJECTION OF MYLERAN-^{35}S

Metabolite	Rat	Mouse	Rabbit
Sulphate	<1	<1	<1
$MeSO_2OH$	8	40	60–70
U1			
Myleran	90	50–60	20–30
U2	—	—	—

The percentage distribution of radioactive components of plasma following administration of Myleran-^{35}S, 2–4 hr after injection.

rat and mouse U1 and U2 were present. There was no evidence of either component in the rabbit urine or in buffer. The unknown U1 may be a product of reaction of methane sulphonic acid with a urinary constituent, as suggested by Peng,[1] but this latter must be lacking in the rabbit. These results also suggest that protracted methods for the extraction of the metabolites e.g. ether extraction in a Soxhlet apparatus over

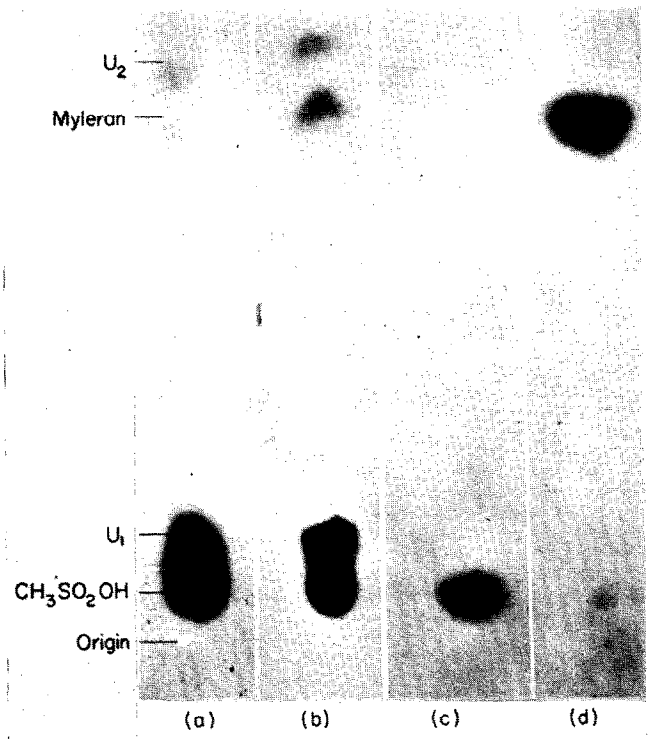

FIG. 1. Radioautographs of chromatograms indicating the 24 hr urinary components in three species after administration of Myleran-^{35}S. Solvent system, *n*-butanol:2N acetic acid (1:1). (a) Rat. (b) Mouse. (c) Rabbit. (d) Myleran alone.

several hours,[2] should be avoided. Myleran alone was stable under the chromatographic conditions employed forming a single spot (Fig. 1(d)).

In summary, methane sulphonic acid was the only material excreted in the urine of a rabbit given ^{35}S-labelled Myleran. Rat and mouse urine contained, in addition to methane sulphonic acid and a little Myleran, two other unidentified components (U1 and U2) which appear to be produced by reaction of labelled material with a substance not present in the rabbit.

More detailed studies of the fate of sulphonoxy alkane groups in similar compounds are in progress.

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