Species Differences in the Metabolism and Excretion of Sulphasomidine and Sulphamethomidine

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1. The excretion of 2,4-dimethyl-6-sulphanilamidopyrimidine (sulphasomidine; Elkosin) and 4-methoxy-2-methyl-6-sulphanilamidopyrimidine (sulphamethomidine) given orally was examined in man, rhesus monkey, rabbit and rat. 2. About 70% of sulphasomidine (0.1 g./kg.) is excreted mainly unchanged in the urine by these species in 24 hr.; less than 15% of the dose is acetylated and there is no marked species difference in the fate of this drug. 3. Sulphamethomidine is excreted more slowly than sulphasomidine, and in the rat, rabbit and monkey the main metabolite is the *N*⁴-acetyl derivative. In man, only 20–30% of the dose is excreted in 24 hr. and nearly 70% of this is sulphamethomidine *N*¹-glucuronide, which is also excreted by the monkey but not by the rat or rabbit. There is therefore a marked species difference in the metabolism of sulphamethomidine. 4. Sulphamethomidine *N*¹-glucuronide was synthesized and shown to be identical with the glucuronide isolated from monkey urine. 5. Sulphasomidine, sulphamethomidine and sulphadimethoxine (2,4-dimethoxy-6-sulphanilamidopyrimidine) were acetylated by rabbit or monkey liver homogenates. Although sulphasomidine is poorly acetylated *in vivo*, it is acetylated *in vitro* at rates comparable with those of the other two drugs. 6. The solubilities, partition coefficients and plasma-protein-binding of the drugs were measured. 7. The results are discussed.

The long-acting sulphonamide drug sulphadimethoxine (2,4-dimethoxy-6-sulphanilamidopyrimidine) shows a remarkable species difference in metabolism (Bridges, Kibby, Walker & Williams, 1968) in that it is excreted in man and other primates, but not in several lower species, mainly as an *N*¹-glucuronide (Adamson, Bridges & Williams, 1966). It was shown that this formation of an *N*¹-glucuronide did not occur in man and the rhesus monkey if the positions of the methoxy groups were altered, for 2-, 4- or 5-methoxy- and 2,5- or 4,5-dimethoxy-6-sulphanilamidopyrimidines did not form appreciable amounts of *N*¹-glucuronide (Bridges, Kibby, Walker & Williams, 1969). It appeared therefore that formation of *N*¹-glucuronide was associated with the 2,4-disubstitution of the pyrimidine ring in this series of drugs. Two other drugs of this series containing 2,4-disubstituted pyrimidine rings have now been examined in man, monkey, rat and rabbit. These are 2,4-dimethyl-6-sulphanilamidopyrimidine (sulphasomidine; Elkosin), a short-acting antibacterial agent, and 4-methoxy-2-methyl-6-sulphanilamidopyrimidine (sulphamethomidine), a medium-long-acting drug in man (Cooper, Madoff & Weinstein, 1962). Sulphasomidine is water-soluble, and is well absorbed in man

and rapidly excreted mainly as the unchanged drug, the extent of acetylation being low (5–15% of the dose) (Prior & Saslow, 1951). The excretion of a glucuronide of sulphasomidine has been claimed (Walter, 1960). Sulphamethomidine is well absorbed in the rabbit and dog, but poorly so in the rat. It is excreted mainly as the acetyl derivative in the rabbit and rat (DiCarlo, Malament, Haynes & Phillips, 1962). In man, about 26% of an oral dose is excreted in the urine in 24 hr., mainly as a glucuronide, which is claimed to be a conjugate of a hydroxysulphamethomidine in which the hydroxyl group occurs in the pyrimidine ring (DiCarlo, Malament & Phillips, 1963). We have reported briefly (Bridges, Walker & Williams, 1967) that this glucuronide is an *N*¹-glucuronide similar to that formed in man from sulphadimethoxine (Bridges, Kibby & Williams, 1965). Sulphasomidine, on the other hand, does not form this type of glucuronide. In the present paper an attempt is made to relate these findings to the physical properties of these drugs.

MATERIALS AND METHODS

4-Methoxy-2-methyl-6-sulphanilamidopyrimidine (sulphamethomidine), m.p. 177–178°, was a gift from Warner-Lambert, Morris Plains, N.J., U.S.A. 2,4-Dimethyl-6-sulphanilamidopyrimidine (sulphasomidine;

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Elkoso), m.p. 240–241°, 2,4-dimethoxy-6-sulphanilamidopyrimidine (sulphadimethoxine; Madribon), m.p. 200–201°, and *N*⁴-acetylsulphadimethoxine, m.p. 210–211°, were given by Dr R. Long, Roche Products Ltd., Welwyn Garden City, Herts. 4-Amino-3-hydroxybenzenesulphonic acid, m.p. 266° (decomp.), and 4-amino-2-hydroxybenzenesulphonic acid, m.p. 275° (decomp.), were given by May and Baker Ltd., Dagenham, Essex. Sulphanilic acid, m.p. 288° (decomp.), sulphanilamide, m.p. 164–166°, and barbituric acid, m.p. 245°, were purchased (Hopkin and Williams Ltd., Chadwell Heath, Essex). Sulphadimethoxine *N*¹-glucuronide (ammonium salt), m.p. 150–160° (decomp.), and sulphadimethoxine *N*⁴-glucuronide (sodium salt), m.p. 149° (decomp.), were synthesized as described by Bridges *et al.* (1965).

Sulphamethomidine *N*¹-glucuronide. Sodium sulphamethomidine (3 g.) and methyl 2,3,4-tri-*O*-acetyl-1-bromoglucuronate (3.8 g.), m.p. 106–109° (Bollenback, Long, Benjamin & Lindquist, 1955), were dissolved in a water (6 ml.) and acetone (25 ml.) mixture and kept at 37° for 4 hr. The resulting dark-red solution was then kept overnight at 0°. It was then treated with propan-2-ol (12 ml.) followed by water (100 ml.) and the tri-*O*-acetyl methyl ester of sulphamethomidine *N*¹-glucuronide separated. This was filtered and sucked dry to a buff powder (3.2 g.), m.p. 115–122°. The ester in acetone (5 ml.) was then passed through a column (2.5 cm. × 20 cm.) of activated alumina (type H; 100–200 mesh) (P. Spence Ltd., Widnes, Lancs.) and eluted with acetone. The first 50 ml. was collected and evaporated to dryness, and the product recrystallized from propan-2-ol to give methyl 2,3,4-tri-*O*-acetyl-1-deoxy-1-[*N*¹-(4-methoxy-2-methylpyrimidin-6-yl)sulphanilamidoglucosid]uronate as white needles, m.p. 108–110° and $[\alpha]_D^{20} + 67^\circ$ (c 1 in methanol) (Found: C, 49.5; H, 5.1; N, 8.8; S, 5.0; C₂₅H₃₀N₄O₁₂S requires C, 49.2; H, 4.9; N, 9.2; S, 5.2%). The above ester (1 g.) in methanol (25 ml.) was treated with 0.5 *N*-barium methoxide in methanol (2.5 ml.) and the mixture kept for 3 days at 0°. Then 2 *N*-H₂SO₄ (0.5 ml.) and water (12.5 ml.) were added, and the solution was passed through a column (2.5 cm. × 10 cm.) of Amberlite CG-120 (H⁺ form; 100–200 mesh) (British Drug Houses Ltd., Poole, Dorset). The column was washed with water (10 ml.) and then eluted with 2 *N*-ammonia (75 ml.) and finally water (50 ml.). The combined eluates were evaporated at 45° *in vacuo* to a white solid. This was dissolved in methanol (5 ml.) and the solution banded on a thin-layer plate of fluorescent silica gel, which was irrigated with propan-1-ol-aq. ammonia (sp.gr. 0.88) (7:3 v/v) for 3 hr. The band corresponding to sulphamethomidine *N*¹-glucuronide was eluted with 0.2 *N*-ammonia and the eluate evaporated to dryness at 45° *in vacuo*. This process was repeated and the ammonium 1-deoxy-1-[*N*¹-(4-methoxy-2-methylpyrimidin-6-yl)sulphanilamidoglucosid]uronate was recrystallized from methanol to give white needles, m.p. 140–150° (decomp.) and $[\alpha]_D^{20} + 4.0^\circ$ (c 5 in water) (Found: C, 42.5; H, 5.1; N, 13.8; S, 6.6; C₁₈H₂₅N₅O₉S, H₂O requires C, 42.8; H, 5.3; N, 13.9; S, 6.3%).

***N*⁴-Acetylsulphamethomidine**, m.p. 218–220°, from 25% (v/v) acetic acid, and ***N*⁴-acetylsulphasomidine**, m.p. 295°, by precipitation from dimethyl sulphoxide solution with water, were prepared by standard methods with acetic acid and acetic anhydride.

Sulphamethomidine *N*⁴-glucuronide. Sulphamethomidine (1.1 g.) in dimethylformamide (10 ml.) was added to a solu-

tion of sodium glucuronate (0.6 g.) in ethylene glycol (20 ml.). The mixture was heated for 10 min. at 70° and then kept in the dark for 24 hr. at room temperature. On addition of excess of acetone a gel formed, so sufficient water to just dissolve the gel was added. The solution was then kept for several days at room temperature and white crystals, m.p. 156–157°, of the sodium salt of sulphamethomidine *N*⁴-glucuronide separated. The compound, which readily reduced Benedict's solution and Fehling's solution on warming, was very labile and was not obtained entirely free of sulphamethomidine. It could, however, be used for chromatographic purposes (see Table 1) and was easily distinguishable from sulphamethomidine *N*¹-glucuronide on paper.

Sulphasomidine *N*⁴-glucuronide and the *N*⁴-sulphates of sulphamethomidine and sulphasomidine were prepared by the methods of Bridges *et al.* (1968, 1969). They were difficult to purify, but could be used to determine their *R*_F values (see Table 1).

Chromatography. The *R*_F values and colour reactions of sulphamethomidine and sulphasomidine and their derivatives are shown in Table 1.

Spectra. The Perkin-Elmer Infracord and Unicam SP. 500 spectrophotometers were used.

Quantitative methods. Free and total amines in urine and bile and metabolites separated on paper chromatograms were estimated essentially as described by Bridges *et al.* (1968).

Animals. Wistar albino rats (Porton strain), New Zealand white rabbits and rhesus monkeys were used. The drugs were given orally. Monkeys received the powdered drugs concealed in honey, rabbits received them by stomach tube suspended in water made slightly alkaline with NaHCO₃ and containing a little bile salt, and rats received them by stomach tube suspended in aq. 0.5% CM-cellulose (1 ml.). Human subjects took the powdered drug in rice-paper sachets. The bile ducts of rats were cannulated as described by Abou-El-Makarem, Millburn, Smith & Williams (1967).

Isolation of metabolites. (a) *N*⁴-Acetylsulphamethomidine. The 24 hr. urine (185 ml.) of a rabbit (5 kg.) that had received sulphamethomidine (0.5 g.) was brought to pH 5.5 with *N*-HCl and then shaken with ethyl acetate (400 ml.). The extract was evaporated at 45° to a paste, which was then dissolved in *m*-Na₂CO₃ (10 ml.). The solution was treated with 2 *N*-HCl (10 ml.) and the solid that formed was collected and dissolved in a little aqueous ethanol. The solution was banded on a thin-layer plate (fluorescent silica gel) and developed for 3 hr. in solvent *B* (Table 1). The area containing the *N*⁴-acetyl compound was located and the metabolite extracted with 0.2 *N*-ammonia. The extract was evaporated at 45° *in vacuo* and the *N*⁴-acetylsulphamethomidine (45 mg.) had m.p. and mixed m.p. 218–220° after recrystallization from 25% acetic acid. It was characterized by *R*_F values and i.r. spectrum.

(b) Sulphamethomidine *N*¹-glucuronide. The 24 hr. urine (300 ml.) of a monkey given the drug (0.5 g.) was concentrated to 50 ml. in a rotary evaporator at 45° *in vacuo*. Ethanol (50 ml.) containing 2 *N*-ammonia (5%, v/v) was added and the solution, filtered from solid material, was freeze-dried. The residue in a little aqueous ethanol was banded on Whatman 3MM paper and chromatographed in solvent *B* (Table 1) for 12 hr. The area containing the *N*¹-glucuronide was cut out and eluted with 0.2 *N*-ammonia. The chromatography and elution was repeated. The eluate

Table 1. *R_F* values and colour reactions of sulphasomidine and sulphamethomidine and related compounds

Whatman no. 1 paper was used. The solvents (descending) were: *A*, butan-1-ol-aq. ammonia (sp.gr. 0.88)-water (10:1:1, by vol.); *B*, propan-1-ol-aq. ammonia (sp.gr. 0.88) (7:3, v/v); *C*, butan-1-ol-water-acetic acid (4:2:1, by vol.), run for 12 hr. DMAC, *p*-Dimethylaminocinnamaldehyde; NR, naphtharesorcinol spray (see Bridges *et al.* 1965). — Means no colour reaction; q means quenching of background fluorescence of paper.

Compound	<i>R_F</i> values			Colour reactions		Fluorescence in 254m μ
	Solvent <i>A</i>	Solvent <i>B</i>	Solvent <i>C</i>	DMAC	NR	
Sulphasomidine (SS)	0.45	0.66	0.92	Purple	—	q
<i>N</i> ⁴ -Acetyl-SS	0.60	0.79	0.93	— (Purple after 24 hr.)	—	q
SS <i>N</i> ⁴ -sulphate (potassium salt)	0.05	0.30	0.53	Purple after 10 min.	—	q
SS <i>N</i> ⁴ -glucuronide (sodium salt)	0.00	0.15	decomp.	Purple	Blue	q
Sulphamethomidine (SM)	0.50	0.65	0.92	Purple	—	q
<i>N</i> ⁴ -Acetyl-SM	0.62	0.76	0.94	— (Purple after 24 hr.)	—	q
SM <i>N</i> ⁴ -sulphate (potassium salt)	0.05	0.32	0.54	Purple after 10 min.	—	q
SM <i>N</i> ⁴ -glucuronide (sodium salt)	0.00	0.12	decomp.	Purple	Blue	q
SM <i>N</i> ¹ -glucuronide (ammonium salt)	0.10	0.39	0.63	Purple	Blue-brown	q
Sulphanilic acid	0.15	0.63	0.41	Red	—	Violet
Sulphanilamide	0.63	0.82	0.76	Red	—	Violet
Barbituric acid	0.09	—	0.54	—	—	q
4-Amino-3-hydroxybenzene-sulphonic acid	0.06	0.36	0.35	Red	—	Violet
4-Amino-2-hydroxybenzene-sulphonic acid	0.11	0.58	0.41	Red	—	Violet
Glucuronic acid (sodium salt)	0.00	0.12	0.23	—	Blue	q

was evaporated to dryness at 45° *in vacuo* and the solid recrystallized from aqueous ethanol. The sulphamethomidine *N*¹-glucuronide was obtained as white needles (8mg.), m.p. 140–150° (decomp.). It was not quite pure, but its chromatographic properties, colour reactions and u.v. and i.r. spectra were in agreement with those of the synthetic compound. It did not reduce Fehling's solution or Benedict's solution.

Hydrolysis of sulphamethomidine and its metabolites. The drug or its *N*⁴-acetyl derivative (0.5g.) dissolved in ethanol (5ml.) was mixed with 2*N*-HCl (5ml.) and heated at 100° for 1hr. The residual solution (2ml.) was placed as a band (10cm.) on Whatman 3MM paper and chromatographed with solvent *A*, *B* or *C* (Table 1). Three main products were detected, namely sulphamethomidine, sulphanilic acid and an unknown suspected to contain the pyrimidine moiety. The areas corresponding to sulphanilic acid and the unknown were eluted with 2*N*-ammonia. The eluates were evaporated to dryness at 45° *in vacuo* and i.r. spectra of the residues examined. The sulphanilic acid residue, m.p. 288° (decomp.), was identified but not the unknown.

Aqueous solutions of biosynthetic or synthetic sulphamethomidine *N*¹-glucuronide (1mg./ml.) were heated with an equal volume of 2*N*-HCl at 100° for 1hr. The hydrolysates were then chromatographed as above and the products identified by *R_F* values and colour reactions.

An aqueous solution (0.8ml.) of the ammonium salt of synthetic sulphamethomidine *N*¹-glucuronide (1mg./ml.) free of sulphamethomidine (by chromatography) was added to each of three tubes. To one tube was added 1000 Fishman units (Fishman & Barnfeld, 1955) of β -glucuronidase (Ketodase; William R. Warner and Co., Morris Plains, N.J., U.S.A.) in sodium acetate buffer, pH 5 (0.2ml.) To the second was added the same amount of boiled enzyme and

to the third 0.2ml. of water. The tubes were plugged and incubated for 24hr. at 37°. The contents of the tubes were then chromatographed in solvents *A*, *B* and *C* (Table 1). The experiment was repeated with the biosynthetic glucuronide.

***pK_a*, solubility and partition coefficients.** These were determined as described in the preceding paper (Bridges *et al.* 1969).

Acetylation by liver homogenates. The rates of acetylation of the drugs by liver homogenates were measured as described by Bridges *et al.* (1969).

Protein-binding. Fresh human blood (40ml.) mixed with heparin (0.4ml. or 2000 units; Weddel Pharmaceuticals, London, E.C.1.) was centrifuged at 2000*g* for 10min. The sulphonamide drug (2.4mg.) was dissolved in the plasma (20ml.) and 4 ml. of 0.1*M*-citrate-0.2*M*-Na₂HPO₄ buffer, pH 7.4, added. The resulting solution (5ml.) was placed in a bag made of cellophane tube tied at both ends (15cm. $\times$ 3cm. flat width). The bag was placed with a flat surface against a sintered-glass disk (20mm. diam.; porosity 1; Pyrex Co., Sunderland) near the bottom of a polythene centrifuge tube (10cm. $\times$ 2.7cm.). The tube was centrifuged at 3000*g* for 2hr. at 18°. The contents of the bag were analysed for total drug (bound and unbound) by adding 20% (w/v) trichloroacetic acid (1ml.) to the plasma (1ml.), then centrifuging at 2000*g* for 5min. and analysing the supernatant by the Bratton & Marshall (1939) method. The ultrafiltrate below the sintered-glass disk in the polythene tube was analysed for unbound drug by the Bratton & Marshall (1939) method.

RESULTS AND DISCUSSION

The excretion of total amine after the administration of 2,4-dimethyl-6-sulphanilamidopyrimidine

(sulphasomidine) and 4-methoxy-2-methyl-6-sulphanilamidopyrimidine (sulphamethomidine) is shown in Table 2. In the four species studied, sulphasomidine and its metabolites are more rapidly excreted in the urine than are those of sulphamethomidine, since about 70% of the former is excreted in 24 hr. and about 80% in 2 days, whereas with the latter drug the 24 hr. excretion is 20–30% in man, 40–50% in the rat, 50–60% in the monkey, but nearly 70% in the rabbit. The nature of the metabolites of the two drugs in urine is shown in Table 3. Sulphasomidine is largely excreted unchanged in the four species, for more than

70% of the 24 hr. excretion is unchanged drug and less than 20% is *N*⁴-acetylated, the other metabolites, i.e. *N*⁴-sulphate and *N*⁴-glucuronide, being of little or no importance. With sulphamethomidine, however, the proportion of unchanged drug excreted is less than 20% of the 24 hr. excretion and the major metabolite in the rat, rabbit and monkey is *N*⁴-acetylsulphamethomidine (60–80% of the 24 hr. excretion). However, in man the acetyl derivative is relatively minor (16%) and the major metabolite is now the *N*¹-glucuronide (68% of the 24 hr. excretion). This glucuronide is also an important metabolite in the monkey (32%), but it is quantitatively less than the *N*⁴-acetyl compound (59%). In the rabbit and rat, no *N*¹-glucuronide was detected in the urine. Small amounts of the *N*⁴-sulphate of sulphamethomidine were found in rat, monkey and human urine.

It appears that, in the four species studied, there is no marked difference in the metabolism and excretion of sulphasomidine (I; R₂=R₄=CH₃) and, although this drug is substituted with methyl groups in the 2- and 4-positions of the pyrimidine ring, there is no formation of *N*¹-glucuronide in man and the monkey as with sulphadimethoxine (I; R₂=R₄=O·CH₃), which has methoxyl groups in these positions (Bridges *et al.* 1968). However,

Table 2. *Excretion of total amine after the administration of sulphasomidine and sulphamethomidine to various species*

The drugs were administered orally (dose 100 mg./kg., except in man where the dose was 30 mg./kg.). The urine was analysed daily for total aromatic amine by the Bratton & Marshall (1939) method (see the text). The results are averages with ranges in parentheses. The number of animals used and their sex (M, male; F, female) are given in parentheses in the first column.

Species	Day after dosing	% of dose excreted after	
		Sulphasomidine	Sulphamethomidine
Rat (3F)	1	77 (70–87)	41 (36–49)
	2	80 (75–90)	49 (43–59)
Rabbit (3F)	1	76 (66–85)	69 (60–78)
	2	80 (70–89)	74 (65–83)
Monkey (6F)	1	63 (58–68)	54 (43–69)
	2	68 (62–73)	58 (50–72)
Man (2M)	1	74, 70	29, 22
	2	86, 80	54, 41

Table 3. *Metabolites of sulphasomidine and sulphamethomidine in urine in various species*

The metabolites of sulphasomidine (SS) and sulphamethomidine (SM) were separated chromatographically and determined as described in the text. The urine collected during the first 24 hr. after dosing was analysed and the amount of each metabolite is expressed as a percentage of the amount excreted in 24 hr. The amount excreted in 24 hr. is given in Table 2. The values are the averages for three animals.

		% of 24 hr. excretion in							
Metabolite	Drug given	Rat		Rabbit		Monkey		Man*	
		SS	SM	SS	SM	SS	SM	SS	SM
Unchanged drug		72	19	82	16	87	5	96	13
<i>N</i> ⁴ -Acetyl		19	79	15	75	11	59	4	16
<i>N</i> ¹ -Glucuronide		0	0	0	0	0	32	0	68
<i>N</i> ⁴ -Glucuronide		6	1	0	9	0	0	0	0
<i>N</i> ⁴ -Sulphate		3	1	3	0	2	4	0	3

* Two subjects.

Table 4. *Acid and enzymic hydrolysis of sulphamethomidine and its metabolites*

The compounds were hydrolysed as described in the text and the products identified by chromatography and colour reactions.

Compound	Hydrolysing agent	R_F values of products in solvent A†	Nature of product
Sulphamethomidine or <i>N</i> ⁴ -acetylsulphamethomidine	N-HCl	0.15	Sulphanilic acid
		0.50	Sulphamethomidine
		0.86	Unknown‡
Sulphamethomidine <i>N</i> ¹ -glucuronide, synthetic or biosynthetic	N-HCl	0.15	Sulphanilic acid
		0.00	Glucuronic acid
		0.86	Unknown‡
	β -Glucuronidase*	0.50	Sulphamethomidine
		0.00	Glucuronic acid
		0.10	Sulphamethomidine <i>N</i> ¹ -glucuronide (small amounts)

* The control tubes gave one spot, R_F 0.10 in solvent A, R_F 0.39 in solvent B and R_F 0.63 in solvent C, i.e. the unchanged glucuronide.

† See Table 1. Solvents B and C were also used to confirm the identifications.

‡ The unknown spot is probably that of the pyrimidine moiety which showed up on paper as a dark spot in u.v. light.

Table 5. *Acetylation of sulphasomidine and related drugs by liver homogenates*

The incubation mixture consisted of 0.3 μ M-drug in 1 ml. of 0.1 M-citrate-0.2 M- Na_2HPO_4 buffer, pH 7.4, and the 2000g supernatant of liver homogenate equivalent to 330 mg. of liver in 1 ml. of 0.25 M-sucrose (see the text). Averages for three animals are given with ranges in parentheses.

Compound	Drug acetylated (μ mole/g. of liver/hr.)	
	Rabbit	Monkey
Sulphasomidine	0.190 (0.165-0.225)	0.175 (0.130-0.205)
Sulphamethomidine	0.165 (0.130-0.235)	0.195 (0.17-0.24)
Sulphadimethoxine	0.235 (0.20-0.27)	0.165 (0.140-0.205)

with sulphamethomidine (I; $\text{R}_2=\text{CH}_3$, $\text{R}_4=\text{O}\cdot\text{CH}_3$), which has a methyl group in position 2 and a methoxyl group in position 4, a marked species difference again appears, for *N*¹-glucuronide formation occurs in man and the monkey but not in the rat and rabbit. With 2-methoxy-6-sulphanilamidopyrimidine (I; $\text{R}_2=\text{O}\cdot\text{CH}_3$, $\text{R}_4=\text{H}$) and sulphamonomethoxine (I; $\text{R}_2=\text{H}$, $\text{R}_4=\text{O}\cdot\text{CH}_3$), in which one methoxyl group occurs in the 2- or the 4-position respectively, formation of *N*¹-glucuronide does not occur (Bridges *et al.* 1969).

Sulphamethomidine *N*¹-glucuronide is slowly hydrolysed by β -glucuronidase to sulphamethomidine and glucuronic acid (Table 4) and by N-hydrochloric acid to sulphanilic acid, glucuronic acid and an unidentified pyrimidine derivative. Its infrared spectrum shows an SO_2 stretching frequency at 1160cm^{-1} and therefore, by using the same arguments as Bridges *et al.* (1965) for sulphadimethoxine *N*¹-glucuronide, it is suggested that the glucuronic acid residue in the sulphamethomi-

dine glucuronide formed in man and the monkey is attached to the sulphonamide nitrogen atom. The 2- and 3-hydroxysulphanilic acids (see Table 1) were not found as acid-hydrolysis products, and β -glucuronidase converted the glucuronide into sulphamethomidine, and therefore it is concluded that the glucuronide was not an *O*-glucuronide of a hydroxy-sulphamethomidine in which the hydroxyl group is somewhere in the pyrimidine moiety (cf. DiCarlo *et al.* 1963). The glucuronide is easily distinguishable from the unstable and reducing *N*⁴-glucuronide (see Table 1).

Biliary excretion. The biliary excretion of sulphasomidine and sulphamethomidine and their acetyl derivatives was examined in rats. The compounds were injected intraperitoneally into biliary-cannulated rats at a dose of 100 mg./kg. With sulphasomidine (mol.wt. 278) 6.5% appeared in the bile in 24 hr. (2.1% free and 4.4% acetylated), whereas with *N*⁴-acetylsulphasomidine (mol.wt. 320) 4.6% appeared in the bile unchanged. With

Table 6. pK_a , solubility and distribution coefficients of some sulphonamide drugs

Solubility was determined in 0.1 M-citrate-0.2 M- Na_2HPO_4 buffer, pH 7.4. Partition coefficients were between chloroform and the same buffer. pK_a values were determined as described by Krebs & Speakman (1945).

Compound	pK_a	Solubility in buffer, pH 7.4 (mg./l.)	Partition coefficient $\text{CHCl}_3/\text{buffer}$
Sulphasomidine	7.62	1615	0.15
Sulphamethomidine	7.04	843	1.00
Sulphadimethoxine	6.26	343	5.25
N^4 -Acetylsulphasomidine		44	0.10
N^4 -Acetylsulphamethomidine		260	0.18
N^4 -Acetylsulphadimethoxine		190	0.38

Table 7. Binding of sulphonamide drugs in human plasma

See the text for the method. The substrate concentration was 100 $\mu\text{g./ml.}$

Substrate	% bound
Sulphasomidine	66
Sulphamethomidine	90
Sulphadimethoxine	90
N^4 -Acetylsulphasomidine	78
N^4 -Acetylsulphamethomidine	88
N^4 -Acetylsulphadimethoxine*	87
Sulphamethomidine N^1 -glucuronide	28
Sulphadimethoxine N^1 -glucuronide	33

* Concentration 33 $\mu\text{g./ml.}$

sulphamethomidine (mol.wt. 294) 6.5% also appeared in the bile in 24 hr., made up of 1.3% of unchanged drug, 3.3% of N^4 -acetyl derivative, 0.3% of N^4 -glucuronide and 1.6% of N^1 -glucuronide. Thus in the rat, although no N^1 -glucuronide appears in the urine, a small amount is formed and excreted in the bile. N^4 -Acetylsulphamethomidine (mol.wt. 336) appeared in the bile to the extent of 4% of the dose as the unchanged compound. The values given for the biliary excretion of these compounds are averages for three animals. The biliary excretion of these compounds in the rat is of a low order, and this would have been expected on the hypotheses put forward by Millburn, Smith & Williams (1967) on polarity and molecular weight and the extent of biliary excretion.

Acetylation. Sulphasomidine is poorly acetylated *in vivo* whereas sulphamethomidine is well acetylated in the rat, rabbit and monkey (Table 3). However, *in vitro* sulphasomidine is acetylated in rabbit or monkey liver homogenates at a rate not very different from that of sulphamethomidine or sulphadimethoxine (see Table 5). It would appear that *in vivo* sulphasomidine may be excreted at a rate that does not allow extensive acetylation. At pH 7.4, sulphasomidine is twice as soluble as sulpha-

methomidine and nearly five times as soluble as sulphadimethoxine (see Table 6) in phosphate buffer, whereas its distribution coefficient (chloroform/buffer, pH 7.4) is only 15% of that of sulphamethomidine and about 3% of that of sulphadimethoxine (Table 6). Sulphasomidine is thus more water-soluble and less lipid-soluble at pH 7.4 than the other two drugs, and therefore would have less tendency to be reabsorbed by the renal tubules, whereas the other two would be more readily reabsorbed and undergo further metabolism. The N^4 -acetyl derivatives of the three drugs are relatively poorly lipid-soluble and would tend to be excreted once formed.

The extent of the binding of the drugs and some of their metabolites to human plasma proteins *in vitro* is shown in Table 7. Compared with sulphamethomidine (90%) and sulphadimethoxine (90%), sulphasomidine (66%) is less bound to protein and this suggests that more is immediately available for excretion by the kidney. It is to be noted that the N^1 -glucuronides are poorly bound whereas the N^4 -acetyl derivatives are relatively highly bound.

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