The Metabolism of Sulphamezathine, 4:6-Dimethylpyrimidine and 2-Amino-4:6-dimethylpyrimidine in the Rabbit with Observations on the Hydroxylation of some other Sulphonamides

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Previous investigations (e.g. Gilligan, 1945; Krebs, Sykes & Bartley, 1947) have suggested that the metabolism of sulphamezathine (4:6-dimethyl-2-sulphanilamidopyrimidine, I) was in general similar to that of other sulphonamides, acetylation being

the predominant reaction. Gilligan's observations, however, suggested that in man sulphamezathine gave rise to a considerably increased excretion of glucuronide. Smith & Williams (1948) reported that 10–16% of sulphamezathine was excreted by the rabbit as glucuronide and none as ethereal sulphate. From analogy with the metabolism of sulphapyridine in the rabbit, which we were studying at the time (Bray, Neale & Thorpe, 1946), we expected that the increased glucuronide excretion would be due to the occurrence of hydroxylation of the sulphamezathine and subsequent conjugation with glucuronic acid, since the analytical evidence suggested that the glucuronide was of the ether type which did not reduce alkaline cupric solutions. The relatively large amounts of sulphamezathine excreted as glucuronide encouraged us to attempt the isolation and characterization of the hydroxylation product, a task which proved unexpectedly difficult. In the work now reported the effect of sulphamezathine upon the excretion of diazotizable material, ether-soluble acid, glucuronide and ethereal sulphate in the rabbit has been examined. Attempts to isolate the glucuronide have been unsuccessful, but evidence as to the position of hydroxylation in sulphamezathine has been obtained. 4:6-Dimethylpyrimidine and its 2-amino derivative have also been examined for their effect upon the excretion of ether-soluble acid, glucuronide and ethereal sulphate and some metabolites have been isolated. Because evidence that sulphamezathine was hydroxylated to a small extent in the benzene ring was obtained, some other sulphonamides have been examined for evidence of this type of hydroxylation.

MATERIALS

Compounds previously known. These were prepared by the following methods: 2-hydroxysulphanilic acid and its amide, 3-hydroxysulphanilamide (Thorpe & Williams, 1941). 4:6-Dimethylpyrimidine, m.p. 25°, 4-methylpyrimidine-6-carboxylic acid, m.p. 166°, pyrimidine-4:6-dicarboxylic acid, m.p. 226° (Angerstein, 1901). 2-Amino-4:6-dimethylpyrimidine, m.p. 153–154° (Combes & Combes, 1892). 2-Hydroxy-4:6-dimethylpyrimidine, m.p. 197° (Angerstein, 1901; Evans, 1893).

3-Hydroxysulphanilic acid (4-amino-3-hydroxybenzenesulphonic acid). Sodium benzoxazalone-6-sulphonate (8 g.) was boiled with NaOH (80 ml., 30% w/v) for 20 min. (cf. Thorpe & Williams, 1941). The solution was adjusted to pH 3–4, evaporated to dryness and the residue extracted with ethanol for 24 hr. After removal of solvent the material in the extract was crystallized (charcoal) from water. Yield, 1.2 g. colourless needles. (Found: C, 38.6; H, 3.6; N, 7.6%: equivalent (by titration) 191. Calc. for $C_6H_7O_4NS$: C, 38.1; H, 3.7; N, 7.4%: equivalent 189.)

Complex of urea and 2-amino-4:6-dimethylpyrimidine. Urea (0.6 g.) and 2-amino-4:6-dimethylpyrimidine (1.2 g.) were dissolved in water and evaporated to dryness. The residue was recrystallized (charcoal) from aqueous ethanol. The complex formed colourless prisms, m.p. 186°. (All melting points are uncorrected.) Yield, 1.1 g. (Found: N, 38.0; $C_7H_{13}ON_5$ requires N, 38.2%.)

2-Amino-5-hydroxy-4:6-dimethylpyrimidine. 2-Amino-4:6-dimethylpyrimidine (17.4 g.) was dissolved in acetic acid (50 ml.) and treated with bromine (7.7 ml.) (cf. Curd, Richardson & Rose, 1946). After standing 0.5 hr. the mixture crystallized. The solid was collected, washed with ether and dissolved in water (150 ml.). On addition of excess $NaHCO_3$, 2-amino-5-bromo-4:6-dimethylpyrimidine was precipitated as small colourless prisms almost insoluble in water, soluble in acetone and ethanol, m.p. 184°. (Huber & Hölischer (1938) give m.p. 183–184°.) (Found: N, 20.8; Br, 39.7. Calc. for $C_8H_9N_5Br$: N, 20.8; Br, 40.2%.) Oxidation to a dicarboxylic acid containing bromine confirmed that the bromine atom is in position 5 (see below). Acetylation with acetic anhydride and fused sodium acetate gave 2-acetamido-5-bromo-4:6-dimethylpyrimidine, as small colourless prisms, m.p. 192°. (Found: N, 17.2. $C_8H_{10}ON_5Br$ requires N, 17.2%.) Attempts to replace the bromine atom in 2-amino-5-bromo-4:6-dimethylpyrimidine by a hydroxyl group using NaOH under varying conditions were unsuccessful, but the change was finally effected by means of $Ba(OH)_2$ with Cu bronze as catalyst. 2-Amino-5-bromo-4:6-dimethylpyrimidine (5 g.), $Ba(OH)_2 \cdot 8H_2O$ (15 g.), Cu bronze (0.1 g.) and water (50 ml.) were heated in a sealed tube at 180–200°

for 10 hr. The reaction mixture, after addition of water (60 ml.), was boiled to remove NH_3 and filtered. After removal of Ba from the filtrate as BaSO_4 , the neutral Ba-free solution was evaporated to dryness *in vacuo* and extracted with boiling ethanol. The ethanol extract was evaporated to dryness and the residue extracted with hot acetone to give a solution from which crude 2-amino-5-hydroxy-4:6-dimethylpyrimidine separated on cooling. This was recrystallized from acetone, giving pale-grey needles, m.p. 222° , soluble in water and ethanol. Yield, 0.4 g. (Found: C, 51.8; H, 6.7; N, 30.3. $\text{C}_8\text{H}_{10}\text{ON}_3$ requires C, 51.8; H, 6.5; N, 30.2%.) 2-Amino-5-hydroxy-4:6-dimethylpyrimidine hydrochloride, prepared by addition of a slight excess of HCl to the base, formed colourless needles, m.p. 187° , very soluble in water and ethanol, sparingly in acetone and ether. (Found: N, 23.5. $\text{C}_8\text{H}_{10}\text{ON}_3\text{Cl}$ requires N, 23.9%.)

2:5-Dihydroxy-4:6-dimethylpyrimidine hydrochloride. 2-Amino-5-hydroxy-4:6-dimethylpyrimidine (1 g.) was boiled under reflux with HCl (25 ml., 10N) for 5 hr. The solution was evaporated to dryness and the residue dissolved in a minimum volume of hot ethanol. After standing overnight at 5° light brown prisms of 2:5-dihydroxy-4:6-dimethylpyrimidine hydrochloride separated, m.p. 260° (decomp.) after recrystallization from ethanol. Yield, 0.2 g. (Found: C, 40.7; H, 5.3; N, 16.2; Cl, 19.8. $\text{C}_8\text{H}_8\text{O}_2\text{N}_2\text{Cl}$ requires C, 40.8; H, 5.1; N, 15.9; Cl, 20.1%.) Attempts to prepare the free base were unsuccessful.

2-Amino-5-bromopyrimidine-4:6-dicarboxylic acid. This acid was prepared in order to confirm the constitution of 2-amino-5-bromo-4:6-dimethylpyrimidine described above, since Huber & Hölscher (1938) gave no satisfactory proof that bromination had occurred in position 5. Price, Leonard & Whittle (1945), using more vigorous conditions of bromination, showed that a bromomethyl compound could be formed as well as Huber & Hölscher's compound, but accepted Huber & Hölscher's constitution for the latter. 2-Amino-5-bromo-4:6-dimethylpyrimidine would be expected to yield 2-amino-5-bromopyrimidine-4:6-dicarboxylic acid on oxidation. If the bromine atom had been introduced into position 4 or 6, giving a bromomethyl group, oxidation should have given a Br-free dicarboxylic acid. 2-Amino-5-bromo-4:6-dimethylpyrimidine (1 g.) was boiled with KMnO_4 (3 g.) in 30 ml. N-NaOH. After filtration and cooling, the solution was extracted with ether for 3 hr. to remove unchanged 2-amino-5-bromo-4:6-dimethylpyrimidine. The aqueous solution was adjusted to pH 3 and left to stand 24 hr. at 5° . The precipitate formed was purified by dissolving in NaOH, treating with charcoal and reprecipitating by addition of HCl. Yield, 0.6 g. small yellow prisms sparingly soluble in water, ether and acetone, m.p. 204° (decomp.). (Found: C, 27.5; H, 1.7; N, 16.0; Br, 29.9%: equivalent (by titration), 128. 2-Amino-5-bromopyrimidine-4:6-dicarboxylic acid, $\text{C}_8\text{H}_6\text{O}_4\text{N}_2\text{Br}$, requires C, 27.5; H, 1.5; N, 16.0; Br, 30.5%: equivalent, 131.)

METHODS

Diet and dosage. The rabbits used were does of 2–3 kg. and were maintained on a diet of rabbit pellets and water (Bray, Ryman & Thorpe, 1947). The compounds were administered by stomach tube as suspensions in water. No toxic effects were observed with sulphamezathine at dose levels up to 1 g./kg. 4:6-Dimethylpyrimidine had no toxic effects with a dose of 1 g./kg., but 2-amino-4:6-dimethylpyrimidine (dose, 1 g./kg.) caused deep narcosis of some hours' duration from which the animals invariably recovered.

The human sulphamezathine urines used were pooled samples collected from hospital patients receiving the drug.

Estimation of metabolites. Etheral sulphate, ester and ether glucuronide (for definition see Pryde & Williams, 1933), free and total diazotizable material were estimated as previously (Bray *et al.* 1946; Bray, Lake, Neale, Thorpe & Wood, 1948). Ether-soluble acid from sulphamezathine urine was estimated by continuous extraction with ether at pH 2 and titration of the extract (after removal of solvent) with NaOH (0.02N) to pH 5 using bromocresol green as indicator. For 2-amino-4:6-dimethylpyrimidine urine extraction was carried out at pH 3–4, and titration to pH 4.5 using bromophenol blue or, alternatively, extraction at pH 6 and titration in presence of formaldehyde (Bray, James, Ryman & Thorpe, 1948). 4:6-Dimethylpyrimidine urine was extracted at pH 2 and the extract titrated to pH 4.5 using bromophenol blue.

Detection of possible metabolites by paper chromatography. The method was that used previously in this laboratory (Bray, Thorpe & White, 1950). Compounds related to sulphanic acid were detected either by coupling with diazotized *p*-nitraniline as described by Bray *et al.* (1950) except that NaOH (2N) was used as the final spray in place of Na_2CO_3 , or by diazotizing on the paper by spraying with HCl (2N) followed by NaNO_2 (0.2% w/v) and coupling with *N*-ethyl- α -naphthylamine (0.02% w/v in ethanol). 3-Hydroxysulphanilic acid or its amide could also be detected by ammoniacal AgNO_3 . The R_F values in six solvent mixtures and the colours observed with detecting reagents are recorded in Table 1. The duration of run was 16 hr. in all solvents except C (50 hr.). Hydroxydimethylpyrimidines were detected by their reducing action upon ammoniacal AgNO_3 either on standing at room temperature or after heating at 110° for 5–10 min. R_F values in five solvent mixtures of compounds reducing AgNO_3 are given in Table 2. The duration of run was 16 hr. in each solvent. Compounds identified by paper chromatography were always run alongside authentic specimens and compared in at least three different solvent mixtures. Extracts of normal urine prepared like those of the metabolic urine were examined as controls.

RESULTS

Excretion of normal metabolites. The average daily excretion of reducing material by the rabbits used in these experiments ranged from 97 to 238 mg. (calculated as glucuronic acid). The average percentage by which the daily individual normal values differed from the corresponding weekly averages used as 'baseline' for purposes of calculation was $\pm 11\%$. The corresponding values for the normal excretion of etheral sulphate were 14–51 mg. SO_3 and $\pm 17\%$ (± 6 mg. SO_3) and for normal excretion of ether-soluble acid 436–757 mg. (calculated as hippuric acid) and $\pm 8\%$.

Paper chromatograms of normal rabbit urine. Examination by paper chromatography of extracts of normal rabbit urine prepared in the same way as metabolic urines revealed no substances which could be confused with the metabolites under investigation. When, however, the urine had been hydrolysed a silver spot was obtained on the

Table 1. R_F values and colour reactions of some derivatives of sulphanilic acid

(Solvent mixtures: *A*, *n*-butanol (4 vol.), pyridine (8 vol.), saturated aqueous NaCl (5 vol.), ammonia (sp.gr. 0.880, 3 vol.); *B*, collidine saturated with water; *C*, *n*-butanol (20 vol.), ammonia (sp.gr. 0.880, 3 vol.) (monophase); *D*, isopropanol (4 vol.), ammonia (sp.gr. 0.880, 2 vol.), saturated aqueous NaCl (3 vol.); *E*, ethanol (96%, 20 vol.), ammonia (sp.gr. 0.880, 1 vol.) (monophase); *F*, *n*-butanol (5 vol.), acetic acid (1 vol.), water (4 vol.). Paper Whatman no. 4.)

Compound	Colour with diazotized <i>p</i> -nitraniline	Colour after diazotization and coupling with <i>N</i> -ethyl- α -naphthylamine	Reduction with ammoniacal AgNO ₃	R_F value in solvent mixture					
				<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>F</i>
Sulphanilic acid	Pale red	Red-purple	—	0.63	0.51	0.10	0.44	0.50	0.21
Sulphanilamide	Very pale red	Red-purple	—	0.96	0.95	0.77	0.88	0.90	0.71
2-Hydroxysulphanilic acid	Blue-purple	Red-purple	—	0.73	0.74	0.08	0.18	—	0.27
2-Hydroxysulphanilamide	Blue	Red-purple	—	0.81	0.93	0.06	0.28	0.21	0.61
3-Hydroxysulphanilic acid	Grey-purple	Orange	+	0.62	0.57	0.04	0.13	0.31	0.26
3-Hydroxysulphanilamide	Grey-purple	Orange	+	0.86	1.00	0.22	0.41	0.40	0.63
Sulphamezathine	Pale red	Red-purple	—	0.80	0.95	0.55	0.60	0.69	0.90

Table 2. R_F values of some compounds reducing ammoniacal silver nitrate

(Solvent mixtures: *A* and *C* as in Table 1; *G*, ethyl acetate (10 vol.), acetic acid (3 vol.), water (7 vol.); *H*, ethanol (96%, 1 vol.), acetone (3 vol.) (monophase); *J*, *n*-butanol (1 vol.), pyridine (1 vol.), saturated aqueous NaCl (2 vol.), as used by Evans, Parr & Evans (1949). Paper Whatman no. 4.)

Compound	Colour with ammoniacal AgNO ₃ after			R_F value in solvent mixture				
	Standing 5 min.	Standing 30 min.	Heating at 110°*	<i>A</i>	<i>C</i>	<i>G</i>	<i>H</i>	<i>J</i>
2-Hydroxy-4:6-dimethylpyrimidine	None	Pale grey	Red-brown	0.69	0.45	0.60	0.72	1.00
2:5-Dihydroxy-4:6-dimethylpyrimidine hydrochloride	None	Pale grey	Bright silver	0.36	0.10	0.10	0.23	0.15
2-Amino-5-hydroxy-4:6-dimethylpyrimidine hydrochloride	Dark grey	Dark grey	Merge into background colour	0.74	0.35	0.91	0.83	0.90
3-Hydroxysulphanilic acid	Dark grey	Dark grey		0.62	0.04	0.04	0.35	0.22
3-Hydroxysulphanilamide	Dark grey	Dark grey		0.86	0.22	1.00	0.95	1.00

* Background colour of paper becomes deep yellow-brown.

chromatogram after heating with ammoniacal AgNO₃. The R_F 's in the five solvent mixtures given in Table 2 were identical with those of 2:5-dihydroxy-4:6-dimethylpyrimidine in the same solvent mixtures. A number of compounds known to occur in urine were subjected to the hydrolysis procedure and tested on paper chromatograms. Only allantoin (after hydrolysis) gave spots comparable to those given by 2:5-dihydroxy-4:6-dimethylpyrimidine. Uric acid did not react in this way. The substance formed from allantoin giving the spots on the chromatogram has not been identified. It appeared to be present in very small amount compared to the amount of the allantoin hydrolysed.

Sulphamezathine

Quantitative studies

The results obtained after administration of sulphamezathine are summarized in Table 3. It was possible to account for an average of 88% of the dose. 79% of the dose was acetylated and 12% excreted as ether glucuronide. No increase in the excretion of ester glucuronide, ether-soluble acid or ethereal sulphate was observed. These results are in reasonable agreement with those of Smith & Williams (1948).

Table 3. Excretion products of sulphamezathine in the rabbit

(The doses ranged from 0.3 to 2.5 g./rabbit. In the four experiments where ether-soluble acid was measured the doses were 2.5 g./rabbit.)

	No. of exp.	Percentage of dose excreted	
		Range	Average
Diazotizable material			
Free	32	3-19	9
Acetylated		66-93	79
Total		82-97	88
Ethereal sulphate	7	0-1	0
Ester glucuronide	10	0	0
Ether glucuronide	10	7-17	12
Ether-soluble acid	4	0	0

Qualitative experiments

Hydrolysis of sulphamezathine. Unlike sulphanilic acid, which is hydrolysed to sulphanilic acid and 2-aminopyridine, sulphamezathine (10 g.) on boiling with HCl (5*N*) for 5 hr. gave sulphanilamide (1.3 g., m.p. 167°) sulphanilic acid (3.3 g.) and 2-hydroxy-4:6-dimethylpyrimidine (4.4 g., m.p. 197°). Under

these conditions sulphanilamide does not yield sulphanilic acid so that fission of sulphamezathine occurs at both the N—C and S—N linkages. 2-Hydroxy-4:6-dimethylpyrimidine is formed on boiling 2-amino-4:6-dimethylpyrimidine with HCl (5N) for 2 hr. Sulphamezathine is stable on boiling with NaOH (2.5N) for 5 hr.

Isolation of N⁴-acetylsulphamezathine. This compound was isolated from both human and rabbit sulphamezathine urine by continuous extraction with ether at pH 7. After recrystallization from ethanol (charcoal) the compound had m.p. 248° alone or mixed with an authentic specimen.

Attempted isolation of glucuronide. In spite of many attempts no crystalline glucuronide has been isolated from the urine of either rabbits or human patients dosed with sulphamezathine. Rabbit urine at pH 6 was continuously extracted with ether for 24 hr. and, after adjustment to pH 4, for a further 24 hr. This removed considerable quantities of acetylsulphamezathine and small amounts of sulphamezathine, but no other metabolite could be detected in the ether extracts. The residual urine was treated in the usual way for isolation of glucuronides via the lead salt (e.g. Bray *et al.* 1947). By ether extraction at pH 4 for 72 hr. a further quantity of free and acetylated sulphamezathine was removed from the lead-free glucuronide solution. The ether-extracted solution was evaporated *in vacuo* at 60° and extracted with hot ethanol, but no crystalline glucuronide could be isolated from the syrup left after removal of ethanol. No sulphamezathine or acetylsulphamezathine could be detected in the syrup by paper chromatography.

Hydrolysis of glucuronide syrup. Alkaline hydrolysis (cf. Bray *et al.* 1946) failed to yield a crystalline hydroxysulphamezathine. The syrup was, therefore, hydrolysed by boiling with HCl (5N) for 3 hr. The resulting solution, after filtration, was evaporated to dryness, and the residue exhaustively extracted with ethanol. Examination of the concentrated extract by paper chromatography showed the presence of sulphanilic acid, 3-hydroxysulphanilic acid and their amides, 2-hydroxy-4:6-dimethylpyrimidine and 2:5-dihydroxy-4:6-dimethylpyrimidine. The identifications were confirmed using all the solvent mixtures given in Tables 1 and 2. The amount of 2:5-dihydroxy-4:6-dimethylpyrimidine was very much greater than that of the compound in normal urine giving a similar spot on the chromatograms. When the syrup was hydrolysed with 2N instead of 5N-HCl, 2-amino-5-hydroxy-4:6-dimethylpyrimidine was detected in addition to the hydrolysis products mentioned above. This disposes of the possibility that the spot on the chromatogram corresponding to 2:5-dihydroxypyrimidine was entirely due to a compound derived from normal urine.

4:6-Dimethylpyrimidines

Quantitative studies

The results obtained after administration of 4:6-dimethylpyrimidine and 2-amino-4:6-dimethylpyrimidine are summarized in Table 4. More than half the dose of 4:6-dimethylpyrimidine was accounted for as ether-soluble acid and about 10 % appeared to be hydroxylated and conjugated, mainly as ethereal sulphate. The excretion of metabolites of 2-amino-4:6-dimethylpyrimidine was slow, and the values given in Table 4 represent 48 hr. excretion. This

Table 4. *Excretion products of 4:6-dimethylpyrimidine and 2-amino-4:6-dimethylpyrimidine in the rabbit*

<i>rabbit</i>	(Dose 1.0 g.)	Percentage of dose excreted	
	No. of exp.	Range	Average
4:6-Dimethylpyrimidine			
Ethereal sulphate	4	4-12	8
Ester glucuronide	17	0-5	2
Ether glucuronide	9	0	0
Ether-soluble acid	17	47-64	55
2-Amino-4:6-dimethylpyrimidine			
Ethereal sulphate	5	13-27	22
Ester glucuronide	12	0	0
Ether glucuronide	12	7-20	13
Ether-soluble acid	5	0	0

compound appears to be hydroxylated more readily than 4:6-dimethylpyrimidine, about 35 % of the dose being excreted as ethereal sulphate and ether glucuronide. None was excreted as ether-soluble acid.

Qualitative experiments

4:6-Dimethylpyrimidine. The 24 hr. urine of six rabbits which had each received 1 g. 4:6-dimethylpyrimidine was adjusted to pH 4 and continuously extracted with ether for 24 hr. The extracted material was crystallized from water (charcoal) and gave 0.3 g. small yellow prisms (m.p. 167-168°) darkening on exposure to air. There was no depression of melting point on admixture with 4-methylpyrimidine-6-carboxylic acid, m.p. 166°. (Found: N, 20.6. Calc. for C₆H₈O₂N₂: N, 20.3 %.) The ether extract gave no precipitate with saturated aqueous mercuric chloride. This suggests that no appreciable amount of 4:6-dimethylpyrimidine is excreted unchanged.

2-Amino-4:6-dimethylpyrimidine. The ether extract of the 48 hr. urine (pH 7) from ten rabbits which had received a total of 10 g. 2-amino-4:6-dimethylpyrimidine yielded 300 mg. crystalline material, which after recrystallization from water had m.p. 187°. This was identified as an equimolecular complex of 2-amino-4:6-dimethylpyrimidine and urea; mixed m.p. 186° with synthetic complex

(m.p. 187°). (Found: C, 45.6; H, 7.0; N, 38.6. Calc. for $C_7H_{13}ON_5$: C, 45.9; H, 7.1; N, 38.2%.) The residual urine after addition of HCl (0.2 vol. 10N) was boiled for 2 hr., cooled, adjusted to pH 1 and again extracted with ether for 48 hr. The extracted hydrolysed urine was evaporated to dryness *in vacuo* and the residue exhaustively extracted with ethanol. Examination of this extract by paper chromatography indicated the presence of much 2-amino-5-hydroxy-4:6-dimethylpyrimidine. The spots corresponding to 2:5-dihydroxy-4:6-dimethylpyrimidine were not sufficiently intense to be distinguished with certainty from the spots given by hydrolysed normal urine. Acetone (5 vol.) was added to the ethanol extract. The precipitate was discarded and the acetone solution, after concentration, was adsorbed on a paper powder column (57 cm. long, 3.5 cm. diameter, cf. Hough, Jones & Wadman, 1949) and eluted with a mixture of acetone (3 vol.) and ethanol (1 vol.) for 48 hr. Fractions collected between 12 and 27 hr. were combined and concentrated to a syrup which crystallized on standing. Recrystallization from ethanol with addition of acetone or ether gave 200 mg. colourless needles m.p. 187°, identified as 2-amino-5-hydroxy-4:6-dimethylpyrimidine hydrochloride (m.p. 187°) by mixed m.p. (187°) and comparison on paper chromatograms. (Found: C, 41.2; H, 5.6; N, 23.8; Cl, 19.9. $C_8H_{10}ON_5Cl$ requires C, 41.0; H, 5.7; N, 23.9; Cl, 20.2%.)

Other sulphonamides

Sulphadiazine (2-sulphanilamidopyrimidine), sulphamerazine (4-methyl-2-sulphanilamidopyrimidine), sulphapyrazine (2-sulphanilamidopyrazine), sulphapyridine (2-sulphanilamidopyridine) and sulphathiazole (2-sulphanilamidothiazole) were administered to rabbits. For each experiment the pooled urines from four rabbits which had each received 2.5 g. (1 g. in the case of sulphathiazole) were used. Ethanol extracts of the hydrolysed glucuronide syrups were prepared as already described for sulphamezathine and examined by paper chromatography. Each extract was run using the solvent mixtures A, C, D, E and F described in Table 1. The chromatograms from all the sulphonamides except sulphapyridine indicated the presence not only of both sulphanilic acid and its amide but also of small amounts of 3-hydroxysulphanilic acid and its amide. The hydroxylated acid and amide appeared to be present in amounts somewhat smaller than those from sulphamezathine, the spots from sulphamerazine showing most strongly while those from sulphathiazole were comparatively feeble. (The dosage for sulphathiazole was two-fifths that of the other sulphonamides.) Neither products of hydrolysis nor the administered sulphonamide, free or acetylated, could be detected in the glucuronide fractions before hydrolysis. Whilst the hydrolysed glucuronide

syrups from these four sulphonamides each yielded both sulphanilic acid and sulphanilamide on acid hydrolysis, no sulphanilamide or 3-hydroxysulphanilamide could be detected on the chromatograms from the hydrolysed glucuronide syrup from sulphapyridine, although both the corresponding acids were readily seen. The amount of 3-hydroxysulphanilic acid appeared to be comparable with that obtained from sulphamerazine.

DISCUSSION

Our study of the two pyrimidines has proved that oxidation of a methyl group in a 4:6-dimethylpyrimidine can occur in the rabbit since not only was there a large increase in the excretion of ether-soluble acid, but also 4-methylpyrimidine-6-carboxylic acid was isolated from the urine of rabbits receiving 4:6-dimethylpyrimidine. When, however, an amino group is introduced in position 2 both analytical evidence (Table 4) and failure of attempts at isolation suggested that no carboxylic acid was formed. There was, however, a significant increase in excretion of ethereal sulphate and ether glucuronide indicating hydroxylation corresponding to approximately one-third of the dose, compared with the very small increase in excretion of ethereal sulphate after giving 4:6-dimethylpyrimidine. Furthermore, 2-amino-5-hydroxy-4:6-dimethylpyrimidine was isolated from the hydrolysed urine of rabbits dosed with 2-amino-4:6-dimethylpyrimidine.

If sulphamezathine be regarded as a N^2 -substituted 2-amino-4:6-dimethylpyrimidine, hydroxylation in position 5 of the pyrimidine nucleus might be expected as well as absence of oxidation of a methyl group to carboxyl. In fact there is no increase in excretion of ether-soluble acid after giving sulphamezathine (Table 3) and this supports the idea that no oxidation of methyl to carboxyl occurs. Regarding sulphamezathine as a N^1 -substituted sulphanilamide, considerable N^4 -acetylation and a small degree of hydroxylation in position 3 of the benzene nucleus might be expected, since sulphanilamide is known to be largely acetylated in the rabbit, and to be hydroxylated to a very much smaller extent, forming 3-hydroxysulphanilamide (Thorpe, Williams & Shelswell, 1941; Williams, 1946). There is evidence of acetylation of sulphamezathine from the quantitative results (Table 3) and from the isolation of N^4 -acetylsulphamezathine. Unfortunately, we have failed to isolate a glucuronide from sulphamezathine urine, and acid hydrolysis of the glucuronide fraction not only splits off the glucuronic acid but also causes fission of the hydroxylated sulphamezathine molecule. Hydrolysis of sulphamezathine under similar conditions has shown that fission either at the S—N or at the N—C linkage is possible, since both sulphanilic acid and sulphanil-

amide are formed. There is the further complication that, if on boiling with acid any 2-amino-4:6-pyrimidine is formed, it would be readily converted to 2-hydroxy-4:6-pyrimidine. Similarly, 2-amino-5-hydroxy-4:6-dimethylpyrimidine can be converted, although less readily, to 2:5-dihydroxy-4:6-dimethylpyrimidine. Theoretically, then, hydrolysis of a glucuronide of hydroxylated sulphamezathine might yield sulphanilic acid (or amide), 3-hydroxysulphanilic acid (or amide), 2-hydroxy-4:6-dimethylpyrimidine, 2-amino-5-hydroxy-4:6-dimethylpyrimidine or 2:5-dihydroxy-4:6-dimethylpyrimidine. We have actually detected all these compounds in the hydrolysed urine of rabbits dosed with sulphamezathine. This clearly indicates that hydroxylation has occurred in both rings, and raises the question whether two monohydroxysulphamezathines or one dihydroxysulphamezathine are formed as metabolites. The fact that we detected sulphanilic acid and sulphanilamide argues the existence of 5-hydroxy-4:6-dimethyl-2-sulphanilamidopyrimidine (II) and the presence of 2-hydroxy-4:6-dimethylpyrimidine in the hydrolysis mixture is evidence for 2-(3'-hydroxysulphanilamido)-4:6-dimethylpyrimidine (III). The existence of the two monohydroxysulphamezathines does not, however, eliminate the possibility that some 5-hydroxy-2-(3'-hydroxysulphanilamido)-4:6-dimethylpyrimidine (IV) might

also be formed. It should, perhaps, be emphasized that neither sulphamezathine nor any of its hydrolysis products could be detected in the glucuronide syrup before hydrolysis, so that the sulphanilic acid, sulphanilamide and 2-hydroxy-4:6-pyrimidine identified after hydrolysis must have been formed by breakdown of hydroxysulphamezathines. No 3-hydroxysulphanilic acid, 3-hydroxysulphanilamide or 2-amino-5-hydroxy-4:6-dimethylpyrimidine could be detected in the syrup before hydrolysis.

The results of the examination of the hydrolysed glucuronide syrups obtained from the urine of rabbits dosed with the other sulphonamides suggests that they also can be hydroxylated both in the heterocyclic and in the benzene ring. The amounts of sul-

phanilic acid and amide in the hydrolysates were obviously much greater than those of the 3-hydroxy acid and amide so that hydroxylation of the heterocyclic ring appears to be the predominant reaction. It is, perhaps, of interest that of the six sulphonamides only sulphapyridine gave no sulphanilamide or 3-hydroxysulphanilamide on hydrolysis. This confirms earlier work in which sulphanilic acid but no sulphanilamide was isolated after hydrolysis of sulphapyridine or hydroxysulphapyridine (Bray *et al.* 1946). It would appear that the linkage between the sulphonamide nitrogen and the carbon of the pyridine ring is more stable than that with the carbon of the other heterocyclic rings.

SUMMARY

1. The metabolism of sulphamezathine (4:6-dimethyl-2-sulphanilamidopyrimidine), 4:6-dimethylpyrimidine and 2-amino-4:6-dimethylpyrimidine in the rabbit has been studied.

2. Sulphamezathine is excreted mainly acetylated (79 % of the dose). A small amount (9 % of dose) is unacetylated, and 12 % is excreted apparently conjugated as ether glucuronide. Attempts to isolate the hydroxylation product or glucuronide were unsuccessful.

3. The nature of the hydroxylation product of sulphamezathine is discussed. Examination of glucuronide hydrolysates by paper chromatography provides evidence of the formation of both 5-hydroxy-4:6-dimethyl-2-sulphanilamidopyrimidine and 2-(3'-hydroxysulphanilamido)-4:6-dimethylpyrimidine.

4. 4:6-Dimethylpyrimidine is excreted to an extent of 55 % as ether-soluble acid. 4-Methylpyrimidine-6-carboxylic acid has been isolated from metabolic urines. A small amount (8 % of the dose) is excreted as ethereal sulphate.

5. 2-Amino-4:6-dimethylpyrimidine is excreted as *O*-conjugates to an extent of 35 % of the dose (13 % as ethereal sulphate and 22 % as ether glucuronide). 2-Amino-5-hydroxy-4:6-dimethylpyrimidine has been isolated from the hydrolysed urine.

6. There is evidence that hydroxylation in position 3 in the benzene ring occurs to a small extent when sulphadiazine, sulphamerazine, sulphapyrazine, sulphapyridine or sulphathiazole are metabolized in the rabbit.

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Isolated Nuclei from Cells of the Cerebral Cortex. Preparation and Enzyme Content

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Cell nuclei have previously been isolated from the liver and certain other animal tissues (Dounce, 1943), but little is known of the biochemical properties of nerve cell nuclei or of the part played by them in the economy of the cell. The nuclei of nerve cells differ from those previously obtained, in that they come from cells so highly specialized that they have lost their power of mitosis. It may therefore be expected that any biochemical characteristics found in them will reflect, not so much the special requirements of cell division as the part played by the nucleus in the normal functional activity of the cell. The relatively large size of the nerve cell nuclei and their prominent nucleolar apparatus have suggested that they may be specially concerned in the synthesis of the nucleoprotein and other metabolites required for the maintenance of the nerve axons (Caspersson, 1947).

The method now described for the preparation in quantity of isolated nuclei from the cerebral cortex is similar to that used by Dounce for liver: it depends on grinding the tissue with dilute citrate solution at pH 6.0–6.2 to cause extrusion of the nuclei from the cells, followed by repeated differential centrifugation at varying speeds to separate the nuclei from the cytoplasm and cell debris. The method is mild enough to avoid destruction of many of the enzymes present in the tissue, and it has enabled a study of the

enzymes of the nerve cell nucleus to be made. It has been shown that these nuclei contain an active cytochrome oxidase, carbonic anhydrase, acetylcholine esterase and, contrary to some investigators who have used the Gomori histological method, active alkaline and acid phosphatases. These results give confirmation to the view that the nerve cell nucleus is metabolically active.

EXPERIMENTAL

Preparation of isolated nuclei

After removing the meninges from the brain, the grey matter was separated from the white by scraping with a blunt spatula. To 100 g. cortical tissue in a large mortar were added 240 g. crushed ice and 240 ml. water. The distilled water was previously brought to pH 7.0 by adding NaOH to neutralize any CO₂ dissolved in it. While grinding, citric acid solution (20 ml., 1.1%) was added slowly, so that the pH (glass electrode) was kept at 6.0–6.2. Grinding was continued until the ice had melted (45 min.). The mixture (600 ml.) was then strained through four thicknesses of cotton gauze (20 mesh/in.) into four 250 ml. centrifuge bottles.

The centrifuging was carried out in eight stages with a Servall G/l angle centrifuge, to which a fixed resistance of 177 Ω was connected in series to allow a wider range of low speeds to be obtained. At each stage the supernatant