THE ABSORPTION AND ELIMINATION OF METABISULPHITE AND THIOSULPHATE BY RATS

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Rats eliminated 55 per cent of the sulphur of metabisulphite and 23 per cent of that of thiosulphate into the urine in the 4 hours after the oral administration of these compounds. This sulphur was excreted almost entirely as inorganic sulphate.

A search of the literature yielded much information about the formation of sulphite and its detoxification in the animal body, but little evidence about the absorption of sulphite from the gastrointestinal tract and the manner of its excretion. Strong indication exists that orally administered metabisulphite solution is absorbed by rats, for Lockett and Natoff[1] found that the ingestion of 11·1 to 25·5 mg. of sodium metabisulphite per 100 g. weight per day did not increase the wet weight of the faeces excreted. Such metabisulphite would have required 0·57 to 1·3 ml. of water for its isotonic solution. Less than a third of this volume of water would have caused a significant increase in the wet weight of the faeces if added to them, for the average wet faeces excreted was 2·31 $\pm$ 0·01 g. per 100 g. weight per day. The object of the present work has been to make some estimate of the speed and degree of this absorption and discover in what form ingested metabisulphite is excreted by this species.

Experimental

Female Wistar rats were fed diet 41 b of Stein and were housed in the laboratory for at least 1 week before use.

Experiments of Group I

Eight rats weighing 147·8 $\pm$ 1·8 g. were divided into pairs matching in weight. Two pairs drank only sodium metabisulphite (2,000 p.p.m. as SO_2) and two pairs water, for 3 days: all were deprived of food for the last 24 hours of this period. Then each animal received a volume of its drinking fluid equivalent to 5 per cent of weight by stomach tube. Immediately afterwards the bladders were emptied by gentle suprapubic pressure, each pair was put into a separate metabolism cage, and the urine was collected for 4 hours. A cross-over test was made 4 days later in which those pairs which had previously drunk the metabisulphite solution drank the water, and *vice versa*. The whole procedure was repeated.

Experiments of Group II

Twelve rats, weighing 260·5 $\pm$ 1·6 g., were assigned to four groups for tests in which oral treatments were allocated to groups according to

a 4 × 4 Latin Square design and were administered by stomach tube at intervals of 3 days. On each occasion an hydrating volume of water equivalent to 2·5 per cent body weight was given after a fast of 18 hours and was followed, 1 hour later, by a volume of test solution equal to 5 per cent of body weight. Urine was collected from each group separately throughout the next 4 hours (see Experiments Group I). The test solutions used were NaCl, 0·74 per cent; $Na_2S_2O_3$, 5 H_2O, 4·51 per cent; Na_2SO_4,10 H_2O, 5·86 per cent; $Na_2 S_2 O_5$, 3·46 per cent; all w/v in water.

Experiments of Group III

These differed from those of Group II in that the rats used weighed 267·5 ± 2·4 g., and were assigned to six groups. Treatments were given by intraperitoneal injection, in volume equivalent to 3 per cent of body weight, and consisted of 0·9 per cent NaCl w/v; 13·4 ml. 1·46 per cent anhydrous Na_2SO_4, 8·0 ml. 1·95 per cent $Na_2S_2O_5$ and 8·0 ml. 2·54 per

TABLE I

THE EFFECT OF THE INGESTION OF 5 PER CENT WEIGHT OF A SOLUTION OF SODIUM METABISULPHITE ON THE URINARY EXCRETION OF SULPHUR BY RATS IN THE SUCCEEDING 4-HOUR PERIOD

	Water load	Metabisulphite load	*t* calc. n = 14
Urine volume, ml.	8·1 ± 0·68 (16)	9·3 ± 0·64 (16)	1·36
Inorganic sulphur as SO_4'' in mg. ..	10·7 ± 2·13 (16)	33·2 ± 3·35 (16)	5·56
Reducing power as SO_4'' in mg. ..	1·3 ± 0·27 (16)	2·0 ± 0·29 (16)	2·53
Organic sulphur as SO_4''	0·3 ± 0·03 (16)	0·4 ± 0·12 (16)	0·70

The values shown are means for pairs of rats ± S.E. of the mean (number of observations).

cent $Na_2S_2O_3$,5 H_2O all w/v in water, each diluted to 100 ml. with 0·9 per cent NaCl w/v, administered to each group in turn in a four-part cross over test according to a random block design.

Chemical Methods

The total and inorganic sulphur of urine was estimated as sulphate by the method of Bray, Humphris, Thorpe, White and Wood[2], modified only by substitution of a Gallenkamp photoelectric colorimeter N.3615 for a Spekker photoelectric absorptiometer, and replacement of the Chance Neutral Filter H 508 by one with a neutral density of 1·15. Since sulphite, thiosulphate and thiocyanate reduce iodine, they were collectively estimated in urine as an increase in the reduction of iodine above control levels. 1 ml. urine was slowly added to 5 ml. of 0·1N aqueous iodine. After 2 minutes 0·2 ml. HCl (B.P.) was added and the excess iodine was titrated with 0·01N thiosulphate using mucilage of starch as indicator. Blank tests were made throughout.

RESULTS

Changes Induced in the Urinary Excretion of Sulphur by the Oral Administration of Sodium Metabisulphite in Rats

Comparison was made of the sulphur-containing compounds excreted in the urine of rats in the 4-hour period immediately following the

administration by stomach tube of either water or a solution of sodium metabisulphite in Experiments of Group I. The results of the eight tests made are summarised in Table I. The sulphate equivalent of the mean oral dose of metabisulphite administered per rat was 44·4 mg. Of this, 53 per cent was excreted in the urine in the first 4 hours after its administration. Much the larger part of this sulphur was in inorganic form, and was sulphate. There was also a small but significant increase in the reducing power of the urine attributable to the presence of sulphite, thiosulphate or thiocyanate.

Comparison of the Changes Induced in the Urinary Excretion of Sulphur by the Oral Administration of Sulphate, Thiosulphate and Metabisulphate to Rats

Experiments of Group II were used to make comparison of the sulphur containing compounds excreted in the urine in the 4 hours immediately after the administration by stomach tube of solutions of sodium chloride,

TABLE II

THE URINARY EXCRETION OF WATER AND SULPHUR BY GROUPS OF THREE RATS IN THE 4 HOURS AFTER THE ORAL ADMINISTRATION OF AQUEOUS SOLUTIONS OF SODIUM CHLORIDE, SULPHATE, METABISULPHITE AND THIOSULPHATE IN A VOLUME EQUIVALENT TO 5 PER CENT WEIGHT

Salt in loading fluid	NaCl	Na_2SO_4	$Na_2S_2O_5$	$Na_2S_2O_3$
Water load excreted per cent	46·5 ± 9·32 (4)	6·9 ± 0·82 (4)	65·5 ± 4·40 (4)	45·2 ± 11·61 (4)
Inorganic sulphur as SO_4'' in mg.	8·9 ± 0·62 (4)	22·2 ± 3·94 (4)	197·4 ± 6·47 (4)	79·2 ± 11·11 (4)
Organic sulphur as SO_4'' in mg.	1·5 ± 0·21 (4)	3·4 ± 0·37 (4)	2·1 ± 1·61 (4)	4·0 ± 1·42 (4)
Sulphur load excreted per cent	—	7·1 ± 1·15 (4)	55·1 ± 6·24 (4)	23·1 ± 3·11 (4)

The values shown are means + S.E. (number of groups).

metabisulphite, thiosulphate and sulphate in a volume equivalent to 5 per cent of body weight. Equivalent amounts of sodium were present in all these solutions and equal amounts of sulphur in the last three. The results of these experiments are summarised in Table II. The differences between the proportions of the sulphur load excreted in the 4 hours immediately after the ingestion of the three sulphur compounds were highly significant ($P = 0·01$ by t test). Approximately 55 per cent of the sulphur load of metabisulphite, 23 per cent that of thiosulphate and 7 per cent that of sulphate were eliminated. The sulphur load was excreted solely in inorganic form in each case.

Comparison of the Changes Induced in the Urinary Excretion of Sulphur by the Intraperitoneal Injection of Isotonic Solutions of metabisulphite, thiosulphate, and sulphate

Intraperitoneal injections of isotonic aqueous sodium metabisulphite in volume equivalent to 3 per cent of body weight caused an immediate restlessness rapidly followed, in each of 12 rats, by cyanosis, prostration and cardiovascular collapse. Half the animals died at this stage, within 35 minutes of the injection. The remainder had begun to recover at 40 minutes and appeared normal half an hour later. No such toxic

effects resulted from similar intraperitoneal injections of sodium thiosulphate or sulphate. The toxic symptoms of intraperitoneal metabisulphite were not seen when the dose was reduced to one-quarter by dilution of the original isotonic solution 1 part in 4 with isotonic sodium chloride. After trial, one-twelfth the LD50 concentration of metabisulphite was selected for use in comparison of the effect of intraperitoneal injections of isotonic solutions of metabisulphite, thiosulphate and sulphate in volume equivalent to 3 per cent of weight on the urinary excretion of sulphur. The results of one such experiment, typical of three, are shown in Table III. In these Group III experiments the total sulphur injected, expressed as mg. of sulphate per 100 g. weight, amounted to 4·81 when metabisulphite or thiosulphate, and 3·95 when sulphate solutions were used. Neither the form of excretion nor the extent of the excretion of this sulphur differed whether metabisulphite, thiosulphate or sulphate had been given by the intraperitoneal route. In the first 4 hours after injection

TABLE III

COMPARISON OF THE URINARY SULPHUR OUTPUTS IN THE 4 HOURS FOLLOWING THE INTRAPERITONEAL INJECTION OF SODIUM METABISULPHITE, THIOSULPHATE, SULPHATE AND CHLORIDE IN ISOTONIC SOLUTIONS, IN VOLUME EQUIVALENT TO 3 PER CENT WEIGHT

Loading solution	Sodium metabisulphite	Sodium thiosulphate	Sodium sulphate	Sodium chloride
Water load per cent	36·7 ± 5·23 (6)	44·3 ± 9·38 (6)	39·6 ± 4.42 (6)	37·2 ± 5·8 (6)
S.G.	1030 ± 3·43 (6)	1020 ± 3·59 (6)	1022 ± 2·94 (6)	—
Inorganic sulphur as SO_4'' in mg.	28·4 ± 1·56 (6)	27·6 ± 2·88 (6)	21·3 ± 6·73 (6)	6·50 ± 3·11 (6)
Total sulphur as SO_4'' in mg.	30·0 ± 1·35 (6)	29·2 ± 2·95 (6)	23·2 ± 1·84 (6)	7·70 ± 1·58 (6)
Reducing power as SO_3 in mg.	2·0 ± 0·90 (6)	2·4 ± 0·42 (6)	2·4 ± 0·33 (6)	—
Sulphur load excreted per cent	88·6 ± 5·29 (6)	84·9 ± 11·7 (6)	87·7 ± 14·16(6)	—

The values shown are means + S.E. (numbers of groups).

80 to 90 per cent of the administered sulphur was eliminated as inorganic sulphate; this was accompanied by very small amounts of iodine reducing substances. The diureses evoked by these sulphur containing solutions matched that caused by nearly equivalent sodium loading (Table III).

DISCUSSION

The rates of the urinary elimination of sulphur, provided in the forms of metabisulphite, thiosulphate or sulphate, were indistinguishable when these compounds were given by intraperitoneal injection (Table III) but differed when the oral route was substituted (Table II). The form in which the sulphur appeared in the urine was the same whether metabisulphite, thiosulphate or sulphate was administered. Therefore it may be inferred that the rates of absorption of these compounds from the gastrointestinal tract differ. Rats absorb metabisulphite more rapidly than thiosulphate, and sulphate is, as expected, absorbed only to a very limited extent.

Administered thiosulphate and metabisulphite are not excreted as such by rats because the ingestion or injection of these two iodine reducing substances caused no greater increase in the iodine reducing power of the urine during their renal excretion than did that of sulphate (Tables I and

III) and that increase was very small (Table I). All three compounds were excreted in the urine almost solely in the form of sulphate. It must therefore be concluded that the only important detoxification system for metabisulphite and thiosulphate in the rat is an oxidative one (Table III). Fridovich and Handler[3,4], have shown that the oxidation of sulphite by rat livers does take place. An intermediate thiosulphonate is formed and hydrolysed to yield a suphydryl compound and a sulphate. These reactions were shown to involve the reversible participation of a flavo-protein and hypoxanthine proved an obligatory co-factor at pH values of and in excess of 7·6.

The small amount of iodine reducing substance in normal rat urine may be, by analogy, thiosulphate (Table I) for small amounts of thio-sulphate are usually present in the urine of man[5] and in that of cats and dogs[6]. This thiosulphate of normal urine probably arises from the sulphur containing amino acids of the diet[7]. Since rat liver converts mercaptopyruvate rapidly and quantitatively to thiosulphate in the presence of sulphite[8] the slight increase in the iodine reducing power of rat urine during the elimination of the metabisulphite should probably be attributed to the excretion of slightly more than the usual trace of thiosulphate.

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