Biochemical Pharmacology, Vol. 18, pp. 197–203. Pergamon Press. 1969. Printed in Great Britain

THE MODE OF EXCRETION AND METABOLIC FATE OF POTASSIUM NAPHTHYL 2-^{35}S-SULPHATE AND POTASSIUM 5,6,7,8-TETRAHYDRONAPHTHYL 2-^{35}S-SULPHATE*

D. J. HEARSE,† GILLIAN M. POWELL and A. H. OLAVESEN

Department of Biochemistry, University College, Cardiff

(*Received* 7 *June* 1968; *accepted* 15 *August* 1968)

Abstract—The metabolic fates and modes of excretion of naphthyl 2-^{35}S-sulphate and 5,6,7,8-tetrahydronaphthyl 2-^{35}S-sulphate were investigated in the rat. Only small amounts of administered radioactivity were present in the bile of animals receiving the esters and in both cases the major proportion of the radioactive dose appeared in the urine. The bulk of the urinary radioactivity following naphthyl 2-^{35}S-sulphate administration was identified as unchanged parent ester. Urines obtained from animals receiving 5,6,7,8-tetrahydronaphthyl 2-^{35}S-sulphate also contained large quantities of unchanged parent ester. However, such urines also contained significant quantities of other radioactive components. The significance of the results is discussed in relation to the structural difference between naphthyl 2-^{35}S-sulphate and 5,6,7,8-tetrahydronaphthyl 2-^{35}S-sulphate.

INVESTIGATIONS[1] on the metabolism of naphthyl 2-sulphate showed that the ester did not undergo significant metabolic degradation in the rat and was eliminated in the urine. However, the possibility of a biliary circulation has not been explored. It was of interest to determine whether any biliary excretion could be detected and, further, whether modification of the molecular structure of naphthyl 2-sulphate would affect the level of biliary elimination.

Previous studies[2] with aryl sulphate esters containing two six-membered rings have shown that saturation of one of the constituent rings results in extensive changes in the level of biliary excretion. Thus the extent of biliary excretion of radioactivity following the administration of cyclohexylphenyl 4-^{35}S-sulphate was much greater than that observed with biphenylyl 4-^{35}S-sulphate. The only structural difference between these two molecules is that the ring remote from the sulphate moiety is fully saturated in cyclohexylphenyl 4-^{35}S-sulphate. It seemed possible that an analogous modification of naphthyl 2-^{35}S-sulphate would result in an alteration in its mode of excretion. In order to explore this possibility, a comparative study on the mode of excretion and metabolism of naphthyl 2-^{35}S-sulphate and 5,6,7,8-tetrahydronaphthyl 2-^{35}S-sulphate was undertaken.

* D. J. H. is grateful to the Medical Research Council for a Research Studentship. The work was supported by U.S. Public Health Service Research Grant No. AMO1982 from the Arthritis and Metabolic Diseases Division.

† Present address: Department of Pharmacology, New York University Medical Center, 550, First Avenue, New York, N.Y. 10016, U.S.A.

MATERIALS AND METHODS

Aryl sulphate esters

The ^{35}S-labelled sulphate esters of 2-naphthol and 5,6,7,8-tetrahydro 2-naphthol were prepared as described by Hearse, Olavesen and Powell.[3] The specific radioactivities of the preparations were 27 μc/mg and 41 μc/mg respectively.

Thin-layer chromatography (TLC)

The solvent systems employed were A, 2-propanol: chloroform : methanol : water (10:10:5:2 by volume); B,1-butanol : acetic acid : water (60:20:20 by volume); C,benzene : methanol : acetic acid (160:35:10 by volume). TLC and the detection of radioactivity on TLC's was carried out as described by Hearse, Powell, Olavesen and Dodgson.[2]

Measurement of radioactivity

Measurements of radioactivity in urine, bile, faeces and carcasses were made as previously described.[2]

Isotope dilution analysis

Isotope dilution analysis was carried out as described by Hearse *et al.*[2]

Experimental animals

Male and female MRC Hooded rats (approximately 200 g body wt.) were used throughout. Aryl sulphate esters were administered both i.p. and i.v. and urine, faeces and bile were collected under conditions previously described.[2]

RESULTS

Free-range experiments

In preliminary experiments, rats received intraperitoneal injections of aqueous solutions (1 mg/100 g body wt.) of the appropriate esters. Six animals (three male and three female) were used for experiments with any one ester. Urines were collected at intervals of 12 hr, 24 hr and 48 hr after injection and assayed for total and inorganic ^{35}S-sulphate. Faeces were collected after 48 hr and at this time animals were killed by a blow on the back of the head. Faeces and carcasses were taken for the determination of ^{35}S-content. The results are shown in Table 1.

In all experiments, between 87 and 100 per cent of the injected radioactivity appeared in the urine over the 48 hr experimental period, the major proportion appearing during the first 12 hr. Urines contained small amounts of inorganic ^{35}S-sulphate. Thus in urines obtained from animals receiving naphthyl 2-^{35}S-sulphate, between 7 and 9 per cent of the injected radioactivity appeared as inorganic ^{35}S-sulphate. The corresponding figure for animals receiving 5,6,7,8-tetrahydronaphthyl 2-^{35}S-sulphate was between 5 and 13 per cent. In experiments with both compounds the level of inorganic ^{35}S-sulphate was slightly higher in the male than in the female. Only trace amounts of radioactivity were associated with faeces and carcasses.

Cannulation studies

In subsequent experiments each of the ^{35}S-labelled esters was administered (1 mg/100 g body wt.) i.v. to rats (three male and three female) with bile duct and ureter cannulae. Urine was collected in hourly samples and bile as a total fraction over a

TABLE 1. DISTRIBUTION OF RADIOACTIVITY IN URINE, FAECES AND CARCASSES FOLLOWING THE I.P. INJECTION (1 mg/100g BODY WT.) OF ARYL SULPHATE ESTERS TO RATS (MALE AND FEMALE)

M = Male rat; F = Female rat; T = Total ^{35}S-sulphate; I = Inorganic ^{35}S-sulphate; E = Ester ^{35}S-sulphate.

Compound	Animal	% Injected radioactivity recovered in:														
		Urine												Faeces	Carcass	Total
		12 hr			24 hr			48 hr			Total					
		T	I	E	T	I	E	T	I	E	T	I	E	T	T	T
Napthyl 2-^{35}S-sulphate	M_1	80	6	74	10	2	8	2	1	1	92	9	83	1	3	96
	M_2	82	5	77	7	1	6	2	1	1	91	7	84	2	2	95
	M_3	92	7	85	7	1	6	1	1	0	100	9	91	0	1	101
	F_1	94	7	87	2	1	1	1	0	1	97	8	89	1	2	100
	F_2	89	6	83	3	1	2	1	0	1	93	7	86	0	3	96
	F_3	77	5	72	8	1	7	2	1	1	87	7	80	1	2	90
5,6,7,8-Tetrahydro-naphthyl 2-^{35}S-sulphate	M_1	92	6	86	2	1	1	2	1	1	96	8	88	1	1	98
	M_2	85	8	77	8	4	4	1	1	0	94	13	81	1	2	97
	M_3	92	4	88	3	1	2	1	1	0	96	6	90	1	1	98
	F_1	88	4	84	2	1	1	2	0	2	92	5	87	1	2	95
	F_2	90	3	87	4	1	3	1	0	1	95	5	90	0	1	96
	F_3	89	4	85	4	1	3	2	1	1	95	6	89	1	1	97

6-hr experimental period. Bile and urine samples were assayed for total and inorganic ^{35}S-sulphate content (see Table 2).

In experiments with naphthyl 2-^{35}S-sulphate between 48 and 59 per cent of the injected radioactivity appeared in the urine over the 6-hr period and only trace amounts of radioactivity (0·8–1·5 per cent) appeared in the bile. Between 7·7 and 10·5 per

TABLE 2. THE DISTRIBUTION OF RADIOACTIVITY IN BILE AND URINE OF RATS FOR THE 6-hr PERIOD FOLLOWING THE I.V. ADMINISTRATION (1 mg/100 g BODY WT.) OF ^{35}S-LABELLED ARYL SULPHATE ESTERS.

M = Male rat; F = Female rat; T = Total ^{35}S-sulphate; I = Inorganic ^{35}sulphate; E = Ester ^{35}S-sulphate.

Compound	Animal	% Radioactive dose recovered in:					
		Bile		Urine			Total
		T	I	T	I	$\frac{I}{T} \times 100$	T
Naphthyl 2-^{35}S-sulphate	M_1	1·1	0·2	48	4·8	10·0	49
	M_2	1·3	0·3	59	5·7	9·6	60
	M_3	1·2	0·2	52	8·5	10·5	53
	F_1	0·8	0·2	51	3·9	7·7	52
	F_2	1·5	0·3	53	4·2	7·9	55
	F_3	0·9	0·1	55	4·4	8·0	56
5,6,7,8-Tetrahydronaphthyl 2-^{35}S-sulphate	M_1	2·9	0·4	82	8·9	10·9	85
	M_2	4·7	0·6	76	9·4	12·4	81
	M_3	2·5	0·3	87	6·7	7·7	90
	F_1	2·3	0·2	84	7·0	8·3	86
	F_2	1·6	0·1	85	3·5	4·1	87
	F_3	2·0	0·2	81	3·6	4·4	83

cent of the urinary radioactivity appeared as inorganic ^{35}S-sulphate. These figures are in agreement with those previously recorded in free-range rats. Further, as in the free-range experiments, this level of inorganic ^{35}S-sulphate in male urines (mean value 10·0 per cent) was marginally higher than that recorded in urines of females (mean value 7·9 per cent).

In studies with 5,6,7,8-tetrahydronaphthyl 2-^{35}S-sulphate, the major proportion of the injected radioactivity (76–87 per cent) appeared in the urine over the 6-hr experimental period. Between 4·1 and 12·4 per cent of the urinary radioactivity was present as inorganic ^{35}S-sulphate, and an average value of 10·3 per cent was recorded for urine obtained from male rats with a corresponding figure of 5·6 per cent for female rat urines. Only small amounts (1·6–4·7 per cent) of the injected radioactivity appeared in the bile.

In experiments with both naphthyl 2-^{35}S-sulphate and 5,6,7,8-tetrahydronaphthyl 2-^{35}S-sulphate, the major route of elimination of radioactivity was urinary. The percentage of urinary radioactivity appearing as inorganic ^{35}S-sulphate following naphthyl 2-^{35}S-sulphate administration was of the same order as that recorded following the administration of 5,6,7,8-tetrahydronaphthyl 2-^{35}S-sulphate. Further, there appeared to be a sex difference in the inorganic ^{35}S-sulphate content of the urines. In studies with 5,6,7,8-tetrahydronaphthyl 2-^{35}S-sulphate the rate of elimination of

radioactivity was considerably greater than that observed with naphthyl 2-^{35}S-sulphate. However, in both cases, the greatest proportion of the radioactivity eliminated appeared in the urine collected over the first hour (see Fig. 1).

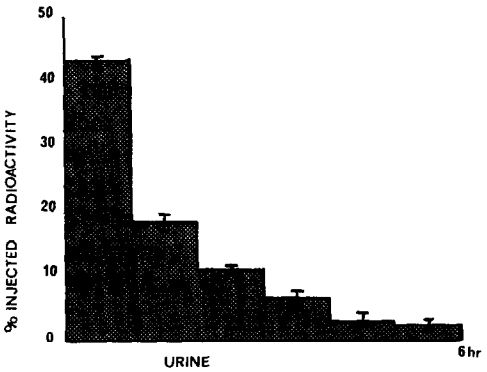

FIG. 1. The appearance of radioactivity in urine with time following the i.v. administration (1 mg/100 g body wt.) of potassium 5,6,7,8-tetrahydronaphthyl 2-^{35}S-sulphate. Each percentage represents the mean of results obtained from three animals. The limits of variation are included.

The experiments in which the esters were administered to rats with bile duct and ureter cannulae were repeated at two further dose levels (0·1 mg/100 g body wt. and 10 mg/100 g body wt.). However, there was no deviation from the excretion patterns obtained at a dose level of 1 mg/100 g body wt.

Further experiments were carried out in which either naphthyl 2-^{35}S-sulphate or 5,6,7,8-tetrahydronaphthyl 2-^{35}S-sulphate was administered (1 mg/100 g body wt.) to male rats with ligated renal pedicles and cannulated bile ducts. Bile was collected for 6 hr and assayed for total ^{35}S-sulphate. In experiments with naphthyl 2-^{35}S-sulphate 7·2 per cent of the administered radioactivity was recovered in the bile compared with an average figure of 1·3 per cent in animals with bile duct and ureter cannulae. In experiments with 5,6,7,8-tetrahydronaphthyl 2-^{35}S-sulphate, 13·9 per cent of the radioactive dose appeared in the bile compared with an average figure of 3·6 per cent in animals with bile duct and ureter cannulae.

Chromatographic analysis of urine samples

Urine samples were collected for 2 hr following intravenous administration of either naphthyl 2-^{35}S-sulphate or 5,6,7,8-tetrahydronaphthyl 2-^{35}S-sulphate (1 mg/100 g body wt.). Aliquots (sufficient to give approximately 2000 cpm measured with a Panax monitor) were applied to thin-layer chromatography plates. Chromatograms of urines from animals receiving naphthyl 2-^{35}S-sulphate were developed in solvent A and chromatograms of urines from animals receiving 5,6,7,8-tetrahydronaphthyl 2-^{35}S-sulphate were prepared by multiple (4) development using solvent C. Radioactive areas were located by scanning and autoradiography.

Urines obtained from animals receiving naphthyl 2-^{35}S-sulphate contained only one major radioactive component whose chromatographic behaviour was identical with authentic naphthyl 2-sulphate. A sample was isolated and purified by preparative

thin-layer chromatography (as described by Hearse *et al.*[2]) and subjected to isotope dilution analysis. Recrystallization of authentic material to a constant specific radioactivity confirmed the identity of the sample. The amounts of inorganic ^{35}S-sulphate detected in chromatograms were of the same order as those recorded previously. In addition, several minor radioactive components (less than 1 per cent of the injected radioactivity) were detected.

Similar chromatographic analysis of urines obtained from animals receiving 5,6,7,8-tetrahydronaphthyl 2-^{35}S-sulphate was carried out. Autoradiograms of chromatograms revealed the presence of four major radioactive areas, one of these was chromatographically identical with authentic parent ester and represented between 50 and 80 per cent of the injected radioactivity. The identity of the material was confirmed by isotope dilution analysis. A further radioactive area (equivalent to less than 10 per cent of the radioactive dose) was chromatographically identical with inorganic ^{35}S-sulphate. The remaining radioactive spots each represented between 10 and 20 per cent of the injected radioactivity.

DISCUSSION

When either naphthyl 2-^{35}S-sulphate or 5,6,7,8-tetrahydronaphthyl 2-^{35}S-sulphate is administered to rats the bulk of the radioactivity is eliminated via the urine and only small amounts (less than 5 per cent) of the radioactive dose appear in the bile. These results are particularly interesting in the light of those recorded by Hearse *et al.*[2] in studies with biphenylyl 4-sulphate and cyclohexylphenyl 4-sulphate where it was found that saturation of the ring remote from the sulphate moiety led to a marked increase in the level of biliary elimination. However, saturation of the ring remote from the sulphate moiety in naphthyl 2-sulphate resulted in only a slight increase in biliary elimination. Therefore, in contrast to the situation with cyclohexylphenyl 4-sulphate the presence of a saturated six-membered ring in 5,6,7,8-tetrahydronaphthyl 2-^{35}S-sulphate does not facilitate biliary elimination.

It seemed possible that rapid clearance of the injected radioactivity by the kidneys might account for the low level of biliary elimination. This hypothesis was explored by administration of the ^{35}S-labelled esters to rats in which renal function had been eliminated by ligation of the renal pedicles. However, although the extent of biliary excretion was increased under these conditions, the amount of radioactivity appearing in the bile was still less than 14 per cent of the injected dose. It therefore seems unlikely that the low level of biliary excretion can be explained solely on the basis of the rapid renal clearance of radioactivity.

Analysis of urines obtained from animals receiving naphthyl 2-^{35}S-sulphate revealed that the ester undergoes little metabolic degradation since the bulk of the urinary radioactivity is in the form of unchanged parent ester while not more than 11 per cent of the urinary radioactivity was in the form of inorganic ^{35}S-sulphate. This observation is in agreement with the work of Hawkins and Young.[1] A similar figure for inorganic ^{35}S-sulphate content was recorded with urines obtained from animals receiving 5,6,7,8-tetrahydronaphthyl 2-^{35}S-sulphate. However, in addition to unchanged parent ester, urines contained significant amounts of other ^{35}S-labelled components. It appears, therefore, that 5,6,7,8-tetrahydronaphthyl 2-^{35}S-sulphate is more susceptible to metabolic degradation than naphthyl 2-^{35}S-sulphate.

Thus while the presence of a saturated six-membered ring in 5,6,7,8-tetrahydronaphthyl 2-^{35}S-sulphate does not result in a marked increase in the biliary elimination it does appear to render the molecule more susceptible to metabolic degradation.

REFERENCES

1. J. B. HAWKINS and L. YOUNG, *Biochem. J.* **56**, 166 (1954).
2. D. J. HEARSE, G. M. POWELL, A. H. OLAVESEN and K. S. DODGSON, *Biochem. Pharmac.* **18**, 181 (1969).
3. D. J. HEARSE, A. H. OLAVESEN and G. M. POWELL, *Biochem. Pharmac.* **18**, 173 (1969).