Biliary Excretion of Foreign Compounds

SULPHONAMIDE DRUGS IN THE RAT

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1. The extent of biliary excretion in the rat of 15 sulphonamide compounds was studied. 2. Most of the sulphonamides studied, with molecular weights from 172 (sulphanilamide) to 352 (*N*⁴-acetylsulphadimethoxine) are poorly excreted in the bile (0–4% of the dose), except sulphapyridine, sulphamethoxypyridazine and sulphadimethoxine. The last three are partly metabolized to glucuronides, whose molecular weights and polarities are such as to allow them to be excreted in the bile in appreciable amounts. 3. Succinylsulphathiazole and phthalylsulphathiazole are polar and have molecular weights (355 and 403) of an appropriate order, and are excreted unchanged in the bile in appreciable amounts. 4. Sulphadimethoxine *N*¹-glucuronide (mol.wt. 487) is extensively excreted in the bile unchanged. 5. The results are examined in the light of the hypotheses put forward in the preceding paper (Millburn, Smith & Williams, 1967).

Sulphonamide drugs have been extensively studied from the point of view of their metabolic transformations, but relatively little is known about their biliary excretion (see Smith, 1966). Sulphanilamide is known to be excreted in small amounts in the bile in man (Bettman & Spier, 1939), dogs (Carrier & Ivy, 1939) and rabbits (Barber, Dible & Haslewood, 1943). Sulphapyridine is also excreted in the bile in man (Hubbard & Butsch, 1941) and rabbits (Barber *et al.* 1943). On intravenous injection, sulphaphenazole, sulphamethoxypyridazine, sulphasomidine and sulphochloropyridazine appear in the bile of rabbits (Neipp, Sackmann & Tripod, 1961). In the present work the biliary excretion of 15 sulphonamides in the rat was examined and the metabolites in the bile were identified in most cases. The results obtained are considered in the light of the suggestions made in the preceding paper (Millburn, Smith & Williams, 1967) on the factors involved in biliary excretion. It is shown that most of the sulphonamides examined are poorly excreted in the bile and that this finding is in accord with the hypotheses elaborated by Millburn *et al.* (1967).

MATERIALS AND METHODS

Chemicals. Most of the sulphonamides used were purchased (May and Baker Ltd., Dagenham, Essex) and purified by recrystallization from aqueous ethanol. The *N*⁴-acetyl derivatives were prepared by the acetylation of the parent compound with acetic anhydride in saturated Na₂CO₃ solution and were recrystallized from aqueous or

absolute ethanol. Melting points agreed with those given in the literature. Ammonium 1-deoxy-1-[*N*¹-(2,4-dimethoxypyrimidin-6-yl)sulphanilamido]glucuronate (sulphadimethoxine *N*¹-glucuronide, ammonium salt), m.p. 150–160° (decomp.), was prepared by the method of Bridges, Kibby & Williams (1965).

Determination of sulphonamides in bile. Sulphonamides containing a free aromatic primary amino group were estimated colorimetrically (at 540mμ) by diazotization and coupling with *N*-(1-naphthyl)ethylenediamine as described by Abou-El-Makarem, Millburn, Smith & Williams (1967). Total sulphonamides (i.e. free plus acetylated) were estimated similarly after a short acid hydrolysis. The recoveries of the sulphonamides and their *N*⁴-acetyl derivatives added to bile (0.1–0.5 mg./ml.) were satisfactory (range 90–100%). Succinyl- and phthalylsulphathiazole were determined as sulphathiazole after acid hydrolysis. Bile samples (3 ml.) obtained from rats treated with either of these compounds were heated in stoppered glass tubes for 1 hr. in a boiling-water bath with an equal volume of 2*N*-HCl. The solution was filtered to remove precipitated bile pigments and a portion of the filtrate (2 ml.) was diluted to 10 ml. Samples (1 ml.) of the latter were diazotized and coupled as described above. The recoveries of succinyl- and phthalylsulphathiazole added to bile samples (0.2 mg./ml.) and assayed as described above were 89% and 91% respectively.

Sulphadimethoxine is excreted in the bile mainly as metabolites and these were estimated individually after separation by paper chromatography. Bile samples (1 ml.), collected from rats injected intraperitoneally with sulphadimethoxine, were chromatographed on Whatman no. 1 paper with solvent *C* (see Table 1). After development and drying of the chromatogram, sulphadimethoxine and its metabolites were located on the paper by viewing under u.v. light. Areas of the chromatogram containing the drug

Table 1. *R_F* values and colour reactions of sulphonamide drugs

Descending chromatography on Whatman no. 1 paper was used. The solvent systems employed were: *A*, butan-1-ol-acetic acid-water (4:1:2, by vol.); *B*, butan-1-ol-aq. NH₃ (sp.gr. 0.88)-water (10:1:1, by vol.); *C*, propan-1-ol-aq. NH₃ (sp.gr. 0.88) (7:3, v/v). All the compounds gave a red colour immediately on spraying with *p*-dimethylaminocinnamaldehyde (0.25% in equal volumes of 2*N*-HCl and ethanol) except the *N*⁴-substituted derivatives, which give a red colour in 6-24 hr.; phthalylsulphathiazole and sulphadimethoxine *N*⁴-glucuronide give the red colour after 3-4 min. In u.v. light all the compounds appear on paper as dark spots, except sulph-anilamide (purple fluorescence), sulphaguanidine and *N*⁴-acetylsulphapyridine (blue fluorescence), and sulphapyridine (blue fluorescence in HCl fumes).

Compound	<i>R_F</i> values in solvents		
	<i>A</i>	<i>B</i>	<i>C</i>
Sulphanilamide	0.59	0.37	0.65
<i>N</i> ⁴ -Acetylsulphanilamide	0.80	0.56	0.73
Sulphacetamide	0.70	0.07	0.45
<i>N</i> ¹ <i>N</i> ⁴ -Diacetylsulphanilamide	0.90	0.29	0.68
Sulphaguanidine	0.62	0.48	0.61
<i>N</i> ⁴ -Acetylsulphaguanidine	0.72	0.73	0.74
Sulphapyridine	0.89	0.51	0.61
<i>N</i> ⁴ -Acetylsulphapyridine	0.91	0.64	0.73
Sulphadiazine	0.77	0.04	0.51
Sulphisoxazole	—	0.40	0.58
Sulphasomizole	0.84	—	0.69
Sulphamethoxy-pyridazine	0.84	0.17	0.58
<i>N</i> ⁴ -Acetylsulphamethoxy-pyridazine	0.89	0.30	0.70
Sulphadimethoxine	0.94	0.51	0.70
<i>N</i> ⁴ -Acetylsulphadimethoxine	0.94	0.72	0.81
Sulphadimethoxine <i>N</i> ¹ -glucuronide	0.72	0.24	0.44
Sulphadimethoxine <i>N</i> ⁴ -glucuronide	Unstable	0.01	0.08
Sulphathiazole	0.79	0.34	0.66
<i>N</i> ⁴ -Acetylsulphathiazole	0.84	0.41	0.74
<i>N</i> ⁴ -Succinylsulphathiazole	0.79	0.05	0.41
<i>N</i> ⁴ -Phthalylsulphathiazole	0.80-0.90	0.11	0.46

Table 2. *Metabolites of sulphonamide drugs in the bile in rats*

The metabolites found in the bile were identified by *R_F* values and colour reactions (see Table 1). The approximate amounts of each metabolite found are given in column 3 (see also Table 3).

Compound administered	Metabolites found in bile	% of dose in 24 hr.
Sulphanilamide	Sulphanilamide	3
	<i>N</i> ⁴ -Acetylsulphanilamide	1
Sulphacetamide	Unchanged drug only	0.5
Sulphaguanidine	Sulphaguanidine	< 0.5
	<i>N</i> ⁴ -Acetylsulphaguanidine	< 0.5
Sulphapyridine	<i>N</i> ⁴ -Acetylsulphapyridine	4
	Unidentified glucuronide	7
Sulphadiazine	Unchanged drug only	2
Sulphathiazole	Unchanged drug only	1
Sulphisoxazole	Unchanged drug only	< 0.5
Sulphasomizole	Unchanged drug only	< 0.5
Sulphamethoxy-pyridazine	Sulphamethoxy-pyridazine + two unidentified metabolites*	8
<i>N</i> ⁴ -Acetylsulphathiazole	Unchanged compound only	2
Sulphadimethoxine	Sulphadimethoxine <i>N</i> ¹ -glucuronide	14
	Sulphadimethoxine	5
	<i>N</i> ⁴ -Acetylsulphadimethoxine	1
	Sulphadimethoxine <i>N</i> ⁴ -glucuronide	0.6
<i>N</i> ⁴ -Acetylsulphadimethoxine	Unchanged compound only	3
Succinylsulphathiazole	Unchanged drug only	43
Phthalylsulphathiazole	Unchanged drug only	11
Sulphadimethoxine <i>N</i> ¹ -glucuronide	Unchanged glucuronide only	78

* These gave the naphtharesorcinol reaction for glucuronic acid and a positive test for free aromatic amine.

Table 3. *Biliary excretion of sulphonamide drugs in rats*

The compounds (50 mg./kg.; R=R'=H unless otherwise indicated) were injected intraperitoneally in aqueous solution as sodium salts; sulphaguanidine was dissolved in propane-1,2-diol and sulphadimethoxine *N*¹-glucuronide was used as its ammonium salt.

No.	Compound	$\text{RNH}-\text{C}_6\text{H}_4-\text{SO}_2\text{NR}'\text{R}''$ $-\text{R}'$	pK_a	Mol.wt.	% of dose in bile in 24 hr.*		
					Free NH_2	Acetylated NH_2	Total NH_2
1	Sulphanilamide	-H	10.4	172	3 (1.7-3.7)	1 (0.4-1.4)	4 (2.1-4.7)
2	Sulphacetamide	-CO·CH ₃	5.4	214	See total	0	0.5 (0.3-0.7)
3	Sulphaguanidine	-C(:NH)·NH ₂	—	214	< 0.5	< 0.5	< 0.5
4	Sulphapyridine		8.4	249	7 (2-14)	4 (2-7)	11 (5-21)
5	Sulphadiazine		6.5	250	See total	0	2 (1.7-2.4)
6	Sulphathiazole		7.1	255	See total	0	1.3 (0.8-1.9)
7	Sulphisoxazole		—	267	See total	0	< 0.5
8	Sulphasomizole		5.0	269	See total	0	< 0.5
9	Sulphamethoxy- pyridazine		6.7	280	See total	0	8 (7.1-8.9)
10	<i>N</i> ⁴ -Acetyl- sulphathiazole†	See 6	—	297	0	See total	2.2 (0-3.6)
11	Sulphadimethoxine		6.3	310	9 (6-11) 19 (17-21)	1.8 (0.4-3.1) 1.3 (0.3-2.1)	11 (7-14) 20 (19-21)¶
12	<i>N</i> ⁴ -Acetylsulpha- dimethoxine†	See 11	—	352	0	See total	3.3 (2.8-3.5)
13	Succinylsulpha- thiazole‡	See 6	—	355	See total	0	43 (16-60)
14	Phthalylsulpha- thiazole§	See 6	—	403	See total	0	11 (6-18)
15	Sulphadimethoxine <i>N</i> ¹ -glucuronide	See 11	3.6	487	See total	0	78 (68-90)

* Mean values with ranges for three or more animals in parentheses, based on the determination of free aromatic NH_2 .

† R = -CO·CH₃.

‡ R = -CO·CH₂·CH₂·CO₂H.

§ R = -CO·C₆H₄·CO₂H(-o).

|| R' = C₆H₉O₆ and dose 100 mg./kg.

¶ Values for a 250 mg./kg. dose.

and its metabolites were cut out, and the material was eluted with 3% (w/v) NaOH (5–10 ml.). The eluates were acidified with 2N-HCl, diluted and assayed as described above.

Cannulation of rats. Female Wistar albino rats (weighing 200 ± 10 g.) were used and cannulated as described in a preceding paper (Abou-El-Makarem *et al.* 1967).

Chromatography. Bile samples (0.05–0.2 ml.) were chromatographed on Whatman no. 1 paper in three solvent systems and the compounds in bile were identified by comparison of their R_f values and colour reactions with those given by authentic reference compounds (see Table 1). The sulphonamides and their metabolites were detected by viewing the paper with u.v. light and by their colour reactions with *p*-dimethylaminocinnamaldehyde and naphtharesorcinol reagents (Table 1).

RESULTS AND DISCUSSION

The nature and the extent of excretion of the metabolites of 15 sulphonamide compounds in the bile in rats are given in Tables 2 and 3. If one considers the first 12 compounds in Table 3, i.e. from sulphanilamide up to and including *N*⁴-acetylsulphadimethoxine, it is seen that the extent of biliary excretion is low and less than 5% of the dose in all cases except sulphapyridine (11%), sulphamethoxypyridazine (8%) and sulphadimethoxine (11%). It was postulated in the preceding paper (Millburn *et al.* 1967) that for appreciable biliary excretion (i.e. 5–10% or more of the dose) the compound should be highly polar and of molecular weight not less than 325 ± 50 or could be converted into such a compound by metabolism. Sulphanilamide, sulphacetamide, sulphaguanidine, sulphadiazine, sulphathiazole, sulphisoxazole, sulphasomizole, *N*⁴-acetylsulphathiazole and *N*⁴-acetylsulphadimethoxine do not fit these criteria and are poorly excreted in the bile. For *N*⁴-acetylsulphadimethoxine the molecular weight, 352, is of the right order, but it is not sufficiently polar and it is not metabolized to a highly polar conjugate.

Sulphapyridine, sulphamethoxypyridazine and sulphadimethoxine, however, are partly metabolized to glucuronides (see Table 2). Such glucuronides would be highly polar and would have molecular weights of 441, 472 and 487 respectively, if they were monoglucuronides. The glucuronides of sulphapyridine (2-sulphanilamidopyrid-5-yl glucuronide; Scudi & Childress, 1956) and of sulphadimethoxine (sulphadimethoxine *N*¹-glucuronide; Bridges *et al.* 1965) are known. The formation of these glucuronides probably accounts for the higher biliary excretion of these three drugs compared with the other nine compounds referred to in the preceding paragraph.

The next two compounds in Table 3 are succinylsulphathiazole (I) and phthalylsulphathiazole. The first is highly excreted (43%) in the bile, and

the second has an appreciable excretion (11%). As shown in Table 2, neither of these compounds is metabolized and both are excreted unchanged in the bile. Their appreciable excretion in the bile in the unchanged state can be explained on the grounds that they meet the criteria for such a biliary excretion (Millburn *et al.* 1967). Their molecular weights (355 and 403) are of the right order and both are polar and contain a free carboxyl group. The lower biliary excretion of phthalylsulphathiazole compared with the succinyl derivative may be due to the very low solubility of the former, which may not be readily absorbed from the peritoneal cavity after injection.

The last compound in Table 3 is sulphadimethoxine *N*¹-glucuronide (II), a compound whose properties fit the criteria of molecular weight (487) and polarity (pK_a about 3.6) for high biliary excretion in the rat and whose biliary excretion was found to be 78% of the dose. This value should be compared with that of sulphadimethoxine, which is 10–20% of the dose (Table 3), but whose major metabolite in the bile is the *N*¹-glucuronide (Table 2). The extent of biliary excretion of sulphadimethoxine probably depends on the extent to which it is metabolized to the *N*¹-glucuronide.

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