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Margaret Moss [a]; Rosemary H. Waring [b]
[a] Nutrition and Allergy Clinic, Stockport, Cheshire SK4 3NP, UK
[b] School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

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LABORATORY RESEARCH

The Plasma Cysteine/Sulphate Ratio: A Possible Clinical Biomarker

MARGARET MOSS MA UCTD DIPION[1] AND ROSEMARY H. WARING PHD[2]

[1]*Nutrition and Allergy Clinic, 11 Mauldeth Close, Heaton Mersey, Stockport, Cheshire SK4 3NP, UK,* [2]*School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK*

Abstract

Purpose: *To explore the possible manifestations of abnormal levels of either cysteine or sulphate, whether high or low, and the ratio between them, in human subjects.*

Design: *A case–control study of the plasma cysteine and sulphate levels and cysteine/sulphate ratio in chronically ill patients.*

Materials and Methods: *Eighty-one chronically ill patients of a nutrition clinic were suspected of having abnormal levels of sulphate and/or cysteine. Their plasma was checked to determine their cysteine and sulphate levels and cysteine/sulphate ratio. These were compared with the results of 177 controls. They were grouped according to their results, and their symptoms were listed.*

Results: *Only one patient had a ratio within the reference range, whereas 175 of the controls did. Patients already being treated with relevant nutritional supplements at the time of testing were less likely to have a ratio over 1000. Some close relatives shared abnormal test results, but manifested different symptoms.*

Conclusion: *Patients with chronic conditions including myalgic encephalomyelitis, irritable bowel syndrome, migraine, arthritis, multiple chemical sensitivity and depression are likely to benefit from tests for cysteine and sulphate, and from treatment designed to improve these levels. Oral fish oil, vitamin B2, pantothenate and molybdenum, and Epsom salt baths may help patients with low sulphate. Vitamins B2 and B6, zinc and magnesium, and a low protein diet may reduce high cysteine levels. N-acetyl cysteine, zinc and vitamin C may help those with low cysteine levels. Patients with abnormal levels of sulphate might be counselled against working in polluted conditions, where efficient sulphate conjugation is required, and against using pesticides. They might be advised to be cautious in their use of drugs, and possibly vaccines too. Further work is suggested, to investigate to what extent abnormalities in cysteine and sulphate levels are genetically determined, and to test the efficacy of the treatments outlined, both at improving the cysteine and sulphate levels, and health.*

Keywords: cysteine, sulphate, autism, chronic fatigue syndrome, fibromyalgia, rheumatoid arthritis, multiple sclerosis, irritable bowel syndrome, chemical sensitivity, food sensitivity.

INTRODUCTION

Sulphation is a major detoxification pathway for a wide range of endogenous compounds, including phenols, bile acids, catecholamines, serotonin and steroids. Exogenous substrates

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such as ascorbic acid, paracetamol and various other drugs are also conjugated with sulphate [1, 2] and the process requires the involvement of the sulphotransferase enzymes (SULT isoforms) together with PAPS (3′-phosphoadenosine-5′-phosphosulphate) as the activated sulphate donor [3]. Neurotransmitters such as dopamine are inactivated by this pathway; sulphation accounts for approximately 80% of dopamine metabolism in man, and also removes dietary neurotransmitters such as tyramine from cheese, serotonin from bananas, and *p*-phenylethylamine from chocolate. Failure to carry out this reaction leads to neurotransmitter imbalances which may have clinical consequences; patients with migraine usually have reduced activity of SULT 1A1 (PST) and sometimes also SULT 1A3 (MST) [4]. Generally, sulphation is a high-affinity but low-capacity process [5] limited by the supply of PAPS. This is in turn controlled by the levels of free organic sulphate. Absorption of this anion across the gut occurs via a $2Na^+/SO_4^{2-}$ sodium/sulphate transporter, which is easily saturated.

The amino acid cysteine is obtained from the breakdown of dietary protein, and from transulphuration of another dietary amino acid, methionine. Most of the sulphate *in vivo* (approximately 80–90%) is formed via oxidation of cysteine. This reaction sequence involves an initial (and rate-determining) step using the enzyme cysteine dioxygenase to convert the cysteine to cysteine sulphinic acid. This is degraded by transamination and subsequent fission to form sulphite, which is oxidized to sulphate by the enzyme sulphite oxidase, which has a molybdopterin complex as cofactor (Fig. 1).

Reduced supplies of sulphate not only affect the removal of drugs and neurotransmitters, they also alter the synthesis of biocomponents such as the mucin proteins which line the gastrointestinal tract. These have a protein core with peptide branches, which have many sialic acid and sulphate residues. The sulphation has been shown to be essential for the initial stages in mucin formation, and also for the twin functions of lubrication and protection of tissues from the gut contents. Sulphation here is controlled by another member of the sulphotransferase family, tyrosyl protein sulphotransferase and it has been shown [6] that reduced sulphation of gut mucin is linked with increased permeability and irritable bowel disease. Other proteins and peptides require sulphation for optimal function.

Both gastrin and the peptide cholecystokinin (CCK) need to be sulphated before being activated. Gastrin is involved in the release of stomach acid and pepsin. CCK is involved in the release of digestive enzymes. CCK and gastrin potentiate the action of secretin, which is also involved in the release of digestive enzymes. If protein digestion is inefficient, peptides can enter the bloodstream before being broken down into amino acids [7]. This process is exacerbated if the gut wall is leaky, as increased numbers of large peptides will reach the blood, causing allergic reactions. It has been proposed that opioid chemicals from foods, like glutamorphine from wheat and β-casomorphine from milk, can pass through the gut wall to reach the blood circulation and the central nervous system, causing psychiatric problems [8].

The sulphotransferase enzymes are potentially modulated by dietary components. Flavonoids, polyphenolic compounds found in fruits and vegetables, inhibit the SULT 1A1 isoform competitively and partially inhibit SULT 1A3, although other compounds may also be involved. Studies with fruit and vegetable cytosols showed that radishes, spinach, broccoli, bananas, elderberries, red cabbage, grapes, and red wine inhibit SULT 1A1 and also SULT 1A3 [9, 10]. Other substances, like chlorophenols and chloro-nitrophenols, also inhibit sulphotransferases, and other chemicals may inhibit PAPS formation.

The ability to conjugate with sulphate is variable, and requires both active sulphotransferase enzymes and adequate supplies of sulphate [11, 12]. Poor ability to conjugate amines and phenols with sulphate appears to be implicated in a variety of conditions, like food sensitivity, chemical susceptibility, Parkinson's disease, motor neurone disease, Alzheimer's disease, chronic fatigue syndrome, migraine, rheumatoid arthritis,

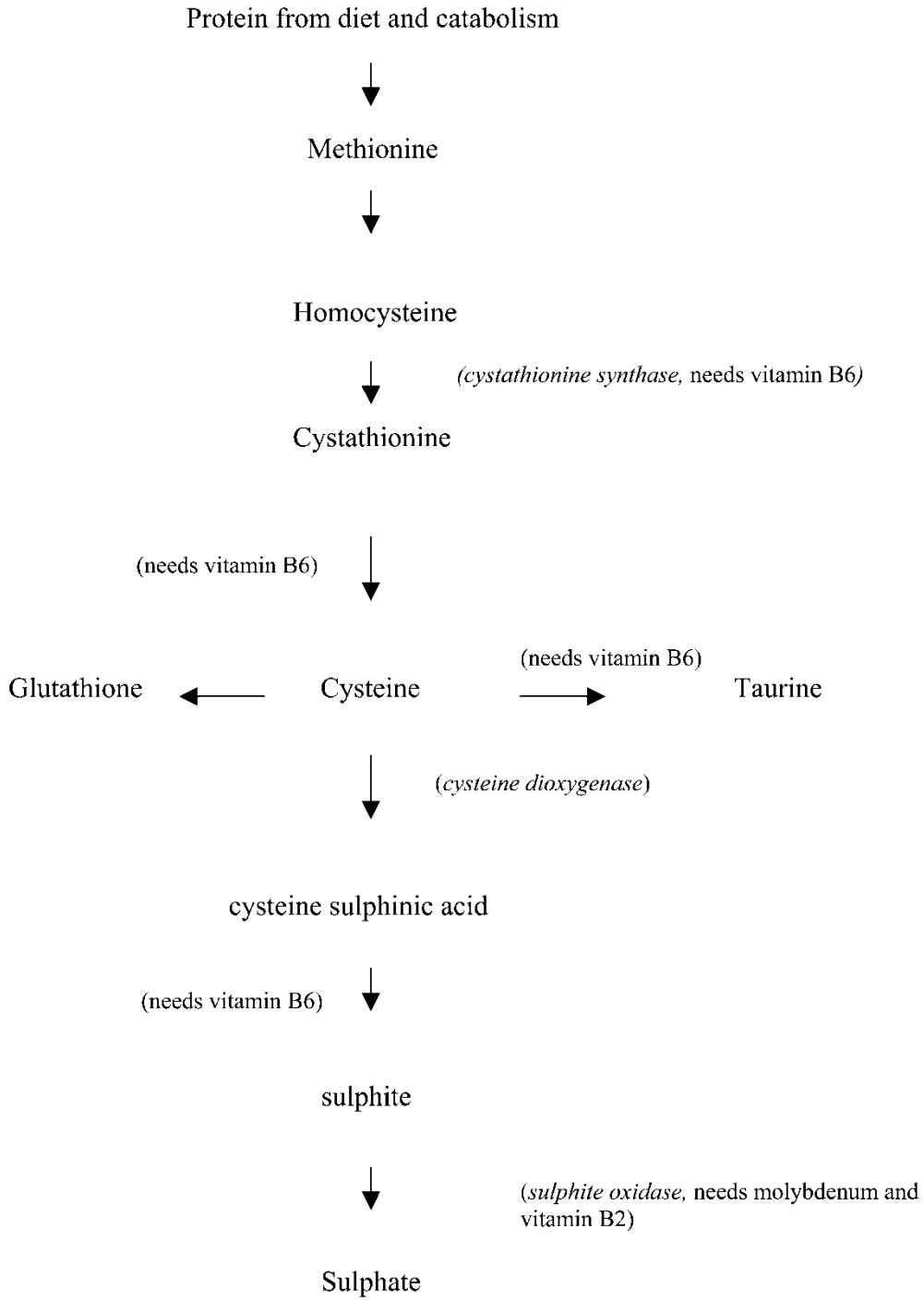

FIG. 1. Metabolism of cysteine, sulphate and taurine.

lupus erythematosus, inflammatory bowel disease, asthma, depression, hyperactivity, systemic primary biliary cirrhosis, adverse drug reactions, and autism [1, 7, 13–21].

The oxidation of cysteine, eventually forming sulphate, appears to be a critical process which is tightly controlled. Cysteine levels are rate determining in glutathione synthesis, so that their concentration effectively determines the major part of the thiol redux system. If cysteine levels are too low, the organism will be more subject to damage from reactive oxygen species, which are generally removed either by thiols or by glutathione-linked enzymes. Viruses can react with thiol-containing compounds, and this may explain why low plasma cysteine levels have been found in patients who are HIV positive [22]. N-acetyl cysteine alone, or combined with vitamin C, was found to be effective in HIV patients [23–26], as was a cysteine-rich whey protein formula [27, 28]. Possible mechanisms for the action of cysteine in HIV patients are the release of anti-HIV cytokines [29], the disruption of membranes by anti-microbial cysteine-rich protegrins [30] and the inactivation of HIV protease [31].

However, other problems may arise if cysteine levels are high, as cysteine is an excitotoxin acting at the N-methyl-D-aspartic acid (NMDA) subtype of the glutamate receptor, and hence alters neuronal transmission [19, 32]. Cysteine levels are raised in Alzheimer's and Parkinson's diseases [14, 33] and have been implicated in memory loss consequent on hippocampal damage. Increased concentrations of plasma cysteine also drive the synthesis of the pro-inflammatory leukotriene–cysteine adducts [34–36] which form complexes with complement C4 and inhibit its action; they are found in rheumatoid arthritis and other autoimmune conditions [37].

Checking plasma levels of sulphate and cysteine and the ratio between them can, therefore, be helpful when assessing how to treat a variety of people with chronic problems, as these parameters may give information on the underlying biochemical disease processes. A group of patients with chronic illness, often affecting multiple body systems, was therefore studied to determine how many were affected by abnormal cysteine and sulphate levels and how many had abnormal cysteine/sulphate ratios.

MATERIALS AND METHODS

Eighty-one patients of a nutrition clinic agreed to have a blood sample taken, centrifuged, and the plasma tested for cysteine and sulphate. Informed consent was obtained from each participant, or, in the case of children, from a parent or guardian, after the nature of the procedure had been fully explained. It was explained to them that the test was to be carried out for clinical reasons, but that the figures might also be used for research. The test was carried out using standard methods [14]. Cysteine was measured in deproteinized plasma by specific reaction with acid ninhydrin reagent and heating. The resultant pink colour was read at 560 nm against a reagent blank. Neither glutathione nor other amino acids interfered with this assay. A calibration curve of increasing concentrations of cysteine was constructed. The assay was validated using a high performance liquid chromatography (HPLC) assay for sulphur-containing amino acids. The intra-assay variation was 6.2%. Sulphate was measured by a turbidimetric assay, using deproteinized plasma. Barium chloride in agarose was added and the sample read at 500 nm, using a calibration curve prepared with potassium sulphate standards. This assay was validated using an (HPLC) assay for inorganic sulphate [38] with a coefficient of variation of 6.8%.

The clinic mostly attracts patients who feel they have failed to obtain a satisfactory diagnosis of their problems, or alternatively, who have obtained a diagnosis, but feel that no satisfactory treatment has been available. They therefore have chronic dysfunction. Patients with diagnoses like multiple sclerosis, myalgic encephalomyelitis or chronic fatigue syndrome may have been told to 'learn to live with it'. Other patients feel that the risks or side-effects of the drugs they have been offered are worse than the original illness. In many

cases, symptoms can be removed by correcting nutritional deficiencies, food elimination, and lifestyle adjustments. However, some more complex cases require more investigation. They may have been written off as 'functional', 'all in the mind', or 'fat file syndrome'. These are the patients who were offered a test. A patient history or family history of illness likely to be related to reduced sulphur metabolism was also considered when deciding whether to offer the test.

The clinic has a narrower range of patients than a general practice, as it tends to specialize in chronic problems. However, it has a wider range of patients than a hospital clinic, as it deals with all body systems and ages. This makes it possible to look at multisystem disorders, and to look at how an inherited biochemical problem may be manifested in different ways in the same family.

Plasma cysteine and sulphate levels were studied in normal adult volunteers, usually staff of academic, secretarial and technical grades, and students at the University of Birmingham, with ages ranging from 18 to 78 years (mean 32.3 ± 14.8). Values are available for 177 adult controls and also for 28 control children, and show the following ranges.

Cysteine levels were between 0.35 and 0.43 nanomols mg protein^{-1}, with a mean ± 2 standard deviations of 0.37 ± 0.04 for adults and 0.38 ± 0.06 for children.

Sulphate concentrations were usually between 1.50 and 3.10 nanomols mg protein^{-1}, mean 2.6 ± 0.9, but levels up to 9.8 have occasionally been encountered. The mean for adults for sulphate was 2.2 ± 2.1. For child controls (age range 5–12 years, mean 8.2 ± 2.9) it was 3.8 ± 2.6. These 28 children were recruited from healthy children admitted to the Birmingham Children's Hospital for routine cold surgery or minor accidents.

The ratio of cysteine $\times 1000$ to sulphate in any individual appears to be approximately constant ($\pm 10\%$), even over a period of years, if samples are taken fasting between 0800 and 0900h. Values in 175 of our 177 volunteers ranged between 89.2 and 121.3. Two were outside this range, with values of 183.6 and 271.4. It is of interest that the former individual had recurrent irritable bowel syndrome, although symptom free at the time of the test, while the latter developed multiple sclerosis 15 months after the test. The remaining volunteers were still healthy 24 months after the test. The mean cysteine/sulphate ratio for controls was 102.8 ± 9.3.

The patients were divided into categories, according to whether their cysteine and sulphate levels and (cysteine $\times 1000$)/sulphate ratio were above, below or within the reference range.

Mean values of cysteine, sulphate and the ratio were calculated for conditions with sufficient patients. Individual patients might be considered in more than one such category, as many had multiple problems.

The percentage of those with a ratio less than 1000 was calculated for two groups:

(1) those on at least some of the supplements designed to deal with poor conversion of cysteine to sulphate;
(2) those not on such supplements.

Those on supplements would also be on a diet designed with their problems in mind, while those not on supplements would be new patients, about to embark on a diet and supplements.

Some patients had already had positive results of other laboratory tests, and where appropriate, further tests were carried out.

RESULTS

None of the patients had all three measures within the reference range.

- Forty-six patients had high cysteine, low sulphate and a high ratio (Table 1).
- Twelve patients had low cysteine, low sulphate and a high ratio (Table 2).

TABLE 1. Group A: patients with high cysteine, low sulphate and high ratio

No.	Sex	Adult or child	Cysteine	Sulphate	Ratio	Taking relevant supplements	Main problem
1	F	A	2.24	1.37	1633	No	MCS
2	M	C	2.43	0.83	2922	No	Asperger
3	F	A	2.56	1.47	1731	No	ME
4	F	A	2.12	1.24	1704	No	ME
5	M	C	0.91	0.63	1444	Yes	Autistic
6	F	A	3.08	1.37	2244	No	ME
7	M	C	0.69	0.51	1350	Yes	Autistic
8	F	A	0.55	0.21	2600	No	MCS
9	F	A	0.95	0.42	2260	No	Lymphadenopathy
10	F	A	0.55	1.06	513	Yes	Asthma
11	F	A	0.60	0.93	646	No	CFS
12	F	A	0.74	0.30	2470	No	Psychosis
13	F	A	1.30	0.45	2970	Yes	Depression
14	F	A	1.29	0.91	1421	No	RA
15	F	A	1.24	0.86	1400	Some	CFS
16	F	A	0.63	0.46	1370	Some	MCS
17	F	A	0.49	0.19	2579	No	Urticaria
18	M	A	0.55	0.34	1618	No	MS
19	F	A	0.82	0.26	3154	No	CFS
20	F	A	0.61	0.12	5083	No	IBS
21	F	C	2.33	0.72	3249	No	Hyperactive
22	F	A	0.47	0.46	1032	No	IBS
23	M	A	0.94	1.13	833	Yes	MCS
24	F	A	0.55	0.73	759	No	Polymyalgia
25	F	A	0.49	0.89	551	No	ME
26	F	A	0.59	0.77	768	No	ME
27	F	A	0.88	0.47	1872	No	CFS
28	M	A	0.65	1.37	470	No	Rhinitis
29	F	A	0.59	0.11	5400	Yes	Migraine
30	M	A	0.894	0.902	991	No	CFS
31	F	A	0.587	1.0782	544	No	IBS
32	F	A	0.6877	0.4202	1637	No	Breast cancer
33	F	A	0.4627	0.1218	3799	Yes	MS
34	M	A	0.54	0.28	1930	No	IBS
35	F	A	0.59	0.87	678	Some	MCS
36	F	A	1.5457	0.3783	4086	No	Hypertension
37	F	A	0.5863	1.4457	406	Yes	MCS
38	F	A	0.5168	1.3622	379	No	RA
39	F	A	0.7311	0.5332	1371	No	Endometriosis
40	F	A	1.1245	0.3485	3227	Yes	Anorexia
41	F	A	1.6061	0.7090	2265	No	RA
42	M	A	0.56	1.20	462	No	MND
43	M	A	0.45	0.35	1282	No	PA
44	M	A	0.48	0.54	882	No	PD
45	F	A	1.4528	1.1730	1239	No	IBS
46	M	A	0.5562	0.4449	1250	Yes	ME

CFS, chronic fatigue syndrome; IBS, irritable bowel syndrome; MCS, multiple chemical sensitivity; ME, myalgic encephalomyelitis; MND, motor neurone disease; MS, multiple sclerosis; PA, pernicious anaemia; PD, Parkinson's disease; RA, rheumatoid arthritis.

- Six patients had cysteine within reference range, low sulphate and a high ratio (Table 3).
- Twelve patients had high cysteine, sulphate within reference range and a high ratio (Table 4).

TABLE 2. Group B: patients with low cysteine, low sulphate and high ratio

No.	Sex	Adult or child	Cysteine	Sulphate	Ratio	Taking relevant supplements	Main problem
1	F	A	0.33	0.18	1833	No	Breast cancer
2	F	A	0.27	0.67	403	No	Fibromyalgia
3	M	C	0.13	0.46	283	Some	Anaemia
4	F	A	0.31	0.37	846	Some	Depression
5	M	A	0.2711	0.3828	708	Some	MCS
6	F	A	0.27	0.73	371	No	Asthma
7	F	A	0.19	0.23	836	No	Ulcers
8	M	A	0.2495	1.1940	209	Yes	Thrombocytopenia
9	F	A	0.3248	1.1339	286	No	MCS
10	F	A	0.3097	0.3118	993	No	IBS
11	F	A	0.2980	0.2628	1134	Yes	CFS
12	M	A	0.1635	0.5709	286	Yes	MS

CFS, chronic fatigue syndrome; IBS, irritable bowel syndrome; MCS, multiple chemical sensitivity; MS, multiple sclerosis.

TABLE 3. Group C: normal cysteine, low sulphate and high ratio

No.	Sex	Adult or child	Cysteine	Sulphate	Ratio	Taking relevant supplements	Main problem
1	F	A	0.38	0.72	528	Yes	MS
2	F	A	0.41	0.31	1322	No	MCS
3	F	A	0.35	0.14	2507	No	IBS
4	M	A	0.4062	0.4129	984	No	Psychosis
5	M	A	0.3767	1.3250	284	Some	ME
6	F	A	0.4012	0.3365	1192	No	ME

IBS, irritable bowel syndrome; MCS, multiple chemical sensitivity; ME, myalgic encephalomyelitis; MS, multiple sclerosis.

TABLE 4. Group D: high cysteine, normal sulphate and high ratio

No.	Sex	Adult or child	Cysteine	Sulphate	Ratio	Taking relevant supplements	Main problem
1	F	A	2.26	2.65	854	No	ME
2	F	A	3.47	4.11	844	No	MS
3	F	A	2.35	5.12	458	No	CFS
4	F	A	0.62	1.76	350	No	CFS
5	F	A	0.63	2.74	229	No	MCS
6	F	A	1.1	3.54	309	Yes	MCS
7	F	A	0.5767	4.3876	131	No	Migraine
8	F	A	0.6211	2.9819	208	Yes	Migraine
9	F	A	0.6333	2.3245	272	Yes	Leukopenia
10	F	A	0.7741	5.2561	147	No	Depression
11	F	A	2.27	1.84	1232	No	Polymyalgia
12	F	A	0.5550	2.0343	273	No	Depression

CFS, chronic fatigue syndrome; MCS, multiple chemical sensitivity; ME, myalgic encephalomyelitis; MS, multiple sclerosis.

TABLE 5. Group E: normal cysteine, normal sulphate and high ratio

No.	Sex	Adult or child	Cysteine	Sulphate	Ratio	Taking relevant supplements	Main problem
1	F	A	0.39	2.27	179	Yes	Spastic paresis

TABLE 6. Group F: low cysteine, normal sulphate and high ratio

No.	Sex	Adult or child	Cysteine	Sulphate	Ratio	Taking relevant supplements	Main problem
1	F	A	0.24	1.68	143	Some	ME
2	F	A	0.33	1.78	184	Yes	Face swelling

ME, myalgic encephalomyelitis.

- One patient had cysteine and sulphate within reference range and a high ratio (Table 5).
- Two patients had low cysteine, sulphate within reference range and a high ratio (Table 6).
- One patient had low cysteine, sulphate within reference range and a low ratio (Table 7).
- One patient had low cysteine, and sulphate and ratio within reference range (Table 8).

Sixty-six per cent of those on at least some of the relevant supplements, and following at least some of the dietary advice, had a ratio less than 1000, in contrast to only 46% of those not yet on supplements.

The last column of Tables 1–8 shows what appears to be the main problem, but most of these patients have multiple problems, and it is not always clear what the main problem is, or even if one problem is more important than the others.

A2 is the son of A1. A14 is the daughter of A13, A39 is the daughter of A38, and A21 is

TABLE 7. Group G: low cysteine, normal sulphate and low ratio

No.	Sex	Adult or child	Cysteine	Sulphate	Ratio	Taking relevant supplements	Main problem
1	F	A	0.131	2.38	55	Some	Demyelination

TABLE 8. Group H: low cysteine, normal sulphate and normal ratio

No.	Sex	Adult or child	Cysteine	Sulphate	Ratio	Taking relevant supplements	Main problem
1	M	A	0.2271	2.0841	109	Yes	MCS

MCS, multiple chemical sensitivity.

the daughter of A22. D7 is the sister of A34. The father of D8 had motor neurone disease. D9 is the mother of B3, and of a hyperactive son. A10 is the mother of a son with severe learning difficulties. A20 is the mother of two children with fragile X autism, and her mother has psoriatic arthritis. The mother of A23 has multiple food and chemical sensitivity and arthritis, and the mother of A33 has multiple food sensitivity. The father of A28 had multiple sclerosis, and the mother had rheumatoid arthritis. The mother of A5 has migraine. The mother of A31 has motor neurone disease. The brother of A12 had leukaemia. A5, A6, A10, A13, A14, B5, C1, D1 and E1 had a high percentage of flat blood cells, suggesting essential fatty acid deficiency. D2 had raised levels of cells with surface changes and early cup forms, suggesting vitamin B12 deficiency. She also had increased gut permeability. D4 had a high percentage of red cells with surface changes and of flat cells. B5 had low phenolsulphotransferase activity and mercury toxicity, shown by a Kelmer test. D6 had low platelet protein, poor phenolsulphotransferase activity, low glutathione, a high percentage of flat red blood cells, and a high level of paradichlorobenzene in blood and fat. A40 and C4 had high urinary kryptopyroles, involving zinc and vitamin B6 deficiency. A5, A13, A14, B5, D2 and F2 had sulphite in urine. A6 had a high level of anti-gliadin antibody, and low intestinal secretory IgA. A10 had low sweat zinc and manganese. A12 had low sweat magnesium and zinc, and low hair zinc. A14 had high blood histamine. A29 had high creatine kinase and erythrocyte sedimentation rate (ESR), and low neutrophils, lymphocytes, monocytes, red cells and packed cell volume. B3 had a low white cell count. A22 had haemolytic anaemia and Cooper's syndrome, which involves inflamed tops of villi, and is related to gluten. B8 had thrombocytopenia.

A5 had convulsions after two different vaccinations in infancy. A23 had a bad reaction to his first triple vaccine. C4 reacted badly, immediately, to a measles, mumps and rubella (MMR) injection. D6 reacted badly to various travel vaccines.

Thirty-three adults had chronic fatigue syndrome or myalgic encephalomyelitis. Individuals had high, normal or low cysteine, but the mean cysteine was high, at 0.89. Their sulphate was normal or low, but the mean was low, at 1.32. They all had a high ratio, the mean being 1257.

Thirty-three adults had sensitivity to foods or other chemicals. Individuals had high, low or normal cysteine, but the mean was high, at 0.90. Their sulphate was normal or low, but the mean was low, at 1.28. All but one had a high ratio, the mean being 1060.

Sixteen adults and two children had irritable bowel syndrome or chronic diarrhoea. Individuals had high, normal or low cysteine, but the mean was high, at 0.85. Their sulphate was normal or low, with a low mean of 1.06. They all had a high ratio, the mean being 1589.

Sixteen adults had arthritis, fibromyalgia, or other aches and pains. Individuals had high, normal or low cysteine, with a high mean of 0.96. Their sulphate was low or normal, with a normal mean of 1.51. They all had a high ratio, with a mean of 1177.

Twelve adults had depression. They had high, normal or low cysteine, with a high mean of 0.66. They had low or normal sulphate, with a low mean of 1.11. They all had a high ratio, with a high mean of 1798.

Nine adults had migraine. They had high or normal cysteine, with a high mean of 0.83. They all had low sulphate, with a mean of 0.77. They all had a high ratio, with a mean of 1922.

These results are compared with those of the controls in Table 9.

DISCUSSION

The combination of high cysteine and low sulphate levels, as seen in group A, the most numerous subset, may reflect the presence of inflammatory cytokines, as tumour necrosis factor-α (TNF-α) and transforming growth factor-β have been shown to inhibit the

TABLE 9. Mean values of cysteine, sulphate and the cysteine/sulphate ratio for patients with different conditions

Group	Mean cysteine	Mean sulphate	Mean ratio
Depression	0.66	1.11	1798
Migraine	0.83	0.77	1922
IBS/diarrhoea	0.85	1.06	1589
CFS/ME	0.89	1.32	1257
Arthritis/fibromyalgia	0.96	1.51	1177
Chemical/food sensitivity	0.90	1.28	1060
Control	0.37	2.20	

CFS, chronic fatigue syndrome; IBS, irritable bowel syndrome; ME, myalgic encephalomyelitis.

expression of cysteine dioxygenase, the enzyme catalysing the initial step in cysteine conversion [39, 40]. Another possibility is that there are mutations in the DNA coding for the enzyme, or in the flanking regions, which lead to a less efficient isozyme of cysteine dioxygenase. Patients in group A often had a family history of chronic illness, suggesting that this could be a susceptibility factor. Research to determine to what extent abnormalities of sulphur metabolism are inherited would be useful. Patients in group B (low cysteine, low sulphate) might have some fault in cysteine synthesis or some viral infection, while group C patients (normal cysteine, low sulphate) could be linked with poor conversion from cysteine to sulphate. Group D, with high cysteine and normal sulphate levels, could reflect increased tissue breakdown or protein consumption; other problems in sulphur metabolism may also be involved here. Groups E, F, G and H had too few individuals to draw any tentative conclusions.

The measles vaccination has been found to increase the levels of the Th1 cytokines, interferon-γ, soluble interleukin-2 receptor (sIL-2R) and TNF-α [41].

Omega 3 fatty acids can be used to reduce the level of cytokines, such as interleukin-1 (IL-1), IL-2, IL-6 and TNF-α [42–46]. This is a function of prostaglandins series 3, formed from the omega 3 fatty eicosapentaenoic acid (EPA). EPA is obtained from oily fish, and the body can also make it from α-linolenic acid in flaxseed oil, and a few other oils. Fish oil has been found to be helpful in treating rheumatoid arthritis [47, 48], possibly because this can reduce cytokine levels and so increase sulphate supply. Corticosteroids upregulate cysteine dioxygenase. As pantothenate is required to produce cortisol, an inadequate level of pantothenate may cause poor conversion of cysteine to sulphate.

If conversion from cysteine to sulphate is poor, molybdenum and vitamin B2 can be supplied, to improve the conversion from sulphite to sulphate by the enzyme sulphite oxidase [49]. Epsom salt baths can also be used to provide sulphate, as it passes through the skin. Vitamin B6 can be supplemented, in order to reduce a high level of cysteine by converting it to taurine. Magnesium, zinc and vitamin B2 are required with vitamin B6.

Foods high in the toxic mineral boron, like tomatoes and apricots, cause excretion of vitamin B2 [50, 51] and may therefore inhibit the enzyme sulphite oxidase that converts sulphite to sulphate. Diets high in purines, caffeine or alcohol may reduce the quantity of sulphite oxidase, by competing for molybdenum and vitamin B2 [49].

In depression there may be high levels of histamine in the brain, and reducing this by inactivation with sulphate may improve mood [52].

Testing cysteine and sulphate levels may become a useful technique for investigating chronic health problems including multiple sclerosis, myalgic encephalomyelitis, irritable bowel syndrome, autism, depression, motor neurone disease, Parkinson's, Alzheimer's, haemolytic anaemia, and also other conditions that are difficult to define. Double-blind clinical trials would be valuable to determine how effective it is to treat patients with

molybdenum, vitamins B2, B6 and C, pantothenate, fish or flax oil, Epsom salt baths, N-acetyl cysteine, zinc and/or magnesium. Testing sulphate levels could be used to determine who is likely to be particularly susceptible to environmental chemicals, so as to advise them against employment in particularly polluted environments, against using unnecessary drugs and against using pesticides. Inoculations may contain phenol [53–55] and might need to be avoided where possible.

The results of the 81 patients tested are summarized in Table 10, together with suggestions for possible treatment. Those with inefficient sulphate production could be advised to use diets with lower quantities of amines and bioflavonoids than the rest of the population. Advice to drink red wine may protect against coronary heart disease, but at the expense of other symptoms in susceptible people. High amine and bioflavonoid foods are listed in Tables 11 and 12. The nutritional and dietary suggestions are based on the biochemistry discussed above.

It is important for patients who do not have a clear diagnosis to have tests that show that their problems are real. The cysteine/sulphate test can contribute to this. Then their families, friends, doctors, employers, or benefit agencies can take them seriously.

Those already on supplements and a diet were less likely to have a very high ratio, suggesting that supplements and a special diet might be helping them. It would therefore be useful to test a series of patients before starting nutritional supplements and a diet, and then to repeat the test a few months later, to determine how successful the programme has been in improving the ratio, and thus the conversion of cysteine to sulphate. It would also be useful to study how closely an improvement in symptoms was associated with an improved ratio. Clinical experience to date confirms that these measures do produce significant clinical improvement.

CONCLUSIONS

Cysteine and sulphate levels may be abnormal in a variety of chronic illnesses, such as depression, migraine, myalgic encephalomyelitis, irritable bowel syndrome, multiple chemical sensitivity and arthritis. Nutritional treatment that improves these levels may also improve the health of patients. Those with abnormal levels may need to restrict amines and phenols in food, medicines, and vaccines, and seek healthy working conditions. Their children may also need to be treated with caution.

TABLE 10. Summary of 81 complex patients

Cysteine	Sulphate	Ratio	Number
High	Low	High	46
Low	Low	High	12
Normal	Low	High	6
High	Normal	High	12
Normal	Normal	High	1
Low	Normal	High	2
Low	Normal	Low	1
Low	Normal	Normal	1

- Only one had the ratio within the normal range.
- Fifty-eight had high cysteine. They could use vitamins B2 and B6 and magnesium.
- Sixty-four had low sulphate. They might benefit from Epsom salt baths, restricting dietary phenols and amines, minimizing drug use, and avoiding creosote, detergent and disinfectant.
- Seventy-nine had a high ratio. They might benefit from ingestion of fish oil, using pantothenate, and using molybdenum and vitamin B2.
- Sixteen had low cysteine. They could use N-acetyl cysteine, zinc and vitamin C.

TABLE 11. Approximate flavonoid content of the fresh edible part of some foods and drinks

Food	mg kg^{-1}	Drinks	mg l^{-1}
Quercetin			
Onion	284–486	Green tea	14–23
Kale	110	Black tea	10–25
French bean	32–45	Tomato juice	13
Apple	36	Red wine	4.1–16
Broccoli	30	Fresh lemon juice	7.4
Slicing bean	28–30	Fresh grapefruit juice	4.9
Apricot	25	Orange juice	3.4–5.7
Broad bean	20	Grape juice	4.4
Cherry	15	Apple juice	2.5
Black grape	15	Chocolate semi-skimmed milk	1.3
Lettuce	1.9–30		
Red currant	13		
White grape	12		
Plum	9		
Strawberry	8.6		
Tomato	4.6–11		
Turnip top	4.6–10		
Pear	6.4		
Red cabbage	4.6		
Kaempferol			
Kale	211	Green tea	9.1–15
Broccoli	72	Black tea	6.3–17
Turnip top	31–64		
Endive	15–95		
Leek	11–56		
Strawberry	12		
French bean	<2–14		
Brussels sprouts	7.4		
Radish	3.9–7.7		
Myricetin			
Broad bean	26	Green tea	5.2–12
White/black grapes	4.5	Black tea	1.7–5.2
		Red wine	6.9–9.3
		Grape juice	6.2
Apigenin			
Celery	108		
Luteolin			
Red bell pepper	11		
Carrot	<1.4		

Olive oil also contains several phenolic compounds.
Source: [56–58].

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TABLE 12. Some amines in foods

Serotonin	Banana, plantain, papaya, passion fruit, pineapple, tomato
Noradrenaline	Banana, plantain
Dopamine	Banana
Tyramine	Cheese, alcoholic drinks, raspberries, red plums, tomatoes, vinegar, sauerkraut, smoked fish, dry fermented sausage, yeast and meat extracts, leftover meat and fish, broad beans, overripe fruit and vegetables, avocado, banana, chicken liver, aubergine
Tryptamine	Cheese
Phenylethylamine	Cheese, chocolate
Octopamine	Lemon
Synephrin	Lemon
Histamine	Sauerkraut, fish, cheese, fermented soya products, vinegar, alcoholic drinks.

Source: [59, 60].

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