The Metabolism of Four Sulphonamides in Cows

By POUL NIELSEN

Department of Pharmacology and Toxicology, The Royal Veterinary and Agricultural University, Copenhagen, Denmark

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1. The metabolism of sulphanilamide, sulphadimidine (4,6-dimethyl-2-sulphanilamidopyrimidine), sulphamethoxazole (5-methyl-3-sulphanilamidoisoxazole) and sulphadoxine (5,6-dimethoxy-4-sulphanilamidopyrimidine) given by intravenous injection has been examined in cows. 2. The sulphonamides were present mainly as unchanged drugs in blood samples collected 2 h after administration. 3. The sulphonamides were excreted in the milk partly as unchanged drugs and partly as conjugated metabolites whereas only small amounts were excreted as the *N*⁴-acetyl derivatives. 4. The unchanged drug and the *N*⁴-acetyl derivative were the major constituents in urine samples after administration of sulphanilamide, sulphamethoxazole and sulphadoxine. 5. Besides the unchanged drug, the *N*⁴-acetyl derivative and the conjugated metabolites, three further metabolites of sulphadimidine were isolated from urine samples and identified. They were 5-hydroxy-4,6-dimethyl-2-sulphanilamidopyrimidine, 4-hydroxymethyl-6-methyl-2-sulphanilamidopyrimidine and sulphaguanidine.

The metabolism of various sulphonamides has been a subject of great interest for many years, and it has been shown that in many cases the *N*⁴-acetyl derivative is the most important metabolite. In the metabolism of a series of methoxysulphapyrimidines Walker & Williams (1972), however, demonstrated great variations from one species to another.

The present work gives a qualitative description of the most important metabolites of sulphanilamide, sulphadimidine, sulphamethoxazole and sulphadoxine, and, further, it gives the results from a quantitative determination of the relative concentrations of metabolites in samples of plasma, milk and urine after intravenous administration of sulphonamides to cows.

Materials and Methods

Drugs

Sulphanilamide, m.p. 165°C, and sulphadimidine (4,6-dimethyl-2-sulphanilamidopyrimidine, sulphamezathine, sulphamethazine), m.p. 197°C, were purchased (Mecobenzon A/S, Copenhagen, Denmark). Sulphamethoxazole (5-methyl-3-sulphanilamidoisoxazole), m.p. 167°C, was a gift from Grindstedvaerket A/S, Aarhus, Denmark, and sulphadoxine (5,6-dimethoxy-4-sulphanilamido-pyrimidine, sulphormethoxine) was a gift from the Wellcome Research Laboratories, Beckenham, Kent, U.K.

Synthesis of reference compounds

The *N*⁴-acetyl derivatives of the four sulphonamides

were synthesized by adding acetic anhydride to a solution of the sulphonamide in question in acetic acid. By heating the solution for a few minutes the *N*⁴-acetyl derivatives were formed and on addition of water they precipitated. After recrystallization from ethanol–water (1:1, v/v) the melting points were: *N*⁴-acetylsulphanilamide, 213–214°C; *N*⁴-acetylsulphadimidine, 247–249°C; *N*⁴-acetylsulphamethoxazole, 210–212°C and *N*⁴-acetylsulphadoxine, 214–215°C. The purity of the synthesized compounds was checked by t.l.c. and paper chromatography. When diazotized and coupled with *N*¹- α -naphthylethylenediamine the synthesized compounds gave no red colour until the acetyl groups had been removed by boiling in acid solution.

The *N*⁴-sulphates of the four sulphonamides were prepared by the method of Bridges *et al.* (1968). The purity of these compounds was also checked by t.l.c. and paper chromatography. The following qualitative identity reactions showed the same results with all four derivatives: (1) acidified solutions of the salts gave no reaction for inorganic sulphate with BaCl₂ until boiled. (2) When diazotized and coupled with *N*¹- α -naphthylethylenediamine they gave a red colour, indicating removal of the sulphonate groups on diazotization. (3) On paper they gave no immediate colour with a spray of 1% *p*-dimethylaminobenzaldehyde in aq. 36% HCl diluted with 3 vol. of methanol (Merck, 1970), but, if the paper was kept for a few minutes, yellow spots developed. Full colour strength was attained in about 30 min. This last test showed that the derivatives did not contain free amino groups until the sulphonate groups had been removed by acid.

Synthesis of the N^4 -glucuronides of the four sulphonamides was performed as described by Bridges *et al.* (1969b). The compounds were very labile and they were never obtained free of uncombined sulphonamide. They were, however, used as such for chromatographic purposes (see Table 1) and on paper they could easily be distinguished from the corresponding N^1 -glucuronides of the sulphonamides. All four derivatives reduced Fehling's solution readily when heated to gentle boiling and gave positive tests with naphthoresorcinol (see Table 1).

N^1 -Glucuronides were prepared by the method of Bridges *et al.* (1969b). The synthesized compounds were difficult to purify, but these too could be used for chromatographic purposes.

Paper chromatography

Table 1 shows the R_F values and colour reactions of the four parent sulphonamides and some of their possible metabolites.

Quantitative determinations

Free and total sulphonamide. The concentration of free sulphonamide in plasma, milk and urine samples was determined by using the method of Bratton & Marshall (1939) modified for milk by Rasmussen (1958). The concentration of total sulphonamide was determined on the same samples after hydrolysis with HCl. The concentration of N^4 -acetylated sulphonamide was calculated as being the

Table 1. R_F values and colour reactions of sulphonamides and some of their derivatives

Descending chromatography on Whatman no. 3 paper was run for 7 h. The solvents are: A, butan-1-ol-aq. NH_3 (sp.gr. 0.88)-water (10:1:1, by vol.); B, propan-1-ol-aq. NH_3 (sp.gr. 0.88) (7:3, v/v); C, butan-1-ol-water-acetic acid (4:2:1, by vol.). Detection: (1), the paper was sprayed with a 1% (w/v) solution of *p*-dimethylamino-benzaldehyde in aq. 36% HCl diluted with 3 vol. of methanol (Merck, 1970); (2), the paper was sprayed with a 0.1% (w/v) solution of naphthoresorcinol in 10% (w/v) trichloroacetic acid and then heated in a water-saturated atmosphere (steam bath) at 70–80°C for 20 min (Merck, 1970). —, Means no colour reaction; q means quenching of background fluorescence of paper.

Compound	R_F in solvent			Colour reactions		Fluorescence in u.v. light (254nm)
	A	B	C	(1)	(2)	
Sulphanilamide	0.48	0.69	0.66	Yellow	—	q
N^4 -Acetylsulphanilamide	0.67	0.80	0.77	None†	—	q
Sulphanilamide N^4 -sulphate	0.05	0.38	0.22	Yellow in 30min	—	q
Sulphanilamide N^4 -glucuronide	0.02	0.10	*	Yellow	Blue	q
Sulphadimidine	0.25	0.57	0.87	Yellow	—	q
N^4 -Acetylsulphadimidine	0.41	0.72	0.87	None†	—	Violet
Sulphadimidine N^4 -sulphate	0.02	0.31	0.50	Yellow in 30min	—	q
Sulphadimidine N^1 -glucuronide	0.06	0.40	0.46	Yellow	Blue-brown	q
Sulphadimidine N^4 -glucuronide	0.02	0.08	*	Yellow	Blue	q
4,6-Dimethyl-5-hydroxy-2-sulphanilamidopyrimidine	0.10	0.35	0.87	Yellow	—	Violet
4-Hydroxymethyl-6-methyl-2-sulphanilamidopyrimidine	0.10	0.46	0.82	Yellow	—	q
Sulphaguanidine	0.35	0.57	0.61	Yellow	—	q
Sulphamethoxazole	0.25	0.54	0.90	Yellow	—	q
N^4 -Acetylsulphamethoxazole	0.42	0.65	0.91	None†	—	q
Sulphamethoxazole N^4 -sulphate	0.03	0.17	0.52	Yellow in 30min	—	q
Sulphamethoxazole N^1 -glucuronide	0.09	0.27	0.54	Yellow	Blue-brown	q
Sulphamethoxazole N^4 -glucuronide	0.01	0.08	*	Yellow	Blue	q
Sulphadoxine	0.19	0.54	0.88	Yellow	—	q
N^4 -Acetylsulphadoxine	0.43	0.69	0.91	None†	—	q
Sulphadoxine N^4 -sulphate	0.03	0.21	0.45	Yellow in 30min	—	q
Sulphadoxine N^1 -glucuronide	0.07	0.33	0.52	Yellow	Blue-brown	q
Sulphadoxine N^4 -glucuronide	0.01	0.07	*	Yellow	Blue	q

* The N^4 -glucuronide decomposes in solvent C.

† If the paper is kept for some hours the spot turns yellow owing to the slow hydrolysis of the acetyl derivative.

difference between the concentration of total and that of free sulphonamide.

The quantitative distribution of the metabolites with a free amino group was determined by means of t.l.c. on glass plates coated with silica gel (0.25mm, silica gel GF₂₅₄; Merck, Darmstadt, West Germany). Before chromatography the proteins in plasma and milk were precipitated by addition of 5vol. of acetone. After 1h at room temperature the solutions were filtered and the filtrate was concentrated to a suitable volume by vacuum distillation at temperatures below 40°C. The concentration of the solutions was adjusted so that 20 μ l maximally was applied per spot.

The thin-layer plates were run to a height of about 15cm. As solvent systems, mixtures of ethyl ether and propan-2-ol were used. The ratio between ethyl ether and propan-2-ol depended on the sulphonamide concerned and was for sulphanilamide 5:1, for sulphadimidine, 4:1, and for sulphamethoxazole and sulphadoxine, 15:2. After being dried the plates were developed by spraying with aqueous solutions of the following reagents in the order shown. (1) 4M-HCl, (2) 1% (w/v) NaNO₂, 3min later (3) 5% (w/v) ammonium sulphamate and 2min later (4) 1% N¹- α -naphthylethylenediamine (w/v). The plates were dried and the spots scraped off with a razor blade and transferred to conical test tubes. By careful stirring the coloured compounds were extracted with 3ml of methanol to which 2 drops of aq. 36% HCl had been added. After centrifugation the extinction of the supernatant at 545nm was measured. The concentration of sulphonamide in the samples was determined by means of a standard curve for the sulphonamide in question.

Isolation and identification of unknown sulphadimidine metabolites

Three metabolites of sulphadimidine (I, II and III) have been isolated from urine samples obtained from cows 24h after intravenous administration of the drug. The following procedures were used.

Free metabolites. Urine was adjusted to pH 5.5 with acetic acid and then extracted three times with equal volumes of ethyl acetate. The extract containing the

total amount of free metabolites was evaporated (all the evaporation processes were performed under reduced pressure at temperatures below 40°C) to a brown paste which was dissolved in a small amount of ethanol and the solution was banded on thin-layer plates (0.5mm, silica gel GF₂₅₄, Merck). The plates were developed in ether-propan-2-ol (4:1, v/v). The area containing the metabolite in question was located under u.v. light. Table 2 shows which zones were used for the isolation of the different metabolites. The silica gel was scraped from the plates and extracted several times with ethanol. The extract was evaporated to dryness and the residue dissolved in a small volume of ethanol. The solution was deposited at the start line of Whatman no. 3 paper which was developed for 7h in propan-1-ol-aq. NH₃ (sp.gr. 0.88) (7:3, v/v) by descending chromatography. The area containing the metabolite, located under u.v. light, was cut out and the paper extracted with ethanol. Table 2 also shows the *R_F* values of the zones used for the isolation of the different metabolites after paper chromatography. The extracts were evaporated to dryness whereupon the metabolites appeared as white or slightly yellow-coloured residues.

Conjugated metabolites. After quantitative removal of the free metabolites from the urine as described above the pH was adjusted to 4.5 by addition of acetic acid. A portion (3ml) of this aqueous phase containing the conjugated metabolites was mixed with 0.4ml of glucuronidase-arylsulphatase (gastric juice from *Helix pomatia* titrating 100000 β -glucuronidase Fishman units+800000 sulphatase Roy units/ml; Industrie Biologique Francaise, 92 Gennevilliers, France) and incubated at 37°C for 24h. Samples of the incubation mixture were then separated by means of t.l.c. and the relative concentrations of the metabolites determined as described above.

To determine the amount of glucuronides present in the conjugates a second portion of the same aqueous phase was made 1mM with D-saccharo-1,4-lactone, a potent and selective inhibitor of β -glucuronidase (Levy, 1952) and incubated and separated as above. The difference between the two incubation mixtures measured the amount of metabolites conjugated to glucuronic acid.

Table 2. *R_F* values of the zones used for isolation of sulphadimidine metabolites

T.l.c. was done in the ascending direction on 0.5mm silica-gel GF₂₅₄ plates to a height of approx. 15cm with ether-propan-2-ol (4:1, v/v) as solvent. Paper chromatography was done in the descending direction on Whatman no. 3 paper for 7h with propan-1-ol-aq. NH₃ (sp.gr. 0.88) (7:3, v/v) as solvent.

	<i>R_F</i> values		
	Metabolite I	Metabolite II	Metabolite III
T.l.c.	0.85–0.95	0.40–0.60	0.20–0.40
Paper chromatography	0.30–0.40	0.40–0.50	0.52–0.62

I.r. and u.v. spectra. These were determined with the Perkin-Elmer Infracord spectrophotometer and the Beckman DB spectrophotometer respectively.

N.m.r. spectroscopy. Spectra at 60 MHz (Instrument: C-60 HL, Japan Electronic Optics Laboratory Co. Ltd., Tokyo, Japan) were taken. The samples were dissolved in $^2\text{H}_2\text{O}$ to which was added a small amount of trifluoroacetic acid. The sodium salt of trimethylsilyltetraduteropropionic acid was used as internal standard and sulphadimidine was used as reference compound.

Mass spectrometry. Mass spectra were obtained with the Perkin-Elmer 270 mass spectrophotometer. The temperature of the solid inlet system was 120–140°C. Here too sulphadimidine was used as a reference compound.

Animals

The animal experiments were performed in three Jersey cows weighing about 400 kg. Each of the four sulphonamides was administered once to each cow. The sulphonamides (60 mg/kg body wt.) were administered to the cows by a single intravenous injection. Samples of plasma, milk and urine were collected 2 and 24 h after the administration of the sulphonamides.

Results and Discussion

The results derived from the thin-layer-chromatographic determination of the distribution of sulphonamide and metabolites in plasma 2 h after the administration are shown in Table 3. The unchanged drugs, with the exception of sulphanilamide, amount to more than 90% of the total contents in plasma, and, with the same exception, only a small part is found as N^4 -acetylated drugs or as conjugated metabolites. The conjugated metabolites include the total amounts of glucuronic acid and sulphuric acid conjugates. The relative amount of unchanged drugs is lower in plasma samples obtained later in the experiment.

Table 3 also shows the distribution of the sulphonamidase in samples of milk collected 2 h after the administration. The sulphonamides are excreted in the milk mainly as unchanged drugs, but considerable amounts of conjugated metabolites were also present in the milk. By paper chromatography it was shown that the conjugated metabolites consisted mainly of the sulphonamide N^4 -glucuronide. These compounds may be either synthesized in or actively excreted through the mammary gland. The relative amounts of the N^4 -acetylated drugs in milk are similar to those found in the plasma. In experiments with the

Table 3. *Relative concentrations of sulphonamides and their metabolites in plasma, milk and urine from cows*

The sulphonamides (60 mg/kg body wt.) were administered to cows by a single intravenous injection. Samples of plasma, milk and urine were collected as indicated in the Table. The metabolites were separated chromatographically and estimated as described in the text. The table gives the averages $\pm$ S.E.M. for three cows.

Drug	Sample	Time after administration (h)	1	2	3	Sum (1+2+3)
			Unchanged drug (%)	N^4 -acetylated drug (%)	Conjugated metabolites (%)	
Sulphanilamide	Plasma	2	78 $\pm$ 6	15 $\pm$ 7	4 $\pm$ 1	97
Sulphadimidine			91 $\pm$ 1	2 $\pm$ 1	5 $\pm$ 1	98
Sulphamethoxazole			92 $\pm$ 5	4 $\pm$ 4	3 $\pm$ 1	99
Sulphadoxine*			93	3	4	100
Sulphanilamide	Milk	2	72 $\pm$ 7	7 $\pm$ 2	20 $\pm$ 8	99
Sulphadimidine			57 $\pm$ 15	2 $\pm$ 1	41 $\pm$ 15	100
Sulphamethoxazole			56 $\pm$ 5	3 $\pm$ 1	41 $\pm$ 5	100
Sulphadoxine			62 $\pm$ 10	1 $\pm$ 1	37 $\pm$ 11	100
Sulphanilamide	Urine	2	55 $\pm$ 4	39 $\pm$ 5	6 $\pm$ 1	100
Sulphadimidine			54 $\pm$ 8	15 $\pm$ 6	16 $\pm$ 1	85
Sulphamethoxazole			68 $\pm$ 7	28 $\pm$ 7	2 $\pm$ 0	98
Sulphadoxine			65 $\pm$ 7	29 $\pm$ 7	6 $\pm$ 1	100
Sulphanilamide	Urine	24	15 $\pm$ 6	72 $\pm$ 6	10 $\pm$ 1	97
Sulphadimidine			23 $\pm$ 4	8 $\pm$ 2	32 $\pm$ 2	63
Sulphamethoxazole			—	45 $\pm$ 9	—	45
Sulphadoxine			25 $\pm$ 7	52 $\pm$ 9	23 $\pm$ 5	100

* The average of two cows.

same sulphonamides, Rasmussen (1969) and Jørgensen (1972), however, found higher concentrations of the N^4 -acetyl derivatives in the milk than in the plasma from goats. The disagreement may be due to differences in the experimental conditions and possibly also to the species. The results reported here are obtained from cows receiving the sulphonamides by a single intravenous injection, but the results obtained from the goats originate from clearance experiments with constant plasma concentration owing to continuous infusion of the sulphonamide.

Table 3 further shows that the sulphonamides are excreted mainly as unchanged drugs in the urine during the first 2h just as for the milk, but here the N^4 -acetylated drugs are found to make up a much greater part than the conjugated metabolites. The relative concentrations of unchanged sulphonamide are higher in blood than in urine samples obtained simultaneously (Table 3). This means that the clearance is lower for the parent compounds than for the metabolites, which is in accordance with the results obtained in man by Strauss *et al.* (1941) and Frisk (1943), and with those that Jørgensen & Rasmussen (1972) obtained in goats.

The N^4 -acetylated derivative is by far the most important metabolite of sulphanilamide and sulphadoxine excreted in the urine collected 24h after administration of the sulphonamides to the cows (Table 3).

The concentration of sulphamethoxazole was not high enough to perform a complete determination of the metabolites, but as shown in Table 3 the N^4 -acetylsulphamethoxazole amounted to 45% of the total amount in the 24h urine sample. The extent of N^4 -acetylation is approximately the same as reported by Frisk (1943) and Strauss *et al.* (1941) for sulphanilamide in man, by Bünger (1967) for sulphamethoxazole in man and by Bünger (1967) and Böhni *et al.* (1969) for sulphadoxine in man. Bridges *et al.* (1969a) showed that the N^4 -acetylation of sulphadoxine was to a high degree dependent on the species. In the rabbit 89% of the amount excreted during the first 24h was N^4 -acetylated. The corresponding values were for the monkey 85%, for the rat 66% and for man 29%. The N^4 -acetylation of sulphadimidine is also dependent on the species. Whereas Smith & Williams (1948) and Bray *et al.* (1951) found about 75% of a dose of sulphadimidine excreted as N^4 -acetylsulphadimidine from rabbits, Volini *et al.* (1945) reported 45% in man. By clearance experiments in goats with constant plasma concentration of sulphadimidine Jørgensen & Rasmussen (1972) found only 0–10% excreted in the urine as N^4 -acetylated drug. In the urine samples from cows 15% and 8% of N^4 -acetylsulphadimidine were found respectively in the 2h and the 24h sample (Table 3). Thus the N^4 -acetylation seems to be of somewhat greater importance in the cows than in the goats.

Table 3 shows that sulphanilamide, sulphameth-

oxazole and sulphadoxine are excreted in the urine as unchanged drug, N^4 -acetylated drug or as conjugated metabolites and only very small amounts are found as other non-identified metabolites. For sulphadimidine, however, these three groups amounted only to 85 and 63% respectively in the 2h and the 24h urine sample. The remaining part was therefore isolated and separated as mentioned above and three unknown metabolites I, II and III were identified from their spectra as described below.

Sulphadimidine. The mass spectrum of this compound showed only a very small molecular-ion peak at m/e 278. The dominating peak in the spectrum was obtained at m/e 214 which is $M-64$ corresponding to $M-SO_2$. Most of the other peaks in the spectrum originated from the decomposition of $H_2N-C_6H_5-SO_2^+$ and there were only very few traces from the decomposition of the pyrimidine ring of the molecule.

Similar fractionating patterns with expulsion of SO_2 from the molecules have been described for sulphonylureas by Budzikiewicz *et al.* (1967).

Metabolite I. The quantitative determinations showed that this metabolite amounted to 3 and 4% (mean of three cows) respectively in the 2h and the 24h urine sample.

The mass spectrum of this molecule exhibited a very small molecular-ion peak at m/e 294. As for the sulphadimidine, this spectrum was also dominated by the $M-SO_2$ peak at m/e 230. This means that an O atom has been incorporated into the sulphadimidine molecule. The position of the O atom was established by comparing the n.m.r. spectrum of metabolite I with that of sulphadimidine. The only significant difference between these two spectra was the lack in the spectrum of the metabolite of the CH signal appearing in the spectrum of sulphadimidine at 6.75p.p.m. The structure of metabolite I must then be as shown in Fig. 1.

Bray *et al.* (1951) demonstrated the presence of the same metabolite conjugated to glucuronic acid in rabbits' urine. As shown in Table 4 metabolite I was present partly conjugated to sulphuric acid and partly as free metabolite in cows' urine and the presence of glucuronide was not demonstrated.

Metabolite II amounted to 10 and 31% (mean of three cows) respectively in the 2h and the 24h urine sample.

Mass spectrometry showed this compound to be isomeric with metabolite I. This spectrum also exhibited a large $M-SO_2$ peak at m/e 230, whereas the molecular-ion peak at m/e 294 was very small. The structure of this metabolite was established by comparing the n.m.r. spectra of sulphadimidine and metabolite II. The only significant difference between the two spectra was the replacement of one CH_3 signal at 2.38p.p.m. in the spectrum of sulphadimidine by a CH_2 signal at 4.55p.p.m. in the spectrum of the metabolite. This requires replacement of a methyl

Fig. 1. Structures of sulphadimidine and some of its metabolites

Table 4. Conjugated metabolites of sulphadimidine in cows' urine

Sulphadimidine (60 mg/kg body wt.) was administered to the cows by a single intravenous injection. After quantitative removal of the free metabolites by extraction with ethyl acetate the relative amount of conjugated metabolites was determined after enzymic hydrolysis of the samples with glucuronidase-arylsulphatase $\pm$ addition of D-saccharo-1,4-lactone (a specific inhibitor of β -glucuronidase) as described in the text.

	Free (%)	<i>N</i> ⁴ -Acetylated (%)	Conjugated to	
			Glucuronic acid (%)	Sulphuric acid (%)
Sulphadimidine	80	10	5	5
Metabolite I (5-hydroxy-4,6-dimethyl-2-sulphanilamidopyrimidine)	35			65
Metabolite II (4-hydroxymethyl-6-methyl-2-sulphanilamidopyrimidine)	90		5	5
Metabolite III (sulphaguanidine)	100			

group in sulphadimidine by a primary alcohol as shown in Fig. 1.

Table 4 shows that metabolite II is conjugated to both glucuronic acid and to sulphuric acid but the major part, 90%, is present in the free state.

Smith & Williams (1948) demonstrated increased output of glucuronic acid after administration of sulphadimidine to rabbits. They concluded that oxidation of the molecule had preceded the glucuronide formation and suggested that this could have taken place at one of the methyl groups in the molecule. They were, however, not able to isolate the metabolite (metabolite II).

The amount of metabolite III did not exceed 1–2% in any of the urine samples investigated. As shown in Table 4 this metabolite was neither conjugated to glucuronic acid nor to sulphuric acid in amounts detectable by the method used.

By contrast with the spectra of sulphadimidine and the two above-mentioned metabolites the mass spectrum for metabolite III showed no $M-SO_2$ peak. The molecular-ion peak was found at m/e 214 and this means that the molecular weight has been decreased by 64 from 278 to 214. The n.m.r. spectrum of this metabolite showed only the characteristic signals from the para-disubstituted benzene ring. These observations indicate destruction of the pyrimidine ring leading to the formation of sulphaguanidine which has a molecular weight of 214.

The mass fragment pattern for metabolite III matched that of authentic sulphaguanidine and the identity of the two compounds was confirmed both by their u.v. and the i.r. spectra and the R_F values on t.l.c. and paper chromatography.

T.l.c. and paper chromatography showed that urine samples which had not been subject to any handling, except application to the paper sheets or the thin-layer plates, also contained the sulphaguanidine. This means that sulphaguanidine has not arisen as an artifact of the isolation procedure.

The chemical reactions by which metabolite III, sulphaguanidine, is formed are not known. One possibility is that the pyrimidine ring has been opened and that this reaction has been followed by a cleavage of the molecule. An alternative possibility is cleavage of the bond between the nitrogen and the pyrimidine ring followed by a condensation with urea. The fact that sulphanilamide was not detected after sulphadimidine administration and that sulphaguanidine was not demonstrated as a metabolite of sulphanilamide does not support this second possibility.

The structure of the conjugated metabolites of

metabolite I and metabolite II were not elucidated, but the presence of both N^1 - and N^4 -glucuronides of sulphadimidine was demonstrated by paper chromatography.

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