DEGRADATION TO SULPHATE OF *S*-METHYL-L-CYSTEINE SULPHOXIDE AND *S*-CARBOXYMETHYL-L-CYSTEINE SULPHOXIDE IN MAN

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SUMMARY

A nearly complete recovery of radioactivity was achieved over 14 days following the oral administration of [^{35}S]-*S*-methyl-L-cysteine sulphoxide and [^{35}S]-*S*-carboxymethyl-L-cysteine sulphoxide to four healthy male volunteers. The urine was the major pathway of excretion of radioactivity (*c.* 96% in 0-14 days; *c.* 59% in 0-24 hours), with the faecal route being relatively unimportant (*c.* 1.7% in 0-3 days). Inorganic sulphate was an important degradation product, incorporating a substantial proportion of radioactive sulphur derived from these molecules (*c.* 40% in 0-14 days; *c.* 20% in 0-24 hours). Subtle differences were noted in the pattern of radioactive sulphate excretion following administration of the two cysteine-sulphoxide compounds, suggesting that their sulphur-containing moieties may enter different catabolic routes.

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KEY WORDS

sulphoxide, sulphate, metabolism, cysteine, *S*-methyl-L-cysteine, *S*-carboxymethyl-L-cysteine

INTRODUCTION

Inorganic sulphate has been recognised for many years as a significant excretory product in human urine /1/. Values often quoted for the daily urinary output of inorganic sulphate are between 2.5-3.5 g and account for 75-95% of the total sulphur content, this being remarkably constant for a given healthy individual on a standard diet /2/. The absorption of inorganic sulphate from the gastrointestinal tract is known to be relatively inefficient with the active sulphate transporters easily being saturated /3,4/. Hence, the majority of sulphate required for essential metabolism is generated from sulphur-containing material within the body. The principal source of this inorganic sulphate has been attributed to the degradation and exhaustive oxidation of the sulphur moiety contained within three amino acids, methionine, cysteine and cystine, present as components of dietary protein. Of these, cysteine is thought to be the major provider. Pathways of intermediary metabolism have been established describing the stepwise metabolism of cysteine sulphur to sulphate but data on the ability of chemically modified cysteine molecules to enter these pathways are limited. Early studies aimed at investigating the role of cysteine analogues within growth and maintenance of health have provided some information, suggesting broadly that certain *S*-substituted cysteine molecules may be degraded to yield sulphate in the rabbit and dog /5-8/. However, information concerning man is lacking /9/.

Some of these latter compounds, namely *S*-methyl-L-cysteine and *S*-carboxymethyl-L-cysteine, have been used therapeutically in medicine as mucolytic agents and small amounts of urinary inorganic sulphate have been detected following the oral administration of radiolabelled material /10,11/. However, no data concerning the degradation of analogous sulphoxide derivatives are available. Does the initial presence of an oxygen atom on the sulphur moiety assist or hinder the eventual provision of sulphate from these molecules and is the sulphoxide an intermediate on the oxidation route to such

sulphate? Opportunity has now been taken to study in man the excretion of *S*-methyl-L-cysteine sulphoxide, a dietary component present in *Brassica* crops /12,13/, and *S*-carboxymethyl-L-cysteine sulphoxide with particular reference to the production of urinary sulphate.

MATERIALS AND METHODS

Chemicals

All m.p. are uncorrected. *S*-Methyl-L-cysteine, *S*-carboxymethyl-L-cysteine, methyl iodide, bromoacetic acid and *tert*-butyl perbenzoate were supplied by Sigma-Aldrich Chemical Co. (Gillingham, Dorset). Other chemicals used were of analytical grade, with solvents being of h.p.l.c. quality, and all were readily available within the laboratory.

Radiolabelled [^{35}S]-methyl-L-cysteine (3-(methylsulphenyl)alanine) was synthesized by methylation of L-[^{35}S]-cysteine with methyl iodide in the presence of sodium /14/. L-[^{35}S]-Cysteine hydrochloride (3.95 g, *c.* 1.0-1.5 mCi; Amersham International plc., Amersham, UK) was dissolved in anhydrous ethanol (70 ml) and finely divided pieces of clean sodium metal (2.3 g) were added slowly. When all the sodium had dissolved an excess of methyl iodide (3.1 ml) was stirred into the reaction mixture that was warmed to 40°C, the stirring continuing for a further 30 min. Cold water (*c.* 45 ml) was then added until the cloudy solution became clear and the pH adjusted to 5.5 with glacial acetic acid. The solution was then left overnight at 4°C and the crystals formed removed by filtration and washed with a minimal quantity of acetone. Subsequent recrystallization from ethanol-water gave white crystals that ran as a single spot on t.l.c. (Rf. 0.45; 0.4 mm silica gel G; butan-1-ol-acetic acid-H_2O, 3/1/1 by vol. /10,15/, and did not depress a mixed melting point (m.p. 244-246°C dec.; literature values 238°C, 240-245°C, 245-250°C, 247-248°C, 248°C, 251°C, /8,14,16-19/ with the authentic compound (yield 60%, specific activity of 39.9 mCi/mol, radiochemical purity >99% by t.l.c. as above). Mass spectral analysis gave a molecular ion (M^+) at m/z 135 and other fragmentation ions at m/z 88, 56, 41.

Radiolabelled [^{35}S]-carboxymethyl-L-cysteine (3-[(carboxymethyl) thio]alanine; 3-(carboxymethylsulphenyl)alanine) was synthesized via an identical procedure with the exception that bromoacetic acid (6.95

g, bromoethanoic acid) was employed as the alkylating agent /14,16,20/. Recrystallization from ethanol-water gave white crystals that ran as a single spot on t.l.c. (Rf. 0.38; as above) and did not depress a mixed melting point (m.p. 192-193°C dec.; literature values 190-191°C, 191-192°C, 193-194°C, 204°C /5,16,21,22/ with the authentic compound (yield 68%, specific activity 49.9 mCi/mol, radiochemical purity >99% by t.l.c. as above). Mass spectral examination gave a base peak at m/z 161 (M^+ - H_2O) and other ions at m/z 179, 134, 116 and 88.

(±)-[^{35}S]-Methyl-L-cysteine sulphoxide (3-(methylsulphinyl)alanine) was prepared by the action of peroxide on *S*-methyl-L-cysteine. *S*-Methyl-L-cysteine (1.9 g) was finely suspended in dry acetone (40 ml) and *tert*-butyl perbenzoate (benzenecarboperoxoic acid, 1,1-dimethyl-ethyl ester; 3.3 g) was added slowly with stirring. The resulting mixture was stirred constantly for 60 min at room temperature after which the white precipitate was filtered and washed with a minimal quantity of acetone. Repeated recrystallization from acetone/water gave off-white material that ran as a single spot on t.l.c. (Rf 0.22, details as above; the system did not resolve the enantiomers) and had a relatively sharp melting point (162-165°C dec., literature values 163-164°C, 164°C, 167-168°C /23-26/ as expected (yield 45%, specific activity 39.9 mCi/mol, radiochemical purity >99% by t.l.c. as above). The material also co-chromatographed with authentic *S*-methyl-L-cysteine (as above) following reduction with hydroiodic acid (HIO_4/NaI) /24,27/. Chemical analysis (found C 31.81%, H 6.01%, N 9.21%; expected for $C_4H_9NO_3S$: C 31.79%, H 5.96%, N 9.27%) and mass spectral analysis (M^+ m/z 151, other ions at m/z 88, 63, 41) corroborated the identity. Polarographic examination demonstrated a slight dextrorotatory activity, $[\alpha]_D^{-0}$ + 11 ± 3 (*c*. 1.0 in water) /10,17,26/, denoting this was the unresolved racemate.

(±)-[^{35}S]-Carboxymethyl-L-cysteine sulphoxide (3-(carboxymethyl sulphinyl)alanine) was prepared in an identical manner starting with (±)-[^{35}S]-carboxymethyl-L-cysteine (2.5 g). Repeated recrystallization from acetone/water gave off-white material that that ran as a single spot on t.l.c. (Rf 0.20, details as above; the system did not resolve the enantiomers) and had a relatively sharp melting point (192°C dec., literature value 190-191°C /21,28,29/) indicating purity (yield 38%, specific activity 49.9 mCi/mol, radiochemical purity 99.2% by t.l.c. as above). Chemical analysis (found C 30.80%, H 4.71%, N 7.11%;

expected for $C_5H_9NO_5S$: C 30.77%, H 4.61%, N 7.18%) and mass spectral examination gave a base peak at m/z 177 (M^+ - H_2O) with other fragment ions at m/z 195, 132, 107, 104, 88 that supported the identity assignment. Polarographic measurement showed a small dextrorotatory activity, $[\alpha]_D^{21}$ + 28 ± 2 (*c.* 1.0 in 0.1 M HCl) /28,29/, indicating this was a mixture of the two enantiomers.

Human investigations

Four healthy male volunteers (age 28.5 ± 2.9 years; weight 88.1 ± 7.1 kg) were each given on two separate occasions an oral dose (200 mg) of either [^{35}S]-*S*-methyl-L-cysteine sulphoxide (52.8 μCi) or [^{35}S]-*S*-carboxymethyl-L-cysteine sulphoxide (51.2 μCi). The two doses were administered at least 16 weeks apart and were taken as a powder within a gelatine capsule with a small amount of water (100 ml) during the early morning following a light breakfast. The subjects were non-smokers and no alcohol or medication was permitted for one week before or during the study. Urine was collected into darkened plastic bottles at regular known intervals during the first 24 h and then daily for the next 13 days. Volumes were measured and the total samples divided into three aliquots that were stored at -70°C until analysis. Faeces were collected daily for 3 days into airtight containers and also frozen (-70°C) until examination. Ethical approval for the study was obtained from the local ethics committee, following consultation with the local radiation protection panel.

Measurement of radioactivity

Aliquots of urine (0.1-1.0 ml) were added directly to a toluene-based scintillation fluid mixed with Triton X-100 (2:1, v/v) /10/ and counted by liquid scintillation spectrometry (Philips Automatic Liquid Scintillation Analyser, Holland), internal standards being used for quench correction. Faeces were homogenised in water, lyophilized, ground to a powder and triplicate samples (*c.* 100 mg) combusted in oxygen (Harvey Biological Material Oxidiser, ICN Tracer Labs Ltd, Sussex, UK), the $^{35}SO_2$ so produced being trapped in an amine-based scintillation cocktail designed for this purpose /10/ and then counted as above.

Quantification and identification of inorganic sulphate

Inorganic sulphate was measured as a decrease in radioactivity after centrifugation of a urine sample to which fine powdered barium chloride had been added until no further precipitation had occurred. This radioactive precipitate itself was isolated, washed with water, and examined by infrared spectroscopy and chromatography.

Paper chromatography (Whatman 1; isobutyric acid - 1 M aq. NH_4OH, 5:3, v/v; descending method) was performed, together with standard $Na_2{}^{35}SO_4$ (Amersham International), and the dried papers scanned for radioactivity (Packard model 7200 radiochromatogram scanner, Packard Instruments Ltd., Berks, UK) and then sprayed with the potassium permanganate/barium chloride reagent which gives a diagnostic colouration for sulphate ions /30/[1]. High-pressure liquid chromatography (HPLC) was also employed. The system used consisted of an LC-XPD pump and an LC-UV detector with a Phillips PU 4700 autoinjector and PM 8251 chart recorder (Pye Unicam, Cambridge, UK). The column (250 mm x 4 mm i.d.), a reversed phase Versapack C18 (Alltech Associates, Carnforth, Lancs), was attached to a guard column (20 mm x 4 mm i.d.) packed with octadecyl-bonded glass beads (Pellicular Media, Greyhound Chromatography, Liverpool). The mobile phase was an aqueous solution of potassium hydrogen phthalate (1 mM) and tetrabutyl ammonium hydroxide (1 mM) adjusted to pH 6.1 with sodium hydroxide (0.5 M), being used with a flow rate of 1.5 ml/min. Sample injection size was 10 µl and ultraviolet detection occurred at 266 nm. Potassium hydrogen phthalate normally absorbs strongly at 266 nm but in the presence of sulphate it acts as a counter ion causing absorbance to be reduced and producing a negative peak, that may be inverted by reversing the recorder leads /31/.

Any radioactive sulphate present in the ethereal sulphate pool was determined as above after specific enzyme hydrolysis with sulphatase (1,000 units from *H. pomatia*, type H-1, Sigma Chemical Co., Ltd, Poole, Dorset, UK) in 0.2 M acetate buffer (pH 5.0), saccharo-1,4-lactone (10^{-4} M) being added to inhibit any β-glucuronidase activity /32/.

[1] The characteristic pink colouration for the sulphate ion, distinguishing it from all other ionic sulphur-oxygen combinations, is stable and remains diagnostic for 25 years, if the chromatograms are stored in the dark.

Mass spectrometry, infrared spectrometry and polarimetry

Electron impact (E.I.) mass spectrometry was carried out on a Kratos MS80RF instrument (Kratos Ltd, Urmston, Manchester, UK), employing a Kratos D555 (Data Generator) computerised display and print-out facilities, with a source temperature of 200°C using direct insertion into the ionization chamber at 200 eV. Infrared spectra were obtained from potassium bromide discs using a Nicolet MX-1 Fourier-transform I.R. spectrometer (Nicolet Instrument Corporation, Madison, WI, USA). Measurements of optical rotation were carried out on a Bellingham and Stanley model A polarimeter (Bellingham and Stanley Ltd, Tunbridge Wells, Kent, UK) at ambient temperature (20°C) using the D-line of sodium in aqueous solution at concentrations of 10 mg/ml (*c.* 1.0 g solute per 100 ml of solution).

RESULTS AND DISCUSSION

Radioactive balance studies

Results from the radioactive balance studies employing the [^{35}S]-labelled compounds showed a similar excretion pattern for all four subjects and demonstrated that both compounds were well absorbed from the gastrointestinal tract with the urine being the major route of excretion (Table 1). Over half of the administered dose (60.2 ± 3.9%, mean ± SD, [^{35}S]-*S*-methyl-L-cysteine sulphoxide; 57.5 ± 3.1%, [^{35}S]-*S*-carboxymethyl-L-cysteine sulphoxide) was excreted during the first 0-24 h with declining amounts appearing throughout the next two weeks; a nearly complete recovery of radioactivity being achieved from the urine after 14 days (96.3 ± 1.3%, [^{35}S]-*S*-methyl-L-cysteine sulphoxide; 95.8 ± 1.4%, [^{35}S]-*S*-carboxymethyl-L-cysteine sulphoxide). The faecal contribution to radioactivity elimination was relatively small and only accounted for a few percent (1.8 ± 0.7%, [^{35}S]-S-methyl-L-cysteine sulphoxide; 1.5 ± 1.0%, [^{35}S]-S-carboxymethyl-L-cysteine sulphoxide) over 3 days.

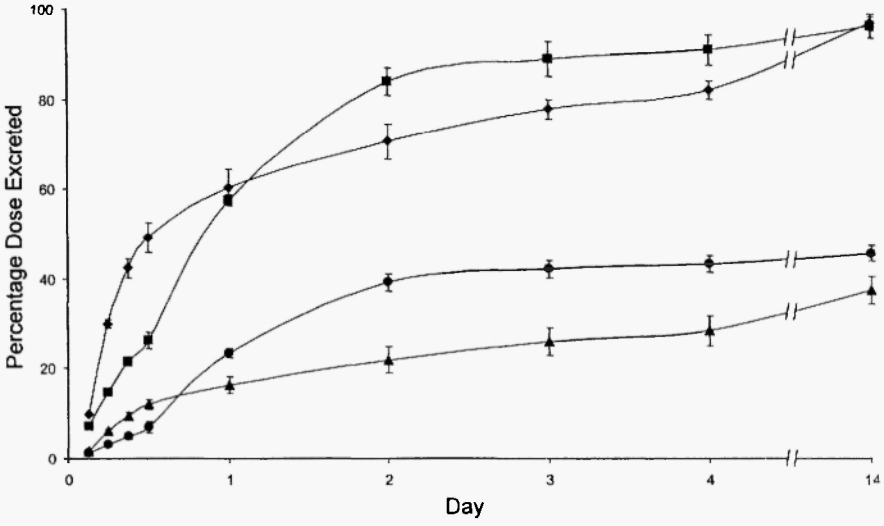
On closer inspection of the urinary data, however, significant differences could be discerned between the excretory patterns of radioactivity for the two compounds. An initial surge in output of radioactivity occurred during the 3-6 h and 6-9 h urine collection periods following [^{35}S]-*S*-methyl-L-cysteine sulphoxide administration

TABLE 1

Recovery of radioactivity expressed as a percentage of the administered dose following the oral ingestion of [^{35}S]-*S*-methyl-L-cysteine sulphoxide and [^{35}S]-*S*-carboxymethyl-L-cysteine sulphoxide by four healthy adult male volunteers

	S-Methylcysteine sulphoxide					*S*-Carboxymethylcysteine sulphoxide				
Volunteer	**1**	**2**	**3**	**4**		**1**	**2**	**3**	**4**	
Urine										
0-3 h	10.8	9.4	8.6	9.8	9.7±0.9	7.2	6.9	7.1	6.7	7.0±0.2
3-6 h	16.6	22.8	20.3	21.2	20.2±2.6	8.7	7.2	7.3	7.1	7.6±0.8
6-9 h	10.0	11.5	14.7	13.3	12.4±2.1	7.9	5.6	6.9	6.9	6.8±0.9
9-12 h	5.7	7.1	7.9	6.7	6.9±0.9	2.9	4.9	5.2	6.2	4.8±1.4
12-24 h	11.4	10.5	12.1	10.2	11.0±0.9	26.3	33.4	32.3	33.1	31.3±3.4
Day 1	54.5	61.3	63.6	61.2	60.2±3.9	53.0	58.0	58.8	60.0	57.5±3.1
Day 2	13.9	8.6	9.8	9.1	10.4±2.4	28.9	22.1	25.0	29.2	26.3±3.4
Day 3	8.2	6.6	7.2	6.3	7.0±0.8	4.8	4.8	6.9	2.8	4.8±1.7
Day 4-14	17.5	20.0	17.0	20.2	18.7±1.7	8.7	9.8	4.6	5.8	7.2±2.4
Total	94.1	96.5	97.6	96.8	96.3±1.5	95.4	94.7	95.3	97.8	95.8±1.4
Faeces										
Day 1-3	2.7	1.8	1.6	1.1	1.8±0.7	1.9	0.1	2.3	1.7	1.5±1.0
Grand total	96.8	98.3	99.2	97.9	98.1±1.0	97.3	94.8	97.6	99.5	97.3±1.9

when compared to that of [^{35}S]-*S*-carboxymethyl-L-cysteine sulphoxide (20.2 ± 2.6 vs 7.6 ± 0.2; 12.4 ± 2.1 vs 6.8 ± 0.9, respectively; $p < 0.5\%$, Gosset's *t*-test). Inversely, higher levels of radioactivity were then voided during the 12-24 h period following the [^{35}S]-*S*-carboxymethyl-L-cysteine sulphoxide dosing (11.1 ± 0.9 vs 31.3 ± 3.4, $p < 0.1\%$), leading to almost equal amounts of excreted radioactivity for both compounds in the overall 0-24 h urine. Again, over twice the amount of radioactivity was detected in the 24-48 h urine sample following the [^{35}S]-*S*-carboxymethyl-L-cysteine sulphoxide dosing (10.4 ± 2.4 vs 26.3 ± 3.4, $p < 0.1\%$). Finally, more radioactivity appeared in the later urine samples (days 4-14) following [^{35}S]-*S*-methyl-L-cysteine sulphoxide administration (18.7 ± 1.7 vs 7.2 ± 2.4; $p < 0.1\%$) (Fig. 1).



■ Total radioactivity following [^{35}S]-*S*-carboxymethyl-L-cysteine sulphoxide
◆ Total radioactivity following [^{35}S]-*S*-methyl-L-cysteine sulphoxide
● Inorganic sulphate following [^{35}S]-*S*-carboxymethyl-L-cysteine sulphoxide
▲ Inorganic sulphate following [^{35}S]-*S*-methyl-L-cysteine sulphoxide

Fig. 1: Cumulative excretion of total radioactivity and radiolabelled sulphate in urine following oral administration (200 mg) of [^{35}S]-*S*-methyl-L-cysteine sulphoxide and [^{35}S]-*S*-carboxymethyl-L-cysteine sulphoxide to four male volunteers. Values are means ± standard deviation (n = 4).

Sulphate identification and quantification

The radioactive precipitate recovered from urine samples following barium chloride treatment gave a characteristic pink spot on a buff ground (becoming white) when examined by paper chromatography and the potassium permanganate/barium chloride reagent /30/. In addition, co-chromatography with authentic sulphate (Rt 6.2 min) was achieved on HPLC /31/ and infrared examination showed a strong band at 1130-1080 cm^{-1}, characteristic of the sulphate ion (sulphites do not absorb in this region) /33,34/.

A substantial proportion of the radioactivity within the urine was present as inorganic sulphate, with around 20% being excreted in this form during the first 0-24 h and 35-45% over the total 14-day period of the study (Table 2). Again, although little variation was seen between volunteers given the same compound, differences were observed in the excretory patterns for inorganic sulphate derived from the two cysteine derivatives and these were reflected partially in the overall urinary radioactivity profile as described above.

The greatest rate of inorganic sulphate excretion (*c.* 1.47% dose/hour) occurred during the 3-6 h urine collection period following [^{35}S]-*S*-methyl-L-cysteine sulphoxide ingestion. This rate then decreased, with 16.3 ± 2.9% being voided as inorganic sulphate during the first 0-24 h and smaller amounts appearing daily until the end of the study. In contrast, little inorganic sulphate was excreted in the 0-12 h urine following [^{35}S]-*S*-carboxymethyl-L-cysteine sulphoxide administration, but after that 16.6 ± 2.5% was voided in the 12-24 h period and another 15.7 ± 3.3% during the second day (24-48 h). These latter amounts (32.3%) accounted for over 70% of the total sulphate voided with no radioactive inorganic sulphate being detected after the 10th day. Over the 0-14 days of the study a significantly greater proportion ($p < 0.5\%$; Gosset's *t*-test) of the dose was excreted as urinary radioactive inorganic sulphate following the administration of [^{35}S]-*S*-carboxymethyl-L-cysteine sulphoxide (45.3 ± 2.4%) when compared to [^{35}S]-*S*-methyl-L-cysteine sulphoxide (37.1 ± 2.4%).

TABLE 2

Recovery of radioactive inorganic sulphate expressed as a percentage of the administered dose following the oral ingestion of [^{35}S]-*S*-methyl-L-cysteine sulphoxide and [^{35}S]-*S*-carboxymethyl-L-cysteine sulphoxide by four healthy adult male volunteers

	***S*-Methylcysteine sulphoxide**					***S*-Carboxymethylcysteine sulphoxide**				
Volunteer	**1**	**2**	**3**	**4**		**1**	**2**	**3**	**4**	
Urine										
0-3 h	2.2	1.5	1.4	1.7	1.7±0.4	1.2	1.3	1.1	1.1	1.2±0.1
3-6 h	4.9	4.1	3.9	4.7	4.4±0.5	2.7	1.5	1.4	1.6	1.8±0.6
6-9 h	2.0	2.6	5.5	3.2	3.3±1.5	2.5	0.7	1.6	2.3	1.8±0.8
9-12 h	1.1	2.7	3.6	2.3	2.4±1.0	1.3	2.1	2.3	2.7	2.1±0.6
12-24 h	3.1	4.4	5.8	4.4	4.4±1.1	13.0	18.9	17.3	17.0	16.5±2.5
Day 1	13.3	15.3	20.2	16.3	16.2±2.9	20.7	24.5	23.7	24.7	23.4±1.9
Day 2	7.6	3.3	5.7	5.5	5.5±1.8	20.0	12.5	14.0	16.3	15.7±3.3
Day 3	4.6	3.2	4.3	3.9	4.0±0.6	3.2	2.5	3.8	1.6	2.8±0.9
Day 4-14	9.8	13.7	10.2	11.4	11.3±1.8	4.5	3.2	2.7	3.3	3.4±0.8
Grand total	35.3	35.5	40.4	37.1	37.0±2.4	48.4	42.7	44.2	45.9	45.3±2.4

DISCUSSION

If the values obtained following the oral dosing of [^{35}S]-carboxymethyl-L-cysteine sulphoxide in the present study are compared with the limited data available in the literature for [^{35}S]-carboxymethyl-L-cysteine administration (dose 750 mg, n = 2; /11/) it can be seen that radioactivity was more slowly excreted following the sulphoxide intake (0-24 h, 57.5 ± 3.1% sulphoxide dose versus 97.0%, 96.8% sulphide dose; 0-48 h, 83.8 ± 3.9% versus 98.0%, 98.4%; 0-72 h, 88.6 ± 3.3% versus 98.2%, 98.5%; /11/). The reverse situation is apparent when the present data for [^{35}S]-methyl-L-cysteine sulphoxide is compared to previous values for [^{35}S]-methyl-L-cysteine administration (dose 150 mg, n = 3; /10/) indicating that radioactivity is more quickly cleared via the urine following the sulphoxide dosing (0-24 h, 60.2 ± 3.9% sulphoxide dose versus 41.1 ± 2.7% sulphide dose; 0-48 h, 70.5 ± 2.1% versus 62.1 ± 5.6%; 0-72 h, 77.6 ± 2.0% versus 70.1 ± 6.3%; /10/).

Some 7-8% of the dose (750 mg) of [^{35}S]-carboxymethyl-L-cysteine was seen as sulphate in urine (0-8 h) /11/ compared to 4.8 ± 1.3% (0-9 h) from [^{35}S]-carboxymethyl-L-cysteine sulphoxide in the present study. However, much more inorganic sulphate was excreted in later periods, with some 39.1 ± 2.0% of the administered sulphoxide radioactivity being found in this form after two days. Similarly, 16.8 ± 2.8% dose (0-24 h) was found as urinary sulphate following [^{35}S]-methyl-L-cysteine administration /10/ compared with 16.3 ± 2.9% dose (0-24 h) following [^{35}S]-methyl-L-cysteine sulphoxide administration in the present study. These values are not significantly different (Gosset's *t*-test).

The production of sulphate from cysteine itself is believed to occur largely via cysteine diooxygenase activity with the formation of cysteine sulphinic acid followed by transamination to β-sulphinylpyruvate and spontaneous fission to form sulphite and thence sulphate. However, alternative pathways appear to exist in which the sulphur is removed before oxidation, such as that thought to occur entirely within the mitochondria and not involve cysteine sulphinic acid as an intermediate but mercaptopyruvate. This latter sulphydryl compound undergoes desulphuration and the reduced sulphur species enters into the sulphane-sulphur pool before being oxidised to sulphate. A direct desulphuration of cysteine to pyruvate has also been proposed /35-37/

The urinary excretion profiles for both total radioactivity and inorganic sulphate were noticeably different following administration of the two sulphoxides suggesting that they were not catabolised at the same rate or even by the same pathways. In particular, *S*-carboxymethyl-L-cysteine sulphoxide was a poorer substrate for the initial metabolism stages (possibly owing to steric factors) while the reverse was the case for *S*-methyl-L-cysteine sulphoxide. It is of interest, however, that clearance of [^{35}S]-material from the body was essentially complete, suggesting that (at least in adults) sulphate turnover was relatively labile and that sulphate derived from these or similar sources was not appreciably incorporated long-term into sulphur-containing biomolecules.

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