Observations on the Fate of Some Aliphatic Sulphonic Acids in the Rat

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Detailed studies on the metabolism of sulphonic acids in the animal have been mainly limited to members of the aromatic series (cf. Williams, 1947). The earlier work on the fate of administered aliphatic sulphonic acids is conflicting and has been discussed by Schmidt & Clark (1922). More recent investigations suggest that, with the possible exception of L-cysteic acid, these compounds are not oxidized to any significant extent to sulphate or thiosulphate, as was claimed by Salkowski (1918) and others, but are probably excreted unchanged. However, the reported excretions of various sulphonic acids administered to animals, determined either from increases in urinary neutral sulphur or by direct isolation of salts of the acids from urine, have not been quantitative and do not preclude the possibility of metabolism of these compounds by other paths.

In the experiments described here, the sodium salts of some aliphatic sulphonic acids, related structurally to carboxylic acids of biochemical interest, have been administered to rats by subcutaneous injection, and a study has been made of their effect on the output of urinary sulphate. These acids were methanesulphonic, iodomethanesulphonic, isethionic (β-hydroxyethanesulphonic), β-sulphopropionic and sulphoacetic acids and taurine. None of these compounds was found to be oxidized to sulphate. Changes in the neutral sulphur excretion of dosed animals and the isolation of two of the acids from urine as their phenylhydrazine salts indicate that these six sulphonic acids are excreted largely if not entirely in unchanged form.

MATERIALS AND METHODS

Preparation of compounds. Sodium methanesulphonate and sodium sulphamate were prepared from commercial samples of the acids; sodium iodomethanesulphonate and disodium sulphoacetate were prepared by the action of Na_2SO_3 on iodoform and monochloroacetic acid respectively (Lauer & Langkammerer, 1935; Stillich, 1906). Aminomethanesulphonic acid and α-aminoethanesulphonic acid were obtained from formaldehyde and acetaldehyde respectively by the action of $NaHSO_3$ and NH_3 (Raschig & Prahl, 1926; Backer & Mulder, 1934). Propionic acid was sulphonated photochemically to β-sulphopropionic anhydride which was converted to disodium β-sulphopropionate (Kharasch, Chao & Brown, 1940). Sodium isethionate was prepared by the action of ethylene oxide on $NaHSO_3$ (Lauer & Hill, 1936). The taurine was a commercial preparation (Eastman Kodak Co.). All compounds were free from sulphate and sulphite.

Plan of metabolism experiments. Pairs of male rats (100–150 g. wt.) were housed in metabolism cages with free access to water and to a standard diet of the following percentage composition: starch 34·8, plain flour 25, casein 10, dried yeast 4, margarine 22, cod-liver oil 2, marmite 2, choline chloride 0·2. The urine was collected by means of a large glass funnel and urine-faeces separator, as described previously (Maw, 1948) and preserved under toluene. Each pair of rats was kept in a metabolism cage for an acclimatization period of 4 days. The urine was collected every 24 hr. for the following 4–5 days. The dose of the appropriate compound, neutralized to pH 7 and dissolved in 2 ml. of water, was then injected subcutaneously and the urine collected every 24 hr. for a further period of 4–5 days. Each 24 hr. urine collection was made up to 100 ml. with washings from the funnel and separator, filtered, and inorganic sulphate, total sulphate and total sulphur estimated on 5 ml. samples of the filtrate. In experiments with sodium iodomethanesulphonate, inorganic iodide and iodate and organic iodine were also estimated.

Estimation of sulphate. The method for the estimation of inorganic and total sulphate was essentially that of Rosenheim & Drummond, as modified by Fiske (1921). In preliminary experiments inorganic phosphate was removed prior to the benzidine precipitation, but this was subsequently omitted when it was found that the results of inorganic sulphate estimations carried out with removal of phosphate on fourteen 24 hr. urines selected at random were not significantly different from the corresponding values obtained without removal of phosphate. The P/S ratios of urines from rats fed on the standard diet described above ranged from 1·4 to 3·6. The urines of rats fed on a diet of rat cubes contained, on the other hand, a much higher proportion of phosphate, and sulphate analyses could not be satisfactorily carried out on these urines unless the phosphate was first removed. The sulphonic acids used had no detectable effect on the estimation of sulphate, whether in pure solution or in urine, when present at concentrations which would be expected in the urines collected in the metabolism experiments.

Estimation of total sulphur. This was estimated by Fiske's (1921) method, with slight modifications in the incineration with Benedict's (1909) reagent to avoid loss by spluttering. This method was also used for estimation of sulphur in the sulphonic acids prepared.

Estimation of iodine. Organically bound iodine in urine was determined by a method based on that of Piria & Schiff (Schiff, 1879), the iodate formed being estimated iodometrically. Normal urines by this method gave titres equivalent to those of blank estimations on the reagents alone. Analysis of sodium iodomethanesulphonate by this procedure was quantitative.

RESULTS

Significance of changes in outputs of urinary sulphate and neutral sulphur. Changes occurring in the outputs of the urinary sulphur fractions have been evaluated in the generally accepted manner, by determining the average normal or basal 24 hr. outputs over the pre-dosage period and comparing these values with successive 24 hr. outputs obtained during the period following administration of the compound under study. Provided that rats were given the preliminary acclimatization period of at least 4 days in the metabolism cages, their normal outputs of inorganic and ethereal sulphate and neutral sulphur became steady and reproducible. Table 1 gives complete data for three typical metabolism experiments in which no oxidation of the administered compounds took place. The inorganic and ethereal sulphate values showed relatively small fluctuations from the mean 24 hr. outputs over the whole period. This was also true of the neutral sulphur values during the pre-dosage period, and increases occurring during the experimental period could be determined with an accuracy of about ± 0·5 mg. sulphur. The direct effect of the various sulphonic acids on endogenous sulphur excretion has not been assessed. Neither sodium isethionate nor sodium iodomethanesulphonate, given by mouth or by injection at the dosage levels employed in the metabolism experiments, produced significant changes in food consumption. Subcutaneous injection rather than ingestion was chosen as the mode of administration. While obviating complicating effects due to the action of intestinal bacteria, etc., this route undoubtedly results in rapid renal excretion of the compounds and consequently a reduction in their availability for possible metabolic reactions.

Changes in urinary inorganic sulphate and neutral sulphur following the administration of sulphonic acids to rats. Table 2 summarizes the results of a series of nine experiments in which four sulphonic acids were administered subcutaneously to pairs of rats at a dosage level of approximately 0·85 m-moles/rat. Following the injection of sodium methanesulphonate, sodium iodomethanesulphonate and taurine, no changes in the outputs of inorganic sulphate and ethereal sulphate occurred. Sodium isethionate produced consistent but very slight increases in inorganic sulphate equivalent to 1–3 % of administered sulphur which were not statistically significant. Preliminary experiments with disodium β-sulphopropionate, disodium sulphoacetate and sodium sulphamate have shown that they are also not metabolized to sulphate. Table 2 shows that the administration of the compounds caused a rise in the neutral sulphur fraction of the urine. Except in one experiment with taurine, this increase invariably occurred only during the first 24 hr. period following the dose, and as illustrated in Table 1, the neutral sulphur outputs then returned to normal. The increases taking place over this 24 hr. period amounted to 92–98 % of the sulphur content of the injected doses. Following the administration of sodium iodomethanesulphonate to rats, no iodide or iodate were found in the urine. Furthermore, organically bound iodine, which appeared in the urine only during the 24 hr. period immediately after injection, corresponded to 101–102 % of the dose. By contrast, the recovery of sulphur in the neutral sulphur fraction in experiments with taurine was far from quantitative (58 and 73·3 %). In one experiment, an increased

Table 1. *Outputs of urinary inorganic and ethereal sulphate and neutral sulphur of pairs of rats before and after administration of some aliphatic sodium sulphonates*

(Values are expressed as mg. S/rat/day. The dose of sulphonate, given in parentheses, was injected into each rat on the day indicated.)

Day	1	2	3	4	5	6	7	8	9
Exp. 3. Sodium methanesulphonate (103 mg.) on the 5th day									
Inorganic sulphate	3·86	3·80	3·90	3·71	3·87	3·79	3·63	3·91	
Ethereal sulphate	0·47	0·24	0·35	0·30	0·29	0·26	0·34	0·44	
Neutral sulphur	1·05	1·56	1·46	1·00	1·27	28·53	1·56	1·48	
Exp. 8. Taurine (107 mg.) on the 4th day									
Inorganic sulphate	1·87	2·08	1·72	1·42	1·57	1·82	2·03	2·15	2·00
Ethereal sulphate	0·39	0·43	0·26	0·38	0·40	0·43	0·45	0·45	0·50
Neutral sulphur	0·75	0·75	0·83	0·80	16·58	0·77	0·64	0·89	0·69
Exp. 9. Sodium isethionate (126 mg.) on the 4th day									
Inorganic sulphate	1·65	1·43	1·68	1·58	2·17	2·03	1·62	1·58	
Ethereal sulphate	0·41	0·44	0·48	0·46	0·34	0·36	0·40	0·40	
Neutral sulphur	0·35	0·43	0·31	0·42	26·00	0·30	0·45	0·48	

Table 2. *Changes in the outputs of inorganic sulphate and neutral sulphur of pairs of rats following the administration of some aliphatic sodium sulphonates*

Compound administered	Dose (mg./rat)	Average pre-injection outputs (mg./S/rat/day)		Outputs in 24 hr. after injection (mg./S/rat/day)		Recovery (% of dose)	
		Inorganic sulphate	Neutral sulphur	Inorganic sulphate	Neutral sulphur	Inorganic sulphate	Neutral sulphur
Sodium methanesulphonate	103	3·81	1·34	3·79	28·53	0	97·3
	103	3·54	1·64	3·45	28·16	0	95·0
Sodium iodomethanesulphonate	157	4·63	2·19	4·31	22·43	0	98·3
	151	4·71	2·20	4·31	21·00	0	95·1
Sodium isethionate	125	3·58	1·76	3·94	27·62	(1·3)	95·3
	125	3·28	1·24	4·07	26·17	(2·9)	91·9
	126	1·58	0·39	2·17	26·00	(2·1)	94·1
Taurine (sodium salt)	107	2·56	0·63	2·34	20·80	0	73·3*
	107	1·77	0·78	1·57	16·58	0	58·0

* Increase in neutral sulphur on 2nd and 3rd days after injection = 5·4 %. Total recovery = 78·7 %.

output corresponding to 5·4 %, continued during the 2nd and 3rd days after dosage, giving a total recovery of 78·7 %.

Isolation of sulphonic acids from urine. Attempts were made to identify the material causing the increase in the neutral sulphur fraction of the urine after administration of sodium iodomethanesulphonate and sodium methanesulphonate to rats. The urines of eight rats, each of which had been injected 24 hr. previously with 125 mg. of sodium iodomethanesulphonate, were pooled and evaporated to dryness under reduced pressure. The residue was continuously extracted for 3 hr. with 50 ml. of boiling 90 % aqueous ethanol and the resulting red solution evaporated to small bulk. This solidified on cooling to a brown crystalline mass, which was washed with absolute ethanol, decolorized with Norite charcoal and recrystallized from 95 % aqueous ethanol. The product, which appeared from its elementary analysis to be impure sodium iodomethanesulphonate, was converted into the phenylhydrazine salt (Latimer & Bost, 1937) by dissolving in 5 ml. of water and adding 1 ml. of concentrated H_2SO_4, followed by 2·5 g. of phenylhydrazine in 1 ml. of ethanol. The mixture was cooled in ice and the resulting crystalline mass washed with cold ethanol and ether and recrystallized from absolute ethanol. The melting point of the crystals (166–166·5°) was not depressed by admixture with authentic phenylhydrazine iodomethanesulphonate, m.p. 166–166·5°. (These and other recorded melting points are uncorrected.) (Found: C, 25·5; H, 3·2; N, 8·1; equiv. (by titration) 331. Calc. for $C_7H_{11}O_3N_2SI$: C, 25·5; H, 3·4; N, 8·5 %; equiv. 330.) The yield was 1·16 g. (71 % recovery of the sulphonic acid administered).

In a similar experiment, in which sodium methanesulphonate was administered to rats at the same dosage level, the phenylhydrazine salt isolated had m.p. 195–195·5°; the mixed melting point with authentic phenylhydrazine methanesulphonate (m.p. 195–196°) was 195–196°. (Found: C, 41·0; H, 5·6; N, 13·6; equiv. (by titration) 204. Calc. for $C_7H_{12}O_3N_2S$: C, 41·2; H, 5·9; N, 13·7 %; equiv. 204.) The yield was 1·47 g. (70 % recovery of the sulphonic acid administered). The recoveries of the two unchanged sulphonic acids from rat urine thus accounted in each case for about three-quarters of the increased neutral sulphur output. Attempts to isolate the acids as their *S*-benzyl-ψ-thiuronium salts were unsuccessful, owing to the appreciable solubility of these derivatives in water and ethanol.

Experiments with aminomethanesulphonic acid and α-aminoethanesulphonic acid. α-Aminosulphonic acids are generally quoted as being metabolic antagonists of the α-aminocarboxylic acids (Woolley 1947, 1952; Winzler, 1949). Initially, McIlwain (1941*a*) reported that the sulphonic acid analogues of glycine, alanine, valine and leucine inhibited the growth of a number of organisms. Subsequently, Spizizen (1943) found aminomethanesulphonic acid to inhibit the multiplication of a bacteriophage of *Escherichia coli*, and Ackermann (1952) found aminophenylmethanesulphonic acid and α-aminophenylethanesulphonic acid to be active inhibitors of the growth of influenza virus in tissue cultures. The fate of such compounds in the animal body would therefore be of particular interest from a chemotherapeutic standpoint. A complicating factor in studies with α-aminosulphonic acids, and one to which insufficient attention appears to have been paid, is the ease of their decomposition in solution. In the case of aminomethanesulphonic acid, Reinking, Dehnel & Labhardt (1905) and Salkowski (1918) reported that decomposition to sulphite occurred spontaneously in neutral aqueous solution, this being accelerated by heat and by the presence of acids and alkalis. McIlwain (1941*b*), however, stated that typical α-aminosulphonic acids, with the exception of the citronellal analogue, showed no appreciable decomposition to sulphite under anaerobic conditions at pH 7·6 and 37°, although breakdown was extensive at 100°.

The stability in solution of two compounds of this type, aminomethanesulphonic acid and α-aminoethanesulphonic acid, was reinvestigated. Solutions (0·02 M) of each compound in 0·1 M phosphate or veronal buffer were kept in stoppered flasks in a

Table 3. *Decomposition of aminomethanesulphonic acid and α-aminoethanesulphonic acid to sulphite and sulphate*

(Solutions (0·02 M) of each sulphonic acid in 0·1 M veronal buffer were kept 24 hr. at 37° before analysis.)

Compound	Conditions	Percentage of sulphonic acid converted		
		To sulphite	To sulphate	Total
Aminomethanesulphonic acid	Anaerobic, pH 7·6	14·8	21·1	35·9
α-Aminoethanesulphonic acid		7·0	25·0	32·0
Aminomethanesulphonic acid	Aerobic, pH 7·0	4·4	25·7	30·1
α-Aminoethanesulphonic acid		0	12·6	12·6
Aminomethanesulphonic acid	Aerobic, pH 8·0	8·0	32·9	40·9
α-Aminoethanesulphonic acid		0	74·1	74·1

thermostat at 37°. When anaerobic conditions were required, nitrogen was bubbled through the buffer during the preparation of the solutions and also throughout the duration of the experiment. Sulphite was determined iodometrically on samples withdrawn at intervals from the flasks. Sulphate was determined on solutions made up in veronal buffer only, as with phosphate buffer preliminary phosphate removal would have been necessary. Under the conditions described by McIlwain (1941*b*), α-aminoethanesulphonic acid was found to decompose slowly to sulphite (7 % breakdown after 24 hr.). Experiments using veronal buffer showed that conversion to sulphate, on the other hand, was high (25 % after 24 hr.). Over the same period aminomethanesulphonic acid was decomposed to a considerable extent to both sulphite and sulphate as shown in Table 3. In the case of either compound, the results obtained for sulphite formation in phosphate buffer and veronal buffer were identical. Further experiments, carried out under aerobic conditions at pH's 7·0 and 8·0, have been summarized in Table 3. The results obtained confirm the previous findings that the two sulphonic acids are unstable in solution. Under both aerobic and anaerobic conditions over the pH range 7–8 aminomethanesulphonic acid gave rise to as much as 9–12 % of sulphite in one hour. Under aerobic conditions this amount diminished during the next 24 hr. to the values given in Table 3, presumably as a result of air oxidation, whereas in nitrogen the amount of sulphite present increased to about 15 %. On account of their instability in aqueous solution at 37° these compounds were considered unsuitable for inclusion in the present series of experiments on rats. The experiments described above serve to emphasize that the mere absence of sulphite in a solution of an α-aminosulphonic acid does not justify the assumption that the compound is stable. The findings on growth inhibition of micro-organisms produced by α-aminosulphonic acids must therefore be accepted with reserve, and a careful study of the stability of individual compounds of this type is essential if studies involving them are to have any validity.

DISCUSSION

Salkowski (1873, 1876, 1918) reported that sodium isethionate, taurine and aminomethanesulphonic acid, when administered orally to the dog and rabbit, were metabolized to sulphate and thiosulphate, whereas sodium ethanesulphonate was excreted unchanged. Schmidt & Clark (1922) were unable to confirm the *in vivo* oxidation of the first two compounds in the dog. White, Lewis & White (1937) found that the rabbit partly oxidized orally administered taurine and L-cysteic acid, but not the injected compounds. Similar results for injected and ingested L-cysteic acid were obtained by Medes (1937) and Medes & Floyd (1942) in man. Among other published data on administered aliphatic sulphonic acids are the experiments of Flaschenträger, Bernhard, Löwenberg & Schläpfer (1934), in which sodium *n*-octanesulphonate, given by mouth to dogs, failed to increase the urinary sulphate output and Bronner & Schueller (1931) found sodium iodomethanesulphonate to be excreted mainly unchanged when administered intravenously to mice, rabbits and dogs. This was based on the absence of inorganic iodide in the urine during such experiments, and the recovery of 93 % of the administered organic iodine in the urine of dogs 24 hr. after the dose.

In the present series of experiments methanesulphonic acid, iodomethanesulphonic acid, isethionic acid, sulphoacetic acid, β-sulphopropionic acid, sulphamic acid and taurine, administered subcutaneously as their sodium salts, were found not to be oxidized to any significant extent to sulphate by the rat. This is in accord with the earlier findings by the workers referred to above, obtained with the rabbit, dog, mouse and man. Recorded instances of increases in sulphate excretion appear to be associated only with the oral administration of these compounds and it seems likely that the increases are the result of the action of intestinal flora (White *et al.* 1937).

Changes in the output of urinary neutral sulphur following the administration of the sodium salts of

methanesulphonic acid, iodomethanesulphonic acid and isethionic acid to rats showed that there was rapid and almost complete elimination of the sulphur of these compounds, indicating the lack of utilization of sulphur in this form. In the case of the first two compounds, it was found that this neutral sulphur increase was due largely, if not entirely, to the excretion of the unchanged acids, which were recovered from the urine as their phenylhydrazine salts. Flaschenträger *et al.* (1934) also recovered *n*-octanesulphonic acid unchanged from the urine of dosed dogs in the form of its barium salt. The behaviour of these sulphonic acids may be contrasted with that of taurine and L-cysteic acid, the sulphur of which is excreted only partly in the urine by the rat and other species.

There is so far no evidence of a direct pathway for the enzymic degradation of chemically stable aliphatic sulphonic acids to sulphate in the animal. Furthermore, with the exception of taurine and L-cysteic acid, these compounds do not appear to undergo any metabolic change *in vivo*.

SUMMARY

1. Six aliphatic sulphonic acids and sulphamic acid, administered to rats by subcutaneous injection as sodium salts in neutral solution, produced no significant increases in the excretion of urinary inorganic and ethereal sulphate.

2. The sulphur of injected methanesulphonic acid, iodomethanesulphonic acid and isethionic acid was excreted almost quantitatively in the neutral sulphur fraction of the urine. Injected methanesulphonic acid and iodomethanesulphonic acid were recovered in about 70 % yield from the urine of dosed rats in the form of the phenylhydrazine salts.

3. Aminomethanesulphonic acid and α-aminoethanesulphonic acid undergo extensive decomposition to sulphite and sulphate in aqueous solution at 37° and are unsuitable for inclusion in biochemical experiments of the type described.

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