Species Differences in the Metabolism of Sulphadimethoxine

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1. The fate of sulphadimethoxine (2,4-dimethoxy-6-sulphanilamidopyrimidine) was studied in man, rhesus monkey, dog, rat, guinea pig and rabbit. 2. About 20–46% of the dose (0.1 g./kg.) of the drug is excreted in the urine in 24 hr. in these species, except the rat, in which only 13% is excreted. 3. In man and the monkey sulphadimethoxine N^1 -glucuronide is the major metabolite in the urine. In the rabbit and guinea pig N^4 -acetylsulphadimethoxine is the main metabolite. In the dog the drug is excreted mainly unchanged. In the rat equal amounts of the unchanged drug and its N^4 -acetyl derivative are the main products. 4. Small amounts of sulphadimethoxine N^4 -glucuronide are found in the urine of all the species. Sulphadimethoxine N^1 -glucuronide occurs in small amounts in the urine of rat, dog and guinea pig; none is found in rabbit urine. 5. Sulphadimethoxine N^4 -sulphate was synthesized and found to occur in small amounts in rat urine. 6. Monkey liver homogenates fortified with UDP-glucuronic acid are able to synthesize sulphadimethoxine N^1 -glucuronide with the drug as substrate. Rat liver has also this ability to a slight extent, but rabbit liver is unable to do so. 7. Sulphadimethoxine N^4 -glucuronide is formed spontaneously when the drug is added to human urine. 8. The biliary excretion of the drug and its metabolites was examined in rats. The drug is excreted in rat bile mainly as the N^1 -glucuronide. The N^1 - and N^4 -glucuronides administered as such are extensively excreted in the bile by rats.

The major metabolite of the long-acting sulphonamide drug sulphadimethoxine (2,4-dimethoxy-6-sulphanilamidopyrimidine; Madribon) in human urine has been shown to be an N -glucuronide (Koechlin, Kern & Engelberg, 1959), the structure of which was shown by Bridges, Kibby & Williams (1965) to be 1-deoxy-1-[N^1 -(2,4-dimethoxypyrimidin-6-yl)sulphanilamidoglucosid]uronic acid, i.e. sulphadimethoxine N^1 -glucuronide. This glucuronide also appears to be the major metabolite of the drug in the urine of some Old and New World monkeys and lemurs and in a tree shrew (Adamson, Bridges & Williams, 1966).

In the present paper the fate of the drug in man, rhesus monkey, dog, rat, guinea pig and rabbit is described. Although this N^1 -glucuronide is a major metabolite in man and the rhesus monkey, it is not in the other four species.

EXPERIMENTAL

Materials. Sulphadimethoxine, m.p. 200–201°, [^{35}S]-sulphadimethoxine (specific radioactivity 35 $\mu\text{C}/\text{mg.}$) and

N^4 -acetylsulphadimethoxine, m.p. 210–211°, were gifts from Roche Products Ltd., Welwyn Garden City, Herts. The ammonium salt of sulphadimethoxine N^1 -glucuronide, m.p. 150–160° (decomp.) and $[\alpha]_{\text{D}}^{25} + 4.5^\circ$ (c 5 in water), and the sodium salt of sulphadimethoxine N^4 -glucuronide, m.p. 149°, were synthesized by the methods described by Bridges *et al.* (1965).

Sulphadimethoxine N^4 -sulphate. Chlorosulphonic acid (2 g.) was added slowly with stirring to dry pyridine (25 ml.) cooled in ice. After 30 min. sulphadimethoxine (6.24 g.) was added and the mixture shaken vigorously until the drug had dissolved. The solution was kept in the dark for 24 hr. and then 0.4 N -KOH (100 ml.) added. The pyridine present was extracted with ether (4 $\times$ 500 ml.) and the aqueous residue evaporated at 45° under diminished pressure to yield a white solid (6.8 g.). This was dissolved in the minimum of water (12 ml.) and acetone (100 ml.) was added. The precipitated inorganic salts were filtered off and the filtrate was evaporated under diminished pressure to yield a white solid residue (2.8 g.), which was recrystallized from ethanol. Dipotassium 4-(2,4-dimethoxypyrimidin-6-yl)-sulphamoylphenyl sulphamate (dipotassium salt of sulphadimethoxine N^4 -sulphate) formed very water-soluble white platelets [Found: C, 29.9; H, 3.0; K, 16.1; N, 11.5; H_2O , 3.5% (loss in *vacuo* at 110°). $\text{C}_{12}\text{H}_{12}\text{K}_2\text{N}_4\text{O}_7\text{S}_2 \cdot \text{H}_2\text{O}$ requires C, 29.7; H, 2.9; K, 16.1; N, 11.6; H_2O , 3.7%]. An acidified solution of the salt gave no test for inorganic sulphate with $\text{Ba}(\text{NO}_3)_2$ until boiled. When diazotized and coupled with ethanolic 0.1% N^1 - α -naphthylethylenediamine it gave a red colour, indicating removal of the sulphonate group on diazotization. On paper it gave no immediate colour with

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a spray of 0.2% *p*-dimethylaminocinnamaldehyde in 0.1N-HCl, but after keeping the paper for 30 min. a purple spot developed. This last test showed that it did not contain a free amino group until the sulphonate group had been removed by acid.

Determination of free and total aromatic amine (Bratton & Marshall, 1939). (a) In urine and bile. Urine or bile (containing 0.01–10 mg. of amine/100 ml.) was diluted with water to contain 10–30 μ g. of sulphadimethoxine/ml. 'Free amine', which includes the free drug, the N^1 - and N^4 -glucuronide and N^4 -sulphate of sulphadimethoxine (the N^4 -glucuronide and N^4 -sulphate groups are removed during diazotization), was determined directly by the Bratton & Marshall (1939) method. The recovery of the drug or its N^1 -glucuronide (2–20 mg./100 ml.) added to urine or bile was 99–100%. For total aromatic amine the diluted urine or bile (1 ml.) was mixed with 2N-HCl (1 ml.) and heated on the boiling-water bath for 20 min., cooled and the amine determined as before. The recovery of N^4 -acetylsulphadimethoxine added to urine (10 mg./100 ml.) was 99%.

(b) In faeces. Faeces (5 g.) were homogenized with 10% NaOH (10 ml.) and centrifuged and the supernatant was analysed for free and total amine as above. The recovery of sulphadimethoxine added to faeces (0.1–10 mg./g. of wet faeces) was 66–92%.

Determination of metabolites on chromatograms. Urine or bile (0.3–2 ml.) was placed as a thin horizontal band across a 6 in.-wide piece of Whatman no. 1 paper, but leaving sufficient space for reference samples of the possible metabolites to be run at the same time. The chromatogram was developed in solvent A (Table 1) for 17 hr. by the descending technique. Strips corresponding to the reference spots (detected by u.v. light, or *p*-dimethylaminocinnamaldehyde and naphtharesorcinol sprays; see Bridges *et al.* 1965) were cut out and eluted with 0.02N-ammonia. The eluates were made up to known volumes with water and free and total amines determined as above. In experiments with [35 S]sulphadimethoxine, 0.1 ml. of urine was banded (1 in. band) on strips of Whatman no. 1 paper (1.5 in. wide) and chromatographed as above. The amount of each metabolite was estimated by running the strip through a Packard radiochromatogram scanner (model no. 7200).

Chromatography. The R_F values and colour reactions (Bridges *et al.* 1965) of sulphadimethoxine and its possible metabolites are given in Table 1.

Animals. The animals used were Wistar albino rats, Chinchilla rabbits, English guinea pigs, mongrel dogs and rhesus monkeys. The human subjects were healthy male volunteers aged 18–26 years. Rats and rabbits were biliary-cannulated as described by Abou-El-Makarem, Millburn, Smith & Williams (1967) and bile was collected for 24 hr. Human subjects took the drug in tablet form. Monkeys received the powdered drug concealed in fruit or honey and dogs received tablets concealed in food. Guinea pigs and rabbits were given the powdered drug suspended in water by stomach tube, and rats were given it suspended in propane-1,2-diol. For intraperitoneal injection in rats the drug was dissolved in warm propane-1,2-diol.

Spontaneous formation of sulphadimethoxine N^4 -glucuronide in urine. Sulphadimethoxine (2–20 mg.) was dissolved in freshly voided normal human urine (100 ml.). Samples (0.5–3.0 ml.) of the urine (pH 6.0–6.5) were immediately banded on Whatman 3MM paper, dried and chromatographed in solvent A (Table 1) against reference spots of

sulphadimethoxine and sulphadimethoxine N^4 -glucuronide. Portions of the chromatogram corresponding to these spots were cut from the paper, eluted with 0.02N-ammonia and the eluates assayed for aromatic amine by the Bratton & Marshall (1939) method.

Synthesis of sulphadimethoxine N^1 -glucuronide by monkey liver. The livers of killed rhesus monkeys were rapidly removed and placed on ice. Portions of the liver (10 g.) were homogenized with ice-cold 0.25M-sucrose (30 ml.) in a Potter tube with a Teflon pestle and centrifuged at either 2000g or 10000g for 10 min. in an MSE Superspeed 40 centrifuge (Measuring and Scientific Equipment Ltd., London, S.W. 1). Liver slices were cut by hand and used immediately. Incubation mixtures consisted of liver homogenate (1 ml.), [35 S]sulphadimethoxine (1.3 μ moles in 0.1 ml. of water containing a little 2N-NaOH to dissolve the drug), 0.5M-tris-HCl buffer, pH 7.4 (0.2 ml.), UDP-glucuronic acid (7 μ moles in 0.4 ml. of water) and water (0.3 ml.) in unstoppered centrifuge tubes. Control tubes contained either boiled liver homogenate or sodium glucuronate (7 μ moles in 1 ml. of water) instead of liver. Additional tubes containing *o*-aminophenol in place of [35 S]sulphadimethoxine were also run. All tubes were incubated at 37° in a shaking water bath (H. Mickle Ltd., Gomshall, Surrey) for 1 hr. The contents of the tubes containing *o*-aminophenol were analysed for *o*-aminophenyl glucuronide (Levy & Storey, 1949). The tubes containing sulphadimethoxine were heated in a boiling-water bath for 1 min., cooled in running water and centrifuged at 2000g for 10 min. Samples (0.1 ml.) of the supernatant were withdrawn, banded on a strip of Whatman no. 1 paper, chromatographed in solvent A (Table 1) and the 35 S was estimated with the radiochromatogram scanner. The peak corresponding to sulphadimethoxine N^1 -glucuronide was cut from the paper, eluted with 0.02N-ammonia, banded on Whatman no. 1 paper, rechromatographed in solvent B, C or D (see Table 1) and estimated for N^1 -glucuronide content in the radiochromatogram scanner (see Table 6). In some experiments the incubation mixture (1 ml.) was extracted with ethyl acetate saturated with water (2 $\times$ 2.5 ml.) to remove free sulphadimethoxine. The residual solution (0.1 ml.) was chromatographed in solvent A or B and the N^1 -glucuronide present determined with the chromatogram scanner. Similar incubations were carried out with rat or rabbit liver homogenates. No N^1 -glucuronide was found with rabbit liver, but with rat liver the N^1 -glucuronide was detected on chromatograms, cut out and counted on the paper in dioxan in a Packard Tri-Carb scintillation spectrometer (model 3214).

RESULTS AND DISCUSSION

Isolation of metabolites. N^4 -Acetylsulphadimethoxine (m.p. 207–208°, mixed m.p. 208–210°) was isolated from rabbit urine brought to pH 5.5 by extraction with ethyl acetate. Free sulphadimethoxine (m.p. and mixed m.p. 197°, from aqueous 50% ethanol) was isolated similarly from dog urine. The isolation of sulphadimethoxine N^1 -glucuronide from human urine has already been described (Bridges *et al.* 1965).

Chromatographic detection of metabolites. Sulphadimethoxine N^1 -glucuronide was detected by its

Table 1. *Chromatography and colour reactions of sulphadimethoxine and its metabolites*

Descending chromatography in Whatman no. 1 paper was used. The solvent systems employed 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*, aq. 80% (v/v) ethanol; *D*, butan-1-ol-acetic acid-water (4:1:2, by vol.). Chromatographs were run for 6 hr. in solvents *A*, *B* and *D* and for 4 hr. in solvent *C*. Abbreviations: DMAC, *p*-dimethylaminocinnamaldehyde reagent; NR, naphtharesorcinol reagent (Bridges *et al.* 1965).

Compound	<i>R_F</i> values				Colour reactions	
	Solvent <i>A</i>	Solvent <i>B</i>	Solvent <i>C</i>	Solvent <i>D</i>	DMAC	NR
Sulphadimethoxine <i>N</i> ⁴ -glucuronide sodium salt	0.00	0.11	0.25	—†	Purple after 5–10 min.	Blue
Sulphadimethoxine <i>N</i> ⁴ -sulphate dipotassium salt	0.05	0.42	0.79	0.66	Purple after 30 min.	None
Sulphadimethoxine <i>N</i> ¹ -glucuronide ammonium salt	0.15	0.54	0.55	0.80	Purple	Blue-brown
Sulphadimethoxine	0.40	0.72	0.95	0.95	Purple	None
<i>N</i> ⁴ -Acetylsulphadimethoxine	0.65	0.84	0.95	0.95	None	None
Sulphanilamide*	0.62	0.75	0.72	0.77	Red	None
Sulphanilic acid*	0.11	0.48	0.57	0.32	Red	None

* These two compounds gave a violet fluorescence on paper in ultraviolet light (254 mμ). The other compounds quenched the background fluorescence of paper and appeared as dark spots.

† Hydrolyses to free amine in this solvent.

Table 2. *Excretion of free and total amine by various species receiving sulphadimethoxine orally*

Species (variety; no. and sex)	Dose (mg./kg.)	% of dose of drug excreted in urine		
		Days 0–1	Days 0–2	Days 0–3
Rabbit (Chinchilla; 3F)	100	Free 4 (3–4)	—	5 (4–6)
		Total 46 (39–52)	—	73 (50–85)
Guinea pig (English; 3M)	100	Free 8 (6–12)	16 (13–18)	41 (34–51)
		Total 26 (20–27)	40 (34–51)	47 (39–59)
Rat (Wistar albino; 8F)	100	Free 5 (3–18)	11 (8–13)	—
		Total 13 (6–21)	31 (17–48)	—
Dog (mongrel; 3F)	200	Free 26 (11–34)	36 (26–42)	—
		Total 26 (11–34)	36 (26–42)	—
Monkey (Macaca; 4F)	100	Free 32 (23–40)	52 (39–60)	53 (46–62)
		Total 39 (32–52)	55 (44–73)	58 (47–75)
	33	Free 24 (10–36)	38 (19–45)	—
		Total 25 (10–36)	39 (19–49)	—
Man (9M)	33	Free 18 (11–28)	—	—
		Total 22 (11–35)	—	—

R_F values and colour reactions (see Table 1) in the urine of the dog, rat, guinea pig and monkey, but not the rabbit. It was a minor metabolite in the urine of dog, rat and guinea pig.

Sulphadimethoxine *N*⁴-glucuronide was detected (see Table 1) in the urine of all the species examined. There are strong reasons for believing that this metabolite is an artifact (see Bridges & Williams, 1962) because it can be detected in fresh normal human urine immediately after the addition of sulphadimethoxine to the urine (see below and Table 4). *N*⁴-Glucuronides of aromatic amines appear to be formed spontaneously from the amines

and glucuronic acid occurring either free or as a labile precursor in normal urine (Bridges, 1963).

Sulphadimethoxine *N*⁴-sulphate was detected (see Table 1) readily in rat urine. Traces of it were found occasionally in human, monkey and rabbit urine.

The free drug itself was detected in all the urines examined.

Quantitative excretion of metabolites. The output of sulphadimethoxine in six species is shown in Table 2. In the species examined, except the rat, 20–40% of the dose (about 0.1 g./kg.) is excreted in the urine in 24 hr. Excretion is most rapid in the

Table 3. *Metabolites of sulphadimethoxine in the urine of various species*

The amount of each metabolite is expressed in two ways. A is the percentage of the dose excreted in 24 hr. B expresses the amount of each metabolite as a percentage of the total metabolites excreted in 24 hr. The values given are averages; the numbers of animals used are given in parentheses in the first column.

Species	Dose (mg./kg.)	Metabolites ...	Sulphadi- methoxine	N ¹ -Glucuronide	N ⁴ -Glucuronide	N ⁴ -Sulphate	N ⁴ -Acetyl
Rabbit (3)	100	A	0.4	0	3.2	0	43
		B	1	0	7	0	92
Guinea pig (2)	100	A	3.2	1	2.6	0	13
		B	16	5	13	0	66
Rat (8)	100	A	5.2	0.9	0.6	0.26	6
		B	40	7	5	2	46
Dog (3)	200	A	15.4	4.6	4.1	0	0
		B	64	19	17	0	0
Monkey (2)	100	A	2.5	29	1.3	Trace	8.8
		B	6	79	3	Trace	21
(4)	33	A	5.2	16	2.7	Trace	1.5
		B	21	62	11	Trace	6
Man (3)	33	A	2	16	2	0	5
		B	8	61	8	0	21

rabbit (46% in 24 hr.) and slowest in the rat (13% in 24 hr.). There is a faecal excretion in the rat, but this is no more than 1% of the dose in 24 hr. However, there is a marked species difference in the amounts of each metabolite excreted (Table 3). In the rabbit and guinea pig the main metabolite is the N⁴-acetyl derivative, the other metabolites being minor ones. In the dog there is no N⁴-acetyl derivative and most of the urinary material is unchanged drug (64% of the 24 hr. excretion). In the rat the unchanged drug and its N⁴-acetyl derivative are excreted in roughly equal amounts and the N⁴-sulphate is present in measurable amounts (0.26% of the dose). In man and the monkey the major metabolite is the N¹-glucuronide, which accounts for more than 60% of the material excreted in 24 hr. after dosing. Some of the drug is also acetylated in these two species. The N¹-glucuronide is a minor metabolite in the urine of guinea pig, rat and dog, but a major one in man and the monkey. It is not present at all in rabbit urine.

The N⁴-glucuronide occurs in the urines of all the species in amounts varying from 0.4% in the rat to 4.1% of the dose in the dog. These variable amounts are probably not significant, since the spontaneous formation of this compound appears to depend on the pH, the amount of glucuronic acid available and also the amount of the free drug in the urine. The largest amount of free drug occurs in dog urine, which also contains the largest amount of N⁴-glucuronide (i.e. 4% of the dose or 17% of the 24 hr. excretion).

The spontaneous formation of the N⁴-glucuronide in normal human urine is shown in Table 4. In this

Table 4. *Spontaneous formation of sulphadimethoxine N⁴-glucuronide in human urine*

Various quantities of sulphadimethoxine were added, with shaking, to 100 ml. samples of freshly voided normal human urine. After 10–15 min. samples of the urine were chromatographed and the N⁴-glucuronide was eluted and estimated (see the text).

Sulphadimethoxine added (mg.)	Drug converted into N ⁴ -glucuronide			
	Urine 1 (pH 6.2)		Urine 2 (pH 6.3)	
	(mg.)	(%)	(mg.)	(%)
2	0.206	10.3	0.218	10.9
10	0.740	7.4	0.72	7.2
15	0.885	5.9	0.83	6.2
20	0.760	3.8	0.90	4.5

particular experiment the largest amount of the glucuronide was formed when the drug concentration was 15 mg./100 ml. The amount found probably depends, among other things, on the free glucuronic acid available in the urine.

Biliary excretion in the rat and rabbit. In biliary-cannulated rabbits only 3% of an intraperitoneal dose (0.1 g./kg.) of sulphadimethoxine was excreted in the bile in 24 hr. Chromatography of the bile showed that it contained free sulphadimethoxine and its N⁴-glucuronide and N⁴-acetyl derivative, but there was no N¹-glucuronide in the bile. In rats, however, about 12% of the dose appeared in the bile in 24 hr. (Table 5), and this material consisted largely of the N¹-glucuronide (11% of the dose or 87% of the biliary material) together with

Table 5. *Biliary excretion of sulphadimethoxine and its metabolites in the rat*

The compounds (100mg./kg.) in propane-1,2-diol (1ml.) were injected intraperitoneally into biliary-cannulated rats. The bile was collected for 24hr. The metabolites in the bile were separated chromatographically and estimated as described in the text. Average values are quoted, the numbers of animals used being given in parentheses. Abbreviation: SD, sulphadimethoxine.

Compound administered	Mol.wt.	% of administered dose appearing in bile in 24hr.				
		As SD	As SD <i>N</i> ¹ -glucuronide	As SD <i>N</i> ⁴ -glucuronide	As <i>N</i> ⁴ -Acetyl-SD	As SD <i>N</i> ⁴ -sulphate
SD (6)	310	1	11	0.5	0	0
SD <i>N</i> ¹ -glucuronide (6)	487	0.5	78	0	0	0
SD <i>N</i> ⁴ -glucuronide (2)	487	2	0	42	0	0
<i>N</i> ⁴ -Acetyl-SD (4)	352	0.2	0	0	3	0
SD <i>N</i> ⁴ -sulphate (6)	390	0.6	0	0	0	2.5

Table 6. *Synthesis of sulphadimethoxine *N*¹-glucuronide by liver preparations from various species*

The incubation mixtures consisted of either [³⁵S]sulphadimethoxine or *o*-aminophenol (1.3 μ mole in 0.1 ml. of buffer), 0.5 M-tris-HCl buffer, pH 7.4 (0.2 ml.), liver (equivalent to 330 mg. in 1 ml. of 0.25 M-sucrose), UDP-glucuronic acid (7 μ moles in 0.4 ml. of water) and water (0.3 ml.). Controls contained boiled liver preparation or sodium glucuronate (7 μ moles in 1 ml. of water).

Animal (no. and sex)	Preparation	Glucuronide formed (μ moles/g. of liver/hr.)	
		From sulphadimethoxine	From <i>o</i> -aminophenol
Monkey	Slices	0.1, 0.09	0.63, 0.67
(2F)	2000g supernatant	1.46, 1.46	1.82, 1.68
	10000g supernatant	1.38, 1.42	1.78, 1.64
	Boiled 10000g supernatant	0, 0	0, 0
	Sodium glucuronate	0, 0	0, 0
Rat	10000g supernatant*	0.08, 0.03	0.46, 0.40
(4F)		0.04, 0.05	0.40, 0.80
Rabbit	10000g supernatant*	0, 0, 0	0.95, 0.87, 0.91
(3F)			

* No activity was found with the boiled preparation.

the free drug (1%) and the *N*⁴-glucuronide artifact (0.0–0.5%). No *N*⁴-acetylsulphadimethoxine was found in rat bile, although it is excreted in the urine by this animal. The extent of formation of the *N*¹-glucuronide in the rat is therefore greater than indicated by that found in the urine (see Table 3). When sulphadimethoxine *N*¹-glucuronide itself is injected intraperitoneally into biliary-cannulated rats some 78% of it appears in the bile in 24hr. (see Table 5). It therefore appears that at least 10% of the dose of sulphadimethoxine is converted in the rat into the *N*¹-glucuronide, which is then largely excreted in the bile and destroyed in the gut. This value of 10% is comparable with that in man, which is 15% in the urine in 24hr. (see Table 3).

Table 5 shows that *N*⁴-acetylsulphadimethoxine (mol.wt. 352) and sulphadimethoxine *N*⁴-sulphate (mol.wt. 390) are not excreted in the bile in the rat to more than about 2–3% of the dose in 24hr. The artifact, sulphadimethoxine *N*⁴-glucuronide (mol.wt. 487), however, has an appreciable biliary

excretion of about 42%. Millburn, Smith & Williams (1967) have suggested that a molecular weight of $> 325 \pm 50$ and the possession of a polar group are factors in causing appreciable biliary excretion of a compound in the rat. The high biliary excretion of the *N*¹- and *N*⁴-glucuronides and the low biliary excretion of the *N*⁴-acetyl derivative appear to conform with these views. However, the low biliary excretion of the *N*⁴-sulphate, which is polar and has a molecular weight of 390, which would suggest moderate biliary excretion, does not conform with these views.

*Enzymic synthesis of the *N*¹-glucuronide.* The results of experiments on the synthesis of sulphadimethoxine *N*¹-glucuronide *in vitro* are shown in Table 6, which shows that glucuronyl transfer to the sulphonimide nitrogen of sulphadimethoxine occurs in the 2000g and 10000g supernatants of monkey liver homogenates fortified with UDP-glucuronic acid. In monkey liver slices the synthesis is small, possibly because the drug does not penetrate

readily under the conditions of the experiment. Synthesis of the glucuronide also occurs to a slight extent in the 10000g supernatant of rat liver homogenates, but no synthesis at all was detected in similar preparations from rabbit liver. The liver homogenates from all three species were able to conjugate *o*-aminophenol. It therefore appears that the enzyme forming the stable *N*¹-glucuronide is different from that forming *o*-aminophenyl glucuronide. Another possibility is that the glucuronyltransferase of monkey liver can catalyse glucuronyl transfer to the sulphonimide nitrogen of sulphadimethoxine and to the hydroxyl group of *o*-aminophenol, whereas that of rabbit liver can only transfer to *o*-aminophenol in these experiments. These findings *in vitro* support those found in the whole animals, especially the rhesus monkey and the rabbit. It should be noted that the stable sulphadimethoxine *N*¹-glucuronide is different from the unstable *N*-glucuronides of aromatic amines, which can be formed spontaneously from glucuronic acid and the free amine. The *N*¹-glucuronide cannot be formed spontaneously in this way. The question of the occurrence of more than one UDP-glucuronyltransferase has been discussed by Dutton (1966) and

our findings suggest that in primates at least two of these may occur.

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REFERENCES

- Abou-El-Makarem, M. M., Millburn, P., Smith, R. L. & Williams, R. T. (1967). *Biochem. J.* **105**, 1289.
Adamson, R. H., Bridges, J. W. & Williams, R. T. (1966). *Biochem. J.* **100**, 71 p.
Bratton, A. C. & Marshall, E. K. (1939). *J. biol. Chem.* **128**, 537.
Bridges, J. W. (1963). Ph.D. Thesis: University of London.
Bridges, J. W., Kibby, M. R. & Williams, R. T. (1965). *Biochem. J.* **96**, 829.
Bridges, J. W. & Williams, R. T. (1962). *Biochem. J.* **83**, 27 p.
Dutton, G. J. (1966). In *Glucuronic Acid*, p. 230. Ed. by Dutton, G. J. London and New York: Academic Press Inc.
Koechlin, B. A., Kern, W. & Engelberg, R. (1959). *Antibiot. Med. (Brit. Ed.)*, **6** (Suppl.), 22.
Levy, G. A. & Storey, I. D. E. (1949). *Biochem. J.* **44**, 295.
Millburn, P., Smith, R. L. & Williams, R. T. (1967). *Biochem. J.* **105**, 1275.