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Research Article

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Transsulfuration in an Adult with Hepatic Methionine Adenosyltransferase Deficiency

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Abstract

We investigated sulfur and methyl group metabolism in a 31-yr-old man with partial hepatic methionine adenosyltransferase (MAT) deficiency. The patient's cultured fibroblasts and erythrocytes had normal MAT activity. Hepatic S-adenosylmethionine (SAM) was slightly decreased. This clinically normal individual lives with a 20–30-fold elevation of plasma methionine (0.72 mM). He excretes in his urine methionine and L-methionine-*d*-sulfoxide (2.7 mmol/d), a mixed disulfide of methanethiol and a thiol bound to an unidentified group X, which we abbreviate CH_3S-SX (2.1 mmol/d), and smaller quantities of 4-methylthio-2-oxobutyrate and 3-methylthiopropionate. His breath contains 17-fold normal concentrations of dimethylsulfide. He converts only 6–7 mmol/d of methionine sulfur to inorganic sulfate. This abnormally low rate is due not to a decreased flux through the primarily defective enzyme, MAT, since SAM is produced at an essentially normal rate of 18 mmol/d, but rather to a rate of homocysteine methylation which is abnormally high in the face of the very elevated methionine concentrations demonstrated in this patient. These findings support the view that SAM (which is marginally low in this patient) is an important regulator that helps to determine the partitioning of homocysteine between degradation via cystathionine and conservation by reformation of methionine. In addition, these studies demonstrate that the methionine transamination pathway operates in the presence of an elevated body load of that amino acid in human beings, but is not sufficient to maintain methionine levels in a normal range.

Introduction

We investigated the sulfur and labile methyl group balances of a 31-yr-old man with a severe, yet incomplete, deficiency in the activity of hepatic methionine adenosyltransferase (MAT)[1] (1). MAT catalyzes the conversion of methionine to S-adenosylmethionine (SAM) (Fig. 1). SAM is a precursor of spermidine and spermine, and the obligatory methyl donor in numerous methyl transfer reactions, forming creatine, phosphatidylcholine, methylated derivatives of DNA, RNA, proteins, and catechol amines, carnitine and other compounds (2). In each of these methyl transfers, SAM gives rise to S-adenosylhomocysteine, which is then converted to homocysteine. These are necessary steps in the transsulfuration pathway responsible for most methionine degradation (3). SAM has also been shown by in vitro studies to inhibit the rate of formation of N^5-methyltetrahydrofolate by methylenetetrahydrofolate reductase (4, 5), to stimulate the activity of cystathionine β-synthase (6), and to inactivate betaine-homocysteine methyltransferase (7). Each of these effects, a priori, endows SAM with the potential to function as an important regulator such that, when SAM is raised, the partitioning of homocysteine would be less toward conservation by methylation to methionine, and more toward conversion to cystathionine for eventual degradation to inorganic sulfate. Although the possibility that the pathway is regulated by the partitioning of homocysteine between these reactions (8) has been studied in intact humans (2, 9, 10), in isolated rat hepatocytes (11, 12), and in model systems reconstituted from cell-free preparations from rats (13, 14), and the topic has been reviewed by various authors (15–17), definitive evidence on the in vivo regulatory role of SAM is not available.

In our patient we have now investigated MAT activity in nonhepatic tissues. Such activity may be normal in subjects with decreased hepatic MAT activity (18). We also identified the compound responsible for the patient's unusual breath odor, as well as several sulfur-containing compounds, in addition to methionine, which were present in abnormally elevated amounts in his urine and tissues. Further, we carried out studies that allowed us to estimate the fluxes of sulfur and of labile methyl groups in this patient. These studies furnish strong new evidence that regulatory effects of SAM, previously demonstrated in vitro only, do indeed help determine the extent to which homocysteine is conserved by the human body. Finally, the findings reported here also provide for the first time quantitative insight into the contribution to methionine degradation provided by transamination to 4-methylthio-2-oxobutyrate (19) under conditions of elevated methionine.

Methods

The patient

This 31-yr-old man with ~ 7% of normal hepatic MAT activity has been described elsewhere (1). Ascertained by the unusual odor to his breath, he had plasma methionine levels of 0.7 mM (25 times normal) with 0.46 mM methionine sulfoxide (see below). Daily urinary excretion of methionine was between 282 and 658 μmol, and steady-state excretion of methionine sulfoxide was between 1,721 and 2,017

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1. *Abbreviations used in this paper:* MAT, methionine adenosyltransferase; SAM, S-adenosylmethionine.

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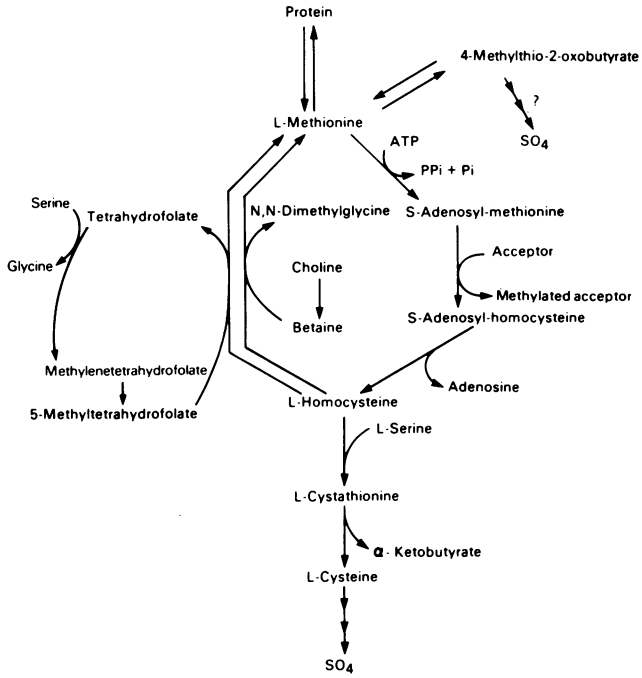

Figure 1. Methionine metabolism and related reactions.

μmol/d, both abnormally elevated. The liver parenchyma contained 25.9 nmol of soluble methionine and methionine sulfoxide/mg of protein. This amounts to 5.5 mmol of methionine/kg wet weight, or 16–80 times normal values (1). The hepatic SAM level was slightly decreased, 18 μmol/kg wet weight, compared to published normal values of 35–70 μmol/kg (9). The patient had normal physical and mental development, and liver histology was normal.

Materials

Polyethylene glycol PEG-6000 was from Koch Chemicals (Colnbrook, Bucks., UK), and from Sigma Chemical Co. (St. Louis, MO), which also supplied indoxyl sulfate, *N*-acetyl-L-methionine, methionine sulfoxide, and 4-methylthio-2-oxobutyrate. Dimethylsulfide was from Merck & Co. (Darmstadt, Federal Republic of Germany). Bio-Rad Laboratories (Richmond, CA) supplied the resin AG 50W-8×, 100–200 mesh, and Pierce Chemical Co. (Rockford, IL) supplied sulfosalicylic acid. All reagents were of analytical grade.

Methods

Clinical studies. Clinical investigations were performed after written, informed consent was obtained from the patient. During the sulfur balance studies, the patient received a constant diet,[2] the methionine and cystine content of which was estimated by the use of published tables (20, 21). His body weight remained essentially unchanged during these studies. 24-h urine collections were started and ended at 8 a.m. Urine specimens were refrigerated immediately after passage and pooled and frozen upon completion of each 24-h collection. Blood samples for determination of amino acids were drawn in the morning with the patient in the fasted state. L-Methionine was made up in gelatin capsules containing 250 mg of the amino acid and administered four capsules at a time, once in the morning and once in the evening.

Cell culture. Normal skin fibroblasts (GM-3349A) and lung fibroblasts (IMR-90) from the Human Mutant Cell Repository (Camden, NJ), and skin fibroblasts from the patient were grown to confluence in Eagle's minimum essential medium containing 10% fetal bovine serum (Gibco, Grand Island, NY), and harvested by trypsinization.

2. The food composition of each meal of this daily diet is available upon request.

Enzyme assays. MAT was assayed as described (22). For MAT measurement in erythrocytes, red blood cells were mixed with 2 vol of distilled water and centrifuged at 2,500 *g* for 10 min. For fibroblasts, cells were washed twice and suspended in 2 ml of 0.1 M potassium phosphate, pH 7.5, sonicated, and centrifuged. Supernatant fluids were assayed for MAT activity using a 120-min incubation period. One unit of enzyme activity formed 1 nmol of SAM in 60 min. Protein was determined by the Bio-Rad protein assay.

Analyses of amino acids, creatinine, creatine, and carnitine. Amino acids were quantitated using an LKB 4150 alpha amino acid analyzer (LKB Biochrom, Ltd., Cambridge, UK). Urine and heparinized plasma were deproteinized with 5% sulfosalicylic acid, filtered through 0.22 μm Millipore membranes (Millipore Corp., Bedford, MA), and stored at −20°C prior to analysis.

"Bound" methionine was taken as the total of methionine plus methionine sulfoxide in a sample of urine which had been subjected to acid hydrolysis (23), minus the methionine plus methionine sulfoxide present in the same urine without acid hydrolysis (23). This quantity represents methionine liberated from ninhydrin-negative forms (e.g., *N*-acetylmethionine) without being confounded by any reduction of methionine sulfoxide to methionine which may occur during the acid treatment (24).

Creatinine plus creatine were assayed according to the Jaffe reaction after treatment of urine with hot acid (25). Carnitine was measured as described (26).

Periodate treatment. A sample of urine (5 ml) was titrated to pH 6.85 with 1 N KOH. To a 0.45-ml aliquot at 0°C was added 0.75 ml of $NaIO_4$, 0.25 M. After 3 min at 0°C, the reaction was stopped by addition of 0.65 ml of ethylene glycol, 2.5 M, and an aliquot was used for amino acid analysis. To a second aliquot of the pH 6.85 sample, authentic L-serine and L-threonine (2 μmol each in a total of 0.088 ml H_2O) were added prior to treatment with periodate.

2-Mercaptoethanol treatment. A sample of urine (5 ml) was titrated to pH 7.5 with 1 N KOH. To a 0.5-ml aliquot was added 0.5 ml of a 1:10 (vol/vol) aqueous dilution of 2-mercaptoethanol. The solution was heated in a screw-cap tube at 100° for 60 min. To a second aliquot of the pH 7.5 sample, authentic L-methionine sulfone (550 nmol in 0.055 ml of H_2O) was added before treatment with 2-mercaptoethanol.

Synthesis and partial resolution of methionine sulfoxides. L-Methionine was treated with H_2O_2 in acetic acid as described by Iselin (27). Under these conditions the oxidation has been reported to proceed with some enantio-specificity, producing a preponderance of the L-*d*-diastereoisomer (28). The crude sulfoxide product was further resolved by use of the picrate salts (29) to yield preparations consisting approximately of either 90% L-*d*-, and 10% L-*l*-methionine sulfoxide or 10% L-*d*-, and 90% L-*l*-methionine sulfoxide.

Sulfur and sulfate determinations. Urinary inorganic sulfate and sulfate esters were measured by the turbidometric method of Lundquist et al. (30), with minor modifications to increase recovery in the case of the sulfate esters. Instead of applying the urine to a column following the removal of inorganic sulfate with $BaCl_2$, the sulfate-free urine (3 ml) was batch-adsorbed in glass tubes with a 1.5-ml packed volume of AG 50W-×8 ion exchange resin. After vortex-mixing twice, the liquid phase was removed and combined with two distilled water washings (3 ml) of the resin slurry. The combined volume (8 ml) was hydrolyzed by boiling in a 100°C water bath for 1 h, and the sulfate content, representing hydrolyzed sulfate esters, was assayed as for free inorganic sulfate. Indoxyl sulfate, 0–6 μmol, treated as above, provided a standard curve for the assay. Total urinary sulfur was measured by oxidation using Benedict's solution (31) and measuring inorganic sulfate, as above.

Breath analysis. Measured volumes of breath (100 and 250 ml) were collected in Tenax tubes and sulfur-containing compounds were analyzed by gas chromatography as previously described (32, 33).

4-Methylthio-2-oxobutyrate assay. Urinary 4-methylthio-2-oxobutyrate was measured directly by the gas chromatographic method of Favier and Caillat (34).

Analysis and identification of CH_3S-SX. The compound abbreviated CH_3S-SX is the mixed disulfide of methanethiol and a thiol bound to an unidentified group X. It was originally identified only as a "base-labile methylthio" compound, and was measured by conversion to methanethiol. To 10 μl of urine in a closed evacuated 15-ml glass vial was added 100 μl of dithiothreitol (10 mg/ml) in 0.1 M Tris-HCl, pH 10.0. After vortexing for 10 s, the mixture was left at room temperature for 90 min and 10 μl of 10 M acetic acid was injected to release the methanethiol into the headspace. The methanethiol was sampled quantitatively and assayed as for serum (33), yielding the concentration of the base-labile methylthio compound.

4-Methylthio-2-oxobutyrate was also measured indirectly by conversion to methanethiol. After the above procedure was performed for measurement of CH_3S-SX, the remaining mixture was flushed with nitrogen to remove traces of free methanethiol, and left at room temperature for 10 min in the opened vial. The vial was again evacuated and 10–15 μl of 10 M NaOH was injected, bringing the pH to 12.5–13.0. After vortexing for 15 s and letting stand for 4 min, 30 μl of 10 M acetic acid was injected and the released methanethiol was assayed (33). The values for 4-methylthio-2-oxobutyrate obtained by this indirect method corresponded to those obtained by the direct method. 4-Methylthio-2-oxobutyrate appeared stable for at least 4 d at 4°C in urine, whether tested by the direct or indirect method of analysis. This finding contrasts with that of Martensson (35), who reported a marked decrease after 6 h at room temperature or 4°C.

The "base-labile methylthio" compound was identified as a mixed disulfide, CH_3S-SX, by virtue of the production of appropriate amounts of CH_3S-SCH_2CH_3 upon addition of a basic solution of ethanethiol to the patient's urine (thiol-disulfide exchange). The chemical identity of the group, "X," is under investigation (Tangerman, A., and H. J. Blom, manuscript in preparation).

3-Methylthiopropionate. This compound was synthesized as described by Kaji et al. (36), and measured in the urine by gas chromatography (36).

Results

Nonhepatic MAT activity. Extracts of the patient's erythrocytes and fibroblasts each displayed a normal MAT activity (Table I).

Identification of the methionine oxidation product. During chromatography of the patient's urine, an unknown ninhydrin-reactive compound appeared in the area near which serine and threonine elute (Fig. 2 *a*). Both methionine sulfone and the two diastereoisomers of L-methionine sulfoxide (L-methionine-*l*-sulfoxide and L-methionine-*d*-sulfoxide), elute close to this area, although the sulfoxides resolve from one another. The compound proved insensitive to periodate treatment (Fig. 2 *b*) and, therefore, is neither threonine nor serine (Fig. 2, *c* and *d*). The unknown was sensitive to 2-mercaptoethanol (Fig. 2 *e*), suggesting it is a methionine sulfoxide rather than methionine sulfone (Fig. 2, *f* and *g*). Finally, the unknown coeluted with authentic L-methionine-*d*-sulfoxide (Fig. 2 *h*), but separated from L-methionine-*l*-sulfoxide (Fig. 2 *i*). These results identified the urinary methionine oxidation product as L-methionine-*d*-sulfoxide.

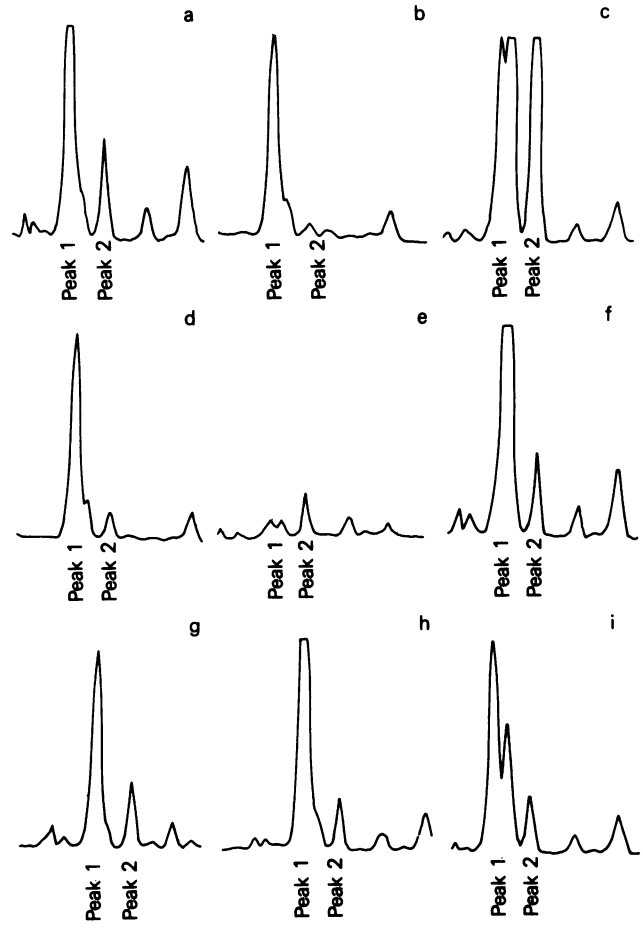

Figure 2. Determination of chemical structure of methionine oxidation product in the urine of MAT-deficient patient. (*a*) Portion of amino acidogram of patient's untreated urine (40 μl). Peak 1 contains unknown compound and threonine. Peak 2 represents serine. (*b*) After periodate treatment. Threonine is gone from peak 1; serine from peak 2. (*c*) Same as *a*, but with added serine and threonine. (*d*) Same as *c*, after periodate treatment. (*e*) Same as *a*, after 2-mercaptoethanol treatment. Peak 1, containing unknown compound, is diminished. (*f*) Same as *a*, but with added methionine sulfone, which elutes with peak 1. (*g*) Same as *f*, after 2-mercaptoethanol treatment. Methionine sulfone is relatively insensitive to 2-mercaptoethanol. (*h*) Same as *a*, but with added L-methionine-*d*-sulfoxide. Note single peak. (*i*) Same as *a*, but with added L-methionine-*l*-sulfoxide. Two peaks indicate that unknown compound is not the *L-l*-diastereoisomer.

Table I. MAT Activity in Erythrocytes and Cultured Fibroblasts from a Patient with Hepatic MAT Deficiency

	Methionine adenosyltransferase activity	
	Erythrocytes	Fibroblasts
	units/ml	*units/mg protein*
Normal controls	5.2, 4.0	0.51, 1.25
Patient	4.4	0.81

Results are means of multiple determinations: erythrocytes (3), fibroblasts (4). Erythrocyte controls were from two men aged 31 and 36 yrs. Fibroblast controls were from a human skin biopsy (first value) and from human fetal lung cells (second value).

Chromatography of the patient's plasma yielded an amino acid profile in the area of interest identical to that for his urine. Therefore, the likely origin of the urinary L-methionine-*d*-sulfoxide was the patient's plasma.

Sulfur balance studies in steady state. The patient, weighing 73 kg, selected a palatable diet, normal for him, and adhered to the same menu for 7 d. The diet was free of meat and, therefore, essentially devoid of creatine and creatinine and

very low in *N*-methylhistidines (37) and carnitine (38). It contained 3,014 calories, 87.7 g of protein, 11.2 mmol methionine, 9.3 mg-atom cystine sulfur, and 3.6 mmol choline. During the final 2 d of the diet, the patient collected his urine, which was analyzed for various sulfur-containing compounds (Table II). Sulfur intake (20.5 mg-atom) was roughly balanced by urinary excretion (22.1 mg-atom). Of the total urinary sulfur, 65% was in the form of sulfate. Free methionine (0.5 mmol), "bound" methionine (0.5 mmol), and methionine sulfoxide (1.7 mmol) accounted for 12% of total sulfur excretion.

Sulfur balance studies during methionine restriction and supplementation. The patient was placed for 11 d on a diet with methionine lowered to 4.8 mmol/d. Cystine was close to normal at 6.8 mg-atom sulfur/d, and the choline content was approximately 3.6 mmol/d. For the last 5 d of this regimen, a supplement of L-methionine (13.4 mmol) brought the methionine intake to 18.2 mmol. Total urinary sulfate did not reflect the changes in methionine intake, falling only slightly during methionine restriction, from 14.0 to 10.4 mmol/d and barely returning to 13.6 mmol/d with methionine supplementation. Plasma methionine and methionine sulfoxide concentrations did not fall substantially under methionine restriction, but did increase marginally during supplementation (Fig. 3). Changes in urinary methionine and methionine sulfoxide mirrored somewhat the changes in plasma levels, starting at 1.8 mmol/d, falling to 1.0 mmol/d on days 6 and 7, and returning to 1.9 mmol/d by day 11 (data not shown). These compounds never contributed > 10% of the total sulfur excretion. Total urinary sulfur, 19.1 mg-atom on the first day, fell only to 15.1 mg-atom during the period of methionine restriction, and reached only 19.4 mg-atom during methionine supplementation. As a result, during the 6 d of methionine restriction the patient had a net negative sulfur balance of 27.8 mg-atom, whereas during the 5 d of methionine supplementation he had a net positive balance of 37.4 mg-atom.

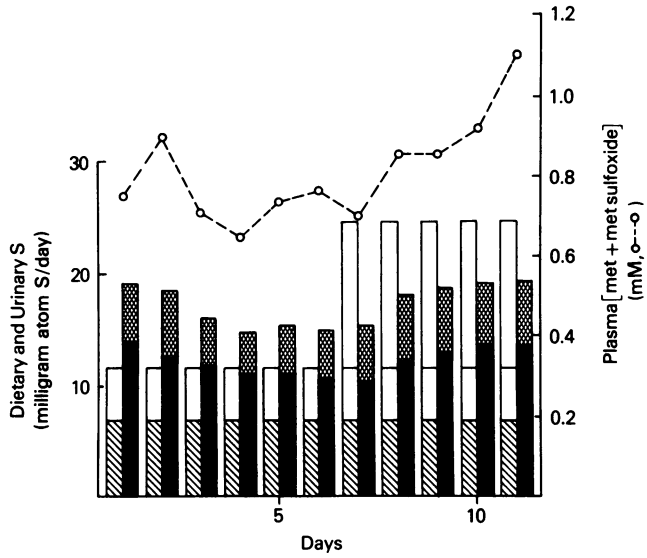

Figure 3. Urinary excretion of total sulfur and sulfate before and after the administration of supplemental L-methionine. Each day is represented by two bars. The left-hand bar represents dietary cystine (*hatched rectangle*) plus methionine (*open rectangle*). The right-hand bar represents urinary excretion of total sulfate (*solid bar*) and of nonsulfate sulfur (*cross-hatched bar*). The patient received a fixed diet throughout the study. Starting on day 7, the diet was supplemented with oral L-methionine (1 gram b.i.d.; total 13.4 mmol) in capsule form. Plasma methionine plus methionine sulfoxide concentration is plotted for each day of the study.

Table II. Urinary Excretion of Sulfur-containing Compounds by MAT-deficient Patient in Steady-State Sulfur Balance

Compound	Daily urinary excretion	
	mg-atom of S	*% of total S*
a. Total sulfur	22.1	100
b. Inorganic sulfate	12.9	58
c. Esterified sulfate	1.5	7
d. Total sulfate ($b + c$)	14.4	65
e. Calculated organic sulfur ($a - d$)	7.7	35
f. Methionine + methionine sulfoxide	2.2	10
g. Bound methionine*	0.5	2
h. 4-Methylthio-2-oxobutyrate‡	0.1	0
i. 3-Methylthiopropionate	0.03	0
j. CH_3S-SX	4.2	19
k. Dimethylsulfide	0.00	0
l. Taurine + cystine	1.6	7
m. Identified organic sulfur ($f + g + h + i + j + k + l$)	8.6	39

The patient received a fixed normal diet containing 11.2 mmol of methionine and 9.3 mg-atom of cystine sulfur daily for 7 d. Values listed are for a 24-h urine collected on day 6 of the regimen. On this day, creatinine excretion was 14.3 mmol. A second 24-h urine collected on day 7 contained similar amounts of each sulfur compound if corrected for the creatinine recovery, which was 72% of that on day 6, and outside the range of creatinine excretions for collections made in the hospital (see Results). Note similarity between calculated and identified organic sulfur.

* Corrected for 85% recovery determined for acetylmethionine after extensive acid hydrolysis.

‡ The degradation product, 4-methylthio-2-hydroxybutyrate (35), was not measured but is expected to be small, i.e., < 0.1 mg-atom of S.

Production of creatinine and other methylated metabolites. Because decreased availability of SAM might limit creatine synthesis and, consequently, creatinine production, we determined whether the MAT-deficient patient produced a normal amount of creatinine. Several facts suggested that he did. His muscle mass appeared grossly normal. He participated in rigorous long-distance running. His total body potassium (140.1 g), determined in a whole-body counting chamber, revealed a lean body mass of 63.8 kg, or 87% of total body weight (73 kg). Standards indicate that this should be reflected by a total daily creatinine production of 14.7 mmol (39). In fact, four 24-h urine collections contained 13.9±0.4 mmol creatinine. During the steady-state balance studies, urinary creatinine + creatine excretion amounted to 14.3 mmol/d (Table III); urinary total carnitine, 0.2 mmol/d; and methylhistidines, 0.4 mmol/d.

Identification of volatile sulfur compounds. The patient's breath, analyzed by gas chromatography, revealed a dimethylsulfide concentration of 5.86±0.11 nM (mean±SD, $n = 5$). Normal values ($n = 20$) are 0.34±0.03 nM (range, 0.13–0.65 nM) (33). No methanethiol or dimethyldisulfide was detected in the breath.

Table III. Steady-State SAM Formation, Methyl Balance, and Methylneogenesis in a MAT-deficient Patient

Compound	Methyl moieties
	meq/24 h
a. Urinary creatinine + creatine	14.3
b. Urinary carnitine	0.2
c. Urinary 3-methylhistidine + 1-methylhistidine	0.4
d. Other methylated compounds in urine*	0.8
e. Sarcosine from SAM‡	2.2
f. Spermidine and spermine	0.5
g. Total SAM formation ($a + b + c + d + e + f$)	18.4
h. Methyls used for methylations ($g - f$)§	17.9
i. Urinary methionine (free + bound) + methionine sulfoxide	2.7
j. Urinary 4-methylthio-2-oxobutyrate	0.1
k. Urinary 3-methylthiopropionate	0.03
l. Urinary CH_3S-SX	2.1
m. Respiratory dimethylsulfide	0.1
n. Total methyl utilization and loss ($h + i + j + k + l + m$)	22.9
o. Dietary methionine	11.2
p. Adjusted dietary choline	2.9
q. Total dietary labile methyls ($o + p$)	14.1
r. Methylneogenesis ($n - q$)	8.8

See legend to Table II.
* Estimated from reference 2, Table 3, with adjustments.
‡ Estimated from reference 8.
§ Estimated from reference 2, Table 4. No net consumption of labile methyls results from the reactions forming spermidine and spermine. The methyls in question are recycled to methionine through methylthioadenosine and methylthioribose (50).

Duplicate blood concentrations of dimethylsulfide were 73 and 96 nM (normal 3–6 nM [33]). No other volatile sulfur compounds were identified. Dimethylsulfide in the patient's urine, collected under conditions which did not ensure against losses due to volatility, was 400 nM (normal, 2 nM). Normal small amounts of methanethiol were also identified in the urine.

Methionine transamination metabolites in urine. For eight 24-h urines collected under different dietary regimens, the patient's mean±SD excretion of 4-methylthio-2-oxobutyrate was 5.6±2.4 mmol/mol creatinine (normal 0.047±0.015, $n = 10$ [Blom, H. J., et al., manuscript in preparation]). During steady state, the patient's daily output of 4-methylthio-2-oxobutyrate amounted to 0.1 mmol (Table II, *h*).

The patient's excretion of 3-methylthiopropionate under steady-state conditions was 2.0 mmol/mol creatinine (normal, 0.022±0.024, $n = 10$ [Blom, H. J., et al., manuscript in preparation]), amounting to 0.03 mmol/day (Table II, *i*).

The "base-labile methylthio" compound, CH_3S-SX, was present in six 24-h collections at 166±14 mmol/mol creatinine (normal, 1.7±0.7, $n = 10$ [Blom, H. J., et al., manuscript in preparation]). During methionine restriction (days 6 and 7, Fig. 3), the patient's value was 66 mmol/mol creatinine. The total amount of CH_3S-SX excreted during steady state was 2.1 mmol/d (Table II, *j*).

Discussion

Our patient with hepatic MAT deficiency has been normal mentally and physically for at least three decades (1). Presumably, this reflects the presence of some residual MAT activity in his liver (1) and the normal MAT activity in his nonhepatic tissues (Table I). The latter finding is similar to that reported for other patients with hepatic MAT deficiency (18, 40), and is consistent with the occurrence of forms of MAT in extrahepatic tissues under genetic control separate from that of the high K_m hepatic form.

A variety of sulfur-containing compounds are excreted by this patient in elevated quantities:

(*a*) Methionine and methionine sulfoxide, now identified as the L-methionine-*d*-sulfoxide diastereoisomer (Fig. 2). At a plasma concentration of 460 μM the latter accounted for ~ 1.7 mg-atom of the daily urinary sulfur excretion. The presence of only one diastereoisomer suggests that the compound is formed enzymatically by a reaction not previously described in human beings.

(*b*) Compounds derived via the methionine transamination pathway:

1. 4-Methylthio-2-oxobutyrate, the immediate product of methionine transamination (41). Direct assay of urine gave a value of 0.1 mmol/d under steady-state conditions. This compound has been similarly increased in other patients with hypermethioninemia (34), and in normal subjects after ingestion of D-, but not L-methionine (42). The latter finding may be attributed to the fact that to enter the mainstream of methionine metabolism the D-isomer, unlike the L-, must undergo either oxidation by D-amino acid oxidase or transamination.

2. 3-Methylthiopropionate, the urinary excretion of which amounted to 0.03 mmol/d. In rat tissues, this compound is formed by oxidative decarboxylation of 4-methylthio-2-oxobutyrate (43). It is increased also in normal humans after administration of D-methionine (36).

3. Dimethylsulfide, the only volatile sulfur-containing compound increased in the breath of our patient, and accounting for the unusual breath odor which was his presenting complaint. With a minute volume of expiration of 8 liters, the patient's 17-fold normal level of expired dimethylsulfide (5.9 nM) would result in a net loss of 0.14 mequivalent of methyl groups and 0.07 mg-atom of sulfur/d. The urinary excretion of dimethylsulfide was small. Dimethylsulfide increases in breath of normal humans after administration of D-, but not L-methionine (44). It may derive its sulfur atom and one methyl group from 4-methylthio-2-oxobutyrate, since methanethiol is a degradation product of 3-methylthiopropionate in rats (45). The second methyl group would then come via a transmethylation reaction involving SAM; an hepatic enzyme that performs this methylation has recently been described (46).

4. The mixed disulfide, CH_3S-SX, quantitatively important, with a urinary excretion of 2.1 mmol/d. To our knowledge this compound has not been found before in humans or other mammals. Although the identity of the -SX portion of the molecule remains to be established, preliminary findings indicate this moiety derives, directly or indirectly, from cysteine (Tangerman, A., and H. J. Blom, manuscript in preparation). The CH_3S- moiety appears to derive from 4-methylthio-2-oxobutyrate, or a metabolite thereof (45).

Total identified urinary sulfur in these organic compounds and in taurine and cystine (excreted in normal quantities by our patient) was 8.6 mmol (Table II, *m*). This value agreed well with the total urinary organic sulfur calculated as the difference between total sulfur and total sulfate (Table II, *e*). Thus the quantitatively important organic sulfur-containing compounds in the patient's urine have been identified, although minor amounts of others may be present. For example, the action of lactate dehydrogenase would be expected to form an amount of 4-methylthio-2-hydroxybutyrate roughly commensurate with the amount of 4-methylthio-2-oxobutyrate (35). The hydroxy compound was not measured directly in the present experiments.

The patient's excretion of sulfur-containing and methyl-containing compounds was measured while he was on several constant dietary intakes. These determinations (Tables II and III, and Fig. 3) were used to estimate the fluxes of sulfur and of labile methyl groups in the presence of a severe deficiency of hepatic MAT (Tables III and IV). Several unexpected and important points emerge from these tables:

(*a*) The patient forms at least 14.9 mmol/d of SAM (Table III, sum of the measured quantities of the methylated excretory products creatinine, creatine, carnitine, and *N*-methylhistidines). To this can almost surely be added another 3.5 mmol estimated by indirect means (Table III, $d + e + f$), giving a total of 18.4 mmol/d. Thus, in spite of his proven defect in hepatic MAT, the patient is forming an amount of the product of this enzyme which is normal for young adult males on normal diets (2, 9). This normal flux is presumably achieved by the buildup of methionine (to 720 μM) which permits any residual activity of the high K_m hepatic form of MAT to function closer to its maximal velocity than is the case at a normal methionine concentration. In normal humans the hepatic methionine concentration is well below half-saturation for the high K_m form of MAT (apparent K_m, 650 μM), the form that provides 91% of the maximal adult capacity to form SAM (47). Flux through the low K_m form(s) of MAT may also be increased at the patient's increased methionine concentration, although current uncertainties as to the kinetic constants of these isoenzymes in humans prevent accurate estimation of the magnitude of this effect.

Table IV. Sulfur Fluxes in MAT-deficient Patient in Steady-State Sulfur Balance

Compound	Sulfur
	mg-atom/24 h
a. Dietary cystine	9.3
b. Urinary cystine and taurine	1.6
c. Urinary CH_3S-SX*	2.1
d. Sulfate from cystine ($a - b - c$)	5.6
e. Dietary methionine	11.2
f. Urinary free and bound methionine and methionine sulfoxide, 2.7; 4-methylthio-2-oxobutyrate and dimethylsulfide, 0.1; CH_3S-SX, 2.1	4.9
g. Respiratory dimethylsulfide	0.1
h. Sulfate from methionine ($e - f - g$)	6.2
i. Homocysteine from transmethylation reactions	17.9
j. Homocysteine recycled ($i - h$)	11.7
k. Adjusted dietary choline	2.9
l. Minimum homocysteine recycled by N^5-methyltetrahydrofolate ($j - k$)‡	8.8

See legend to Table II.

* The −SX portion of this disulfide is presumed to derive from cysteine (Tangerman, A., and H. J. Blom, manuscript in preparation). If it derived from methionine instead, the value of *l* would be even greater, i.e., 10.9.

‡ In making this calculation it is assumed that methyl moieties equivalent to the "adjusted dietary choline" are used for betaine-dependent methylation of homocysteine. This is consistent with evidence from intact humans even under conditions in which SAM is presumably not decreased (9). Thus, normally, there may well be sufficient betaine-homocysteine methyltransferase activity to utilize all available betaine, and any possible increase in the activity of this enzyme due to lower than normal SAM concentrations (7) has not been invoked.

(*b*) In spite of the fact that a normal amount of methionine passes through the primary block at MAT, the patient converts an abnormally low proportion of methionine sulfur to sulfate. In steady state with a normal daily methionine intake of 11.2 mmol, he spilled 4.9 mmol of methionine, methionine sulfoxide, 4-methylthio-2-oxobutyrate, 3-methylthiopropionate, dimethylsulfide, and CH_3S-SX into the urine, leaving only 6.2 mmol of methionine sulfur to be converted to sulfate (Table IV, *h*). His total urinary sulfate contributed only 65% of the total urinary sulfur (normal, 80.1±5.3%). In addition, when given an abnormally high intake of methionine, the patient failed to convert the added sulfur virtually quantitatively to sulfate, as a normal subject would. In 5 d of methionine supplementation (Fig. 3), urinary sulfate increased by a total of 10.5 mmol while methionine intake increased by 68.5 mmol; thus only 15% of added methionine sulfur was converted to sulfate. Contributing to this lack of response by the patient is the damping effect of his large body load of methionine. Given a plasma volume of 3.5 liters with 720 μM methionine, this compartment would contain 2.5 mmol of methionine. A 1,200-g liver with 5.5 mmol of methionine/kg wet weight would contain 6.6 mmol. If plasma and liver have methionine concentrations representative of extracellular fluid (18.3 liters) and intracellular fluid (25.6 liters), respectively, the patient's total body methionine would approximate 154 mmol. Therefore, variations in methionine intake would only slightly perturb the concentration of this amino acid in his bodily fluids.

The patient does convert the bulk of his cyst(e)ine sulfur to sulfate. Of 9.3 mg-atom cystine sulfur taken in, 1.6 mg-atom are excreted as taurine plus cystine (Table IV, *b*). The only abnormal excretory compound in this patient which may derive (in part) from cysteine is the mixed disulfide, CH_3S-SX. If the -SX portion of this compound is formed from cysteine (Tangerman, A., and H. J. Blom, manuscript in preparation), the 2.1 mg-atom of sulfur excreted daily during steady-state balance in the -SX portion of CH_3S-SX (Table IV, *c*) should be subtracted from the cysteine sulfur available for conversion to sulfate. Daily formation of sulfate from cysteine would then be 5.6 mg-atom plus the amount of cysteine formed from methionine.

(*c*) In spite of his high body-load of methionine, the patient conserves a sizable amount of this amino acid by N^5-methyltetrahydrofolate-dependent methylation of homocysteine. Of

the 18.4 mmol of SAM formed daily, virtually all (17.9 mmol) is utilized in transmethylation reactions (Table III, *h*). Thus 17.9 mmol of homocysteine must arise from S-adenosylhomocysteine. Approximately 6.2 mmol of this homocysteine is metabolized to sulfate (Table IV, *h*), leaving 11.7 to be methylated back to methionine (Table IV, *j*). Betaine arising from dietary choline contributes at most 2.9 meq of the methyl groups needed for this recycling of homocysteine, leaving the remainder to be donated by N^5-methyltetrahydrofolate. Thus, methyl groups are being formed by neogenesis at a rate of at least 8.8 mmol/d (Table IV, *l*). The same value is obtained if one calculates the methylneogenesis as the amount by which methyl utilization and loss exceed the total dietary intake of labile methyls (Table III, *r*). Whereas this rate of homocysteine recycling due to new formation of methyls might be regarded as normal, or high normal, for a normal young adult male on a normal methionine intake with a normal methionine body load (2), it is excessively high for the same subject ingesting excess methionine (9). A normal person would dispose of the excess methionine by increasing his rate of SAM formation, using the SAM to methylate glycine to sarcosine, and oxidizing the methyl group of sarcosine; meanwhile the increased homocysteine formed from SAM would be metabolized quickly and quantitatively to sulfate with minimal recycling of homocysteine to methionine (9). This sequence avoids the conservation of methionine in the presence of excess methionine which is a striking feature of our patient. Three mechanisms may contribute to our patient's abnormal methionine conservation. First, since his MAT is probably operating near saturation with respect to methionine, any further increment in methionine may only marginally increase the flux through MAT to produce added amounts of SAM. Second, although methionine is elevated in this patient, SAM is not. Thus the decrease in the rate of formation of N^5-methyltetrahydrofolate by SAM inhibition of methylenetetrahydrofolate reductase (4), expected to occur in a normal subject when methionine concentrations are increased, does not occur in this patient, and he continues to have ample amounts of N^5-methyltetrahydrofolate available for homocysteine methylation. Third, the expected stimulation of cystathionine β-synthase activity by increased SAM (6), which may normally divert a greater portion of available homocysteine toward cystathionine, would not take place in this patient. The latter two effects achieve continued methionine conservation in the face of extreme methionine (but not SAM) excess.[3]

(*d*) Finally, the present studies help define the role in humans of the methionine transamination pathway described by Benevenga and colleagues (19, 41, 43). The contribution of this pathway to methionine catabolism in our patient may be taken as the sum of the amounts of 4-methylthio-2-oxobutyrate and its metabolic products, 3-methylthiopropionate, dimethylsulfide, and the CH_3S-moiety of CH_3S-SX. Since this approximates 2.3 mg-atom sulfur/d (Table II), the net amount of methionine undergoing conversion to 4-methylthio-2-oxobutyrate is at least 20% (2.3/11.2 × 100%) of the dietary intake.[4] This rate is attained under conditions of abnormal methionine accumulation due to the primary block at MAT. The transamination pathway is clearly inadequate to prevent this accumulation.[5]

We note that our conclusions may not pertain in their entirety to patients with isolated hypermethioninemia due to abnormalities other than that of hepatic MAT (3), or to patients with a complete MAT deficiency, who may be more seriously affected both chemically and clinically.

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3. In this interpretation, it is implied that the concentration of SAM is not so low as to produce a functionally significant decrease in the activity of N^5-methyltetrahydrofolate-homocysteine methyltransferase. The latter enzyme depends for its activity upon SAM (48, 49). Billings et al. (12) have reported evidence, however, suggesting this enzyme attains maximal activity at a concentration of SAM somewhat below that in normal liver, whereas a higher than normal concentration of SAM is required to inhibit methylenetetrahydrofolate reductase.

4. This is a minimal estimate since a portion of the sulfur originating in 4-methylthio-2-oxobutyrate may ultimately be converted to sulfate, and would not have been taken into account in the calculation used here. However, the amount of sulfate formed by this route is likely to be small, since only 6.2 mmol of sulfate is derived daily from methionine, an amount very small relative to the ~ 17.9 mmol of homocysteine formed daily, and available for degradation to sulfate by the transsulfuration pathway. We presume, therefore, that most of the sulfate which derives from methionine does so via transsulfuration.

5. The conclusion that the transamination pathway is inadequate to catabolize a normal load of methionine is supported also by the previous demonstration that humans blocked in the transsulfuration pathway due to genetically determined deficient cystathionine-synthase activity are grossly defective in their maximum capacity to convert methionine sulfur to sulfate (23, 31). Furthermore, 4-methylthio-2-oxobutyrate was assayed in the urine of one such patient. Even in a state of marked hypermethioninemia induced by methionine loading, this patient excreted only 0.16 mmol of the keto acid daily (23).

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