Genetic aspects of the polymodally distributed sulfoxidation of S-carboxymethyl-L-cysteine in man

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1 Interindividual variation in the sulfoxidation of S-carboxymethyl-L-cysteine (750 mg p.o.) was investigated in 200 healthy volunteers.

2 Nearly a 100-fold difference was observed between individuals with respect to the amount of sulfoxide metabolites detected in their 0–8 h urine (0.6 to 59.1% recovery). Such a difference was shown to be reproducible over several months in 40 subjects who spanned the entire range of capacities.

3 Cumulative plots and maximum likelihood analysis of the distribution indicated that a bimodal model was most probable.

4 Analysis of pedigree data obtained from 12 families suggested a genetic effect with overlying environmental influences.

Keywords sulfoxidation S-carboxymethyl-L-cysteine polymorphism

Introduction

The past few years have seen an increasing awareness in the recognition of the marked interindividual differences in man's response to drugs, both in terms of their efficacy and in the development of adverse reactions. Such variability arises from differences in both kinetic and dynamic factors which are to a large extent under genetic control. One such major determinant of drug disposition is that of metabolism and a number of reactions involved in the biotransformation of drugs are known to exhibit the phenomenon of genetic polymorphism in man (Eichelbaum *et al.*, 1979; Evans & White, 1964; Mahgoub *et al.*, 1977).

There is evidence within the literature to support the hypothesis that quantitative differences exist between subjects in the extent of oxidation at sulphur centres in various xenobiotic compounds including the phenothiazine derivatives metiazinic acid (Populaire *et al.*, 1969), promethazine (Taylor *et al.*, 1983), thioridazine and trifluoperazine (West *et al.*, 1974) and the histamine H₂-receptor antagonist,

metiamide (Idle *et al.*, 1979a). However, these studies have only involved a few individuals. Recent investigations using larger numbers have shown a wide variation amongst subjects with respect to the amount of sulfoxide metabolites excreted in the urine following the oral administration of S-carboxymethyl-L-cysteine (Waring, 1980; Waring *et al.*, 1982). This compound (Mucodyne®, Mucollex®) is an S-substituted derivative of cysteine which is extensively prescribed in medical practice to facilitate expectoration by reducing sputum viscosity in chronic asthma and bronchitis and consequently plays a role as supportive therapy during the treatment of these respiratory disorders.

The present study was undertaken to look further into the distribution of sulfoxidation potential within a population, to investigate the role of various factors which may influence this capacity and to examine whether or not it is principally an inherited characteristic.

Methods*Human subjects*

Two hundred healthy British white volunteers (90 male, 110 female) were recruited from the staff and students of St Mary's Hospital Medical School, London and the Biochemistry Department of Birmingham University (Table 1).

Their ages ranged from 18 to 53 years (mean 22.6) and their body weights from 45 to 93 kg (mean 63.5). No subject had any history of major organ disease. None consumed more than moderate amounts of alcohol but 43 regularly smoked, nine of whom had 15 or more cigarettes each day. None of the volunteers had been exposed to any recent drug medication, although 44 females were taking oral contracep-

Table 1 Social details and results obtained for the 200 volunteers

Subject	Sex	Age (years)	Body weight (kg)	Social details	Total recovery	% administered dose	
						Sulphoxide recovery	Sulphoxidation index
1	F	24	55	110	91.6	7.7	10.9
2	M	23	67	010	65.1	9.1	6.2
3	F	23	55	010	70.9	7.8	8.1
4	F	24	61	011	56.4	23.4	1.4
5	M	25	78	110	86.4	4.7	17.4
6	M	24	70	010	84.2	24.6	2.4
7	M	26	67	010	71.0	9.8	6.2
8	M	27	70	010	93.0	38.5	1.4
9	F	20	58	010	74.1	33.7	1.2
10	F	21	55	111	33.9	18.3	0.9
11	F	20	59	011	53.4	1.8	28.7
12	F	20	52	010	86.2	23.2	2.7
13	F	25	51	010	78.2	19.7	3.0
14	F	25	48	010	72.5	30.3	1.4
15	F	24	53	010	26.6	14.2	0.9
16	M	21	77	010	32.1	18.2	0.8
17	F	39	62	010	67.6	15.2	3.4
18	F	21	56	010	19.5	1.1	16.7
19	M	21	55	020	47.6	18.9	1.5
20	F	20	52	110	34.7	0.7	48.6
21	F	20	54	011	33.4	16.6	1.0
22	M	43	74	010	21.8	1.1	18.8
23	M	21	66	010	76.0	4.7	15.2
24	M	22	76	110	40.0	2.1	18.0
25	M	20	63	100	66.5	0.4	165.3
26	M	20	72	010	69.0	6.6	9.5
27	F	20	45	000	24.3	1.1	21.1
28	F	20	64	010	97.2	28.4	2.4
29	M	19	62	010	98.2	30.4	2.2
30	F	20	58	011	63.9	21.2	2.0
31	F	21	56	010	55.1	19.5	1.8
32	F	21	53	110	62.5	21.3	1.9
33	F	20	51	010	72.4	3.4	20.3
34	M	21	62	010	37.1	14.7	1.5
35	F	20	57	011	31.4	2.5	11.6
36	M	20	93	000	68.0	13.6	4.0
37	M	20	62	010	97.3	10.0	8.7
38	F	20	63	010	76.6	6.2	11.4
39	F	24	54	011	66.6	8.6	6.7
40	F	23	52	010	95.8	6.8	13.1
41	F	22	57	010	67.9	7.5	8.1
42	M	23	72	120	65.0	27.7	1.3
43	M	22	70	010	60.4	27.4	1.2
44	F	21	61	010	67.5	13.2	4.1
45	F	25	64	010	81.0	4.2	18.3
46	M	21	77	020	71.4	14.4	4.0
47	M	20	72	010	72.4	9.4	6.7

Table 1 (Cont.)

Subject	Sex	Age (years)	Body weight (kg)	Social details	Total recovery	% administered dose	
						Sulphoxide recovery	Sulphoxidation index
48	F	20	51	011	75.3	14.7	4.1
49	M	21	88	020	28.2	7.1	3.0
50	M	21	93	110	30.1	17.8	0.7
51	F	19	55	010	54.0	1.2	44.0
52	F	20	54	011	22.4	6.5	2.4
53	M	20	72	010	96.9	23.6	3.1
54	M	20	73	000	91.6	42.8	1.1
55	M	19	88	010	93.0	30.3	2.1
56	M	18	69	010	78.6	3.9	19.2
57	M	20	72	010	64.7	25.4	1.5
58	M	20	74	120	39.4	9.4	3.2
59	M	21	70	120	77.9	36.2	1.2
60	M	22	65	010	41.8	3.3	11.7
61	M	21	60	010	52.2	15.0	2.5
62	F	26	58	011	53.7	12.6	3.3
63	F	25	56	011	48.2	14.4	2.3
64	M	21	68	010	67.9	13.0	4.2
65	M	21	72	010	70.3	25.9	1.7
66	F	20	59	011	45.3	10.6	3.3
67	F	19	51	010	51.9	13.5	2.8
68	M	20	62	010	69.6	1.6	42.5
69	M	20	92	110	73.8	21.5	2.4
70	M	21	77	010	89.4	28.6	2.1
71	M	19	63	010	30.5	2.8	9.9
72	M	20	79	010	56.5	17.7	2.2
73	F	21	53	111	49.8	8.8	4.7
74	M	24	89	020	47.9	19.1	1.5
75	M	19	67	010	60.1	15.6	2.9
76	M	24	64	010	48.4	16.8	1.9
77	F	21	54	001	30.4	0.4	75.0
78	M	25	72	010	79.3	34.3	1.3
79	M	21	65	010	96.3	40.7	1.4
80	M	21	69	010	30.0	5.6	4.4
81	F	22	55	101	46.9	21.1	1.2
82	F	21	53	011	37.4	1.9	18.7
83	F	21	62	110	17.3	1.1	14.7
84	M	20	68	020	38.6	13.7	1.8
85	M	33	79	210	29.2	9.1	2.2
86	M	25	73	010	39.9	21.1	0.9
87	M	45	79	010	98.0	4.1	22.9
88	F	22	58	010	30.0	2.9	9.3
89	M	26	70	110	41.7	16.4	1.5
90	M	28	70	010	60.6	18.2	2.3
91	M	22	67	010	85.3	18.2	3.7
92	F	24	50	011	27.6	2.6	9.6
93	M	28	61	210	59.3	15.8	2.8
94	F	18	51	011	36.5	8.3	3.4
95	F	19	54	010	30.7	10.2	2.0
96	F	21	53	000	33.4	2.1	14.9
97	F	19	55	011	26.2	2.2	10.9
98	F	22	57	010	26.1	5.0	4.2
99	F	34	63	110	31.6	12.9	1.4
100	F	21	53	110	38.2	11.3	2.4
101	F	21	50	010	48.6	4.5	9.8
102	F	20	60	000	53.4	7.9	5.8
103	F	19	48	000	61.4	7.5	7.2
104	F	22	60	210	51.6	19.9	1.6
105	F	20	57	010	54.4	17.9	2.0

Table 1 (Cont.)

Subject	Sex	Age (years)	Body weight (kg)	Social details	Total recovery	% administered dose	
						Sulphoxide recovery	Sulphoxidation index
106	F	19	47	011	41.5	21.3	0.9
107	F	19	52	010	30.5	11.4	1.7
108	F	19	49	011	33.7	13.9	1.4
109	F	19	54	111	48.3	8.2	4.9
110	F	19	72	111	48.4	11.9	3.1
111	F	20	54	011	49.6	14.9	2.3
112	F	20	56	110	34.9	3.7	8.4
113	F	25	54	010	31.3	8.5	2.7
114	F	19	52	111	36.4	7.5	3.9
115	F	19	59	111	31.2	7.3	3.3
116	F	20	67	211	52.0	10.3	4.0
117	F	31	68	210	38.2	5.1	6.5
118	F	19	62	010	26.4	0.9	28.3
119	F	18	58	211	52.4	17.7	2.0
120	F	22	86	111	82.4	30.9	1.7
121	F	19	54	111	74.4	28.8	1.6
122	F	22	62	110	52.1	1.7	29.6
123	M	21	79	020	78.6	17.0	3.6
124	F	19	58	020	34.7	2.3	14.1
125	F	19	55	010	20.9	4.6	3.5
126	F	21	59	010	65.1	0.7	92.0
127	F	21	58	010	31.0	7.4	3.2
128	M	29	76	010	42.2	13.4	2.1
129	M	25	62	010	34.8	4.8	6.3
130	F	19	56	010	31.6	4.4	6.2
131	M	19	65	010	36.8	0.9	39.9
132	F	18	54	011	69.6	18.9	2.7
133	F	19	51	011	18.7	5.4	2.5
134	F	19	57	010	64.5	13.6	3.7
135	F	19	56	110	42.5	14.8	1.9
136	M	22	67	010	55.2	20.9	1.6
137	M	24	79	220	39.8	1.8	21.1
138	F	22	58	111	51.8	13.5	2.8
139	M	23	75	010	82.6	17.0	3.9
140	M	19	81	010	42.8	11.5	2.7
141	F	19	56	010	43.2	1.2	35.0
142	M	23	66	110	47.2	0.5	93.4
143	M	20	81	010	60.1	23.7	1.5
144	F	19	54	010	26.0	0.4	64.0
145	M	20	57	010	33.5	8.9	2.8
146	F	18	52	010	30.8	5.2	4.9
147	M	23	75	010	33.1	0.7	46.3
148	M	19	76	010	41.0	5.6	6.3
149	M	21	70	010	47.6	10.7	3.4
150	F	18	51	010	66.1	25.4	1.6
151	F	19	54	010	69.9	10.5	5.7
152	F	20	51	000	42.8	9.5	3.5
153	M	20	89	020	75.2	24.4	2.1
154	F	22	60	011	93.5	30.4	2.1
155	F	18	51	011	42.1	18.5	1.3
156	F	18	67	000	56.9	8.1	6.0
157	F	19	57	011	54.1	21.2	1.6
158	F	20	54	010	24.8	10.7	1.3
159	F	20	56	011	38.1	9.3	3.1
160	F	20	58	011	37.6	16.0	1.4
161	M	19	65	010	67.0	15.0	3.5
162	F	18	67	010	46.0	5.6	7.2
163	F	19	45	010	28.0	1.5	17.7

Table 1 (Cont.)

Subject	Sex	Age (years)	Body weight (kg)	Social details	Total recovery	% administered dose	
						Sulphoxide recovery	Sulphoxidation index
164	F	20	54	010	40.7	4.0	9.2
165	M	19	70	010	50.5	13.0	2.9
166	F	19	57	000	45.1	12.6	2.6
167	M	20	76	010	43.6	7.2	5.1
168	F	26	54	011	60.3	2.8	20.5
169	F	20	58	010	39.6	5.9	5.7
170	F	19	50	000	64.6	25.7	1.5
171	M	19	70	010	42.9	11.4	2.8
172	M	21	67	010	40.6	14.7	1.8
173	F	21	52	011	43.7	12.9	2.4
174	F	21	60	021	39.8	16.3	1.4
175	M	22	74	020	68.8	7.7	7.9
176	M	22	74	020	39.6	11.3	2.5
177	M	19	70	010	23.4	0.7	32.4
178	F	23	55	010	26.9	4.7	4.7
179	F	21	51	011	32.9	5.2	5.3
180	F	21	63	010	64.0	16.8	2.8
181	F	24	60	010	38.5	0.4	95.3
182	M	26	76	220	71.0	28.2	1.5
183	M	51	64	010	58.6	0.5	116.2
184	M	26	71	110	52.8	12.8	3.1
185	M	43	73	110	28.0	3.5	7.0
186	M	28	86	020	36.3	7.4	3.9
187	F	25	55	010	32.0	6.1	4.2
188	M	22	92	010	44.0	13.6	2.2
189	F	27	68	020	33.2	14.4	1.3
190	M	53	66	110	71.9	37.6	0.9
191	M	25	78	010	35.6	0.5	70.2
192	M	48	71	010	82.9	12.9	5.4
193	M	28	70	020	26.0	0.5	51.0
194	M	41	69	110	48.6	8.0	5.1
195	M	43	75	010	28.3	3.9	6.3
196	M	42	72	010	97.5	10.6	8.2
197	M	28	88	220	61.6	6.1	9.1
198	F	21	55	011	37.8	2.8	12.5
199	M	20	70	010	39.4	13.7	1.9
200	F	22	55	111	47.4	20.4	1.3

*Social details 1 2 3**column 1* smoking habits

0 = none 1 = 1 to 15 cigs/day 2 = > 15 cigs/day

column 2 alcohol consumption

0 = none 1 = occasional 2 = moderate

column 3 oral contraceptives

0 = no 1 = yes

tives. The families of 12 of these subjects also took part in the study, all of these being in good health. The mono- or dizygosity of the two sets of twins examined had been previously determined by blood grouping, enzyme patterns and HLA tissue typing. All the participants gave full, informed consent before entering the study and appropriate ethical approval was obtained from the local Ethics Committee.

Experimental procedure

Every volunteer took two capsules, each containing 375 mg of S-carboxymethyl-L-cysteine (Mucodyne®; Berk Pharmaceuticals Ltd, Eastbourne, UK), following a light breakfast and was asked to take no other medication either before or during this study. The bladder was emptied before dosing and thereafter all the

urine collected for the next 8 h. The total urine volume was recorded and aliquots stored at -20°C until analysis which was undertaken as rapidly as possible after collection.

Analytical procedure

Reference compounds were obtained or synthesised and the urine samples quantitatively analysed for the parent compound and its metabolites (Figure 1), including the sulphoxides [S-carboxymethyl-L-cysteine sulphoxide, N-acetyl-S-carboxymethyl-L-cysteine sulphoxide, S-methyl-L-cysteine sulphoxide, N-acetyl-S-methyl-L-cysteine sulphoxide] by methods previously described in detail (Brand *et al.*, 1936; Turnbull *et al.*, 1978; Waring, 1978, 1980; Waring & Mitchell, 1982). Briefly, the analysis entailed examining aliquots of neat urine (*circa* 0.1 ml, depending upon total volume, samples with higher volumes or low sulphoxide levels on preliminary runs being concentrated by lyophilisation) by descending paper chromatography (Whatman 3MM) in each of the following four solvent systems:

(a) butan-1-ol / ethanol / water / $\text{NH}_3(\text{aq.})$ [sp.gr. 0.88], 10 / 10 / 4 / 1 by vol.

(b) butan-1-ol / water / acetic acid (glacial) / butyl acetate, 24 / 10 / 5 / 2 by vol.

(c) butan-1-ol saturated with 2N $\text{NH}_3(\text{aq.})$

(d) butan-2-one saturated with 2N $\text{NH}_3(\text{aq.})$

Reference compounds were added to blank urine samples on each chromatogram to act as standards. The development time for solvents (a) and (b) was 16 h whereas that for solvent (c) was 24 h and solvent (d) was 36 h. By preparing four chromatograms for each urine sample it

was possible to effect separation of all the metabolites of S-carboxymethyl-L-cysteine (Barnsley *et al.*, 1969; Waring, 1978, 1980). The stability of both sulphide and sulphoxide compounds was investigated by adding known amounts of the standards to control urine samples and taking them through the above analytical procedure either immediately or after storage at room temperature for 24 h. The sulphide compounds were not oxidised to their corresponding sulphoxides (Sklan & Barnsley, 1968) and the sulphoxides did not show any decomposition.

The sulphur-containing compounds were specifically visualised by utilising the chloroplatinate reaction (Toennies & Kolb, 1951) as modified by Barnsley and co-workers as a chromatographic reagent (Barnsley *et al.*, 1964). The chromatospots obtained were outlined and their areas measured by planimetric densitometry. Calibration curves for each metabolite relating area to amount were constructed in each solvent system by running appropriate concentrations of the standard compound in urine and were shown to be linear over the range employed (*circa* 3 to 80 μg ; Figure 2) (Bray *et al.*, 1964). All of the urines were examined in duplicate on separate occasions and the values obtained for the urinary metabolite profiles were virtually identical.

As there is no evidence for the existence of isoenzymes catalysing the sulphoxidation of S-carboxymethyl-L-cysteine and its sulphide metabolites in man and since repeat tests in subjects had shown that whilst some variation in the amounts of each individual sulphoxide excreted was observed the total amount of sulphoxide metabolites produced did not vary, it was decided for the purposes of the present investigation to use the measurement of total sulphoxides excreted, expressed as a percentage of the total recovery obtained. This also permitted a direct comparison between individuals by standardising the total recovery. In order to skew the distribution obtained and place more emphasis on those individuals who had produced relatively small amounts of sulphoxide by pulling them out from the majority of the population, the results were also expressed in the form of a ratio. A 'sulphoxidation index' (S.I.) for each 0-8 h urine was calculated as follows,

$$\frac{\% \text{ administered dose excreted as sulphide metabolites}}{\% \text{ administered dose excreted as sulphoxide metabolites}}$$

It can be seen that these two parameters are interrelated as follows,

Figure 1 Sulphoxidation of S-carboxymethyl-L-cysteine.

For the parent compound: $\text{R}' = -\text{COOH}$, $\text{R}'' = -\text{CH}(\text{COOH})\text{NH}_2$, sulphur as the sulphide.

For metabolites: $\text{R}' = -\text{COOH}$ or $-\text{H}$, $\text{R}'' = -\text{CH}(\text{COOH})\text{NH}_2$ or $-\text{CH}(\text{COOH})\text{NHCOCH}_3$, sulphur as sulphide or sulphoxide.

Figure 2 A typical planimetric densitometry calibration curve obtained in solvent b.

● = S-methyl-L-cysteine

○ = S-carboxymethyl-L-cysteine, *N*-acetyl-S-carboxymethyl-L-cysteine, *N*-acetyl-S-methyl-L-cysteine.

■ = S-carboxymethyl-L-cysteine sulfoxide, *N*-acetyl-S-carboxymethyl-L-cysteine sulfoxide, S-methyl-L-cysteine sulfoxide, *N*-acetyl-S-methyl-L-cysteine sulfoxide.

All curves in each of the four solvent systems employed are similar.

$$\text{S.I.} = \frac{100 - \% \text{ recovery as sulfoxides}}{\% \text{ recovery as sulfoxides}}$$

$$\% \text{ recovery as sulfoxides} = \frac{100}{(1 + \text{S.I.})}$$

Certain precautions must be taken during the storage of the urine samples. The decomposition of S-carboxymethyl-L-cysteine and its non-acetylated metabolites has been shown to occur if urine samples are left for long periods at room temperature, although they are stable for

at least 6 months when stored at -20°C . The samples show an initial decrease in the amount of the parent compound present followed by an increase in unidentified sulphur-containing material near the origin of the chromatogram which eventually moves upwards over the chromatospots leading to streaking. Since an increase in pH is a rough guide to the age of the urine samples, and may be a determinant in the decomposition observed, although compounds are relatively stable in aqueous alkaline solu-

tions, all urine samples with an initial pH above 6.8 were rejected.

Microbiological examination of three low volume samples subsequently rejected from the study revealed the presence of *E. coli* in two cases and *C. albicans* in the third, probably arising from urogenital infections. The bacterial contamination was shown to lead to a rapid rise in urinary pH with subsequent decomposition of the parent compound and non-acetylated metabolites and the sample infected with *Candida* showed considerable streaking near the origin; many substituted cysteines have been previously shown to be substrates for yeastlike organisms which form thiolactic acids and many other degradation products not usually seen in man (Harris *et al.*, 1968; Maw & Coyne, 1966).

Statistical methods

A bimodal or trimodal phenotype distribution may be evidence for a segregating locus with a major influence on the character under study, although this type of distribution is neither necessary nor sufficient evidence for a major locus effect (Murphy, 1964). In order to detect multimodality, a maximum likelihood tech-

nique has been advocated (Everitt & Hand, 1981; Murphy & Bolling, 1967). This technique allows the testing of alternative hypotheses concerning the number of modes, and the estimation of the mean and variance and proportion in each mode. However, non-normality in the data, for reasons other than distribution comingling, will often result in the spurious conclusion of multimodality. For this reason a power transform was fitted to the data as an alternative to a multimodal model (see Maclean *et al.*, 1976) and although this may subsequently obscure genuine multimodality, this is preferable to being misled by non-normality in the data. The transform employed was of the form $y = r/p[(x/r + 1)^p - 1]$, where y is the transformed datum, x is the original datum, p is the power of the transform and r is a constant used to ensure that every $x/r + 1$ is positive.

The maximum likelihood method was implemented in a FORTRAN programme which used the routine E04 JBF (Numerical Algorithms Group, 1982). All the raw data was analysed without any prior classification and the maxima obtained appeared global within the permissible range of parameter values, as confirmed by exploration of the likelihood surface.

Figure 3 Values obtained for the percentage of recovery excreted as sulfoxide metabolites in 40 non-related volunteers on whom complete biological repeat studies were performed. The small number 2 inside the circles indicates two individuals occupying the same position.

Results

Biological repeatability

The value obtained for the percentage recovery present as sulphoxide metabolites was found to be reproducible in 40 subjects on whom complete repeat tests were performed on two separate occasions. For 18 of these individuals the separation between tests was 12 months or more. When the first estimate of sulphoxide production was compared to the second estimate, the non-parametric Spearman rank correlation gave $r_s = +0.95$, $P < 0.001$ (Figure 3). In addition, four subjects were challenged with the drug on five separate occasions over a period of several months and the values obtained for their sulphoxide production were almost identical (Table 2).

Contributing factors

Statistical analysis of the results (Table 1) showed that no significant association (Spearman rank correlation) existed between the percentage of the administered dose excreted as sulphoxide metabolites (or the S.I.) and the total recovery, age or body weight of the subjects. Both poor and extensive sulphoxidisers were found amongst the three categories of individuals with regard to alcohol consumption and the smoking and non-smoking groups. In addition, the production of sulphoxide metabolites was not dependent upon the other major metabolic routes available to S-carboxymethyl-L-cysteine, namely *N*-acetylation and decarboxylation. In terms of impaired sulphoxidation both males and females were affected and were present amongst the volunteers recruited from both Institutes.

Of the 110 females examined, 44 were taking oral contraceptives. When these individuals were examined as a sub-group they were shown to produce significantly lower 0–8 h urine volumes ($2P < 0.05$; Wilcoxon rank sum test) and excreted more of the total dose as sulphoxide metabolites ($2P < 0.02$) than those females not taking oral contraceptives, although notable individual exceptions did occur. The total drug recoveries obtained for both groups were not significantly different. Such a water-retaining effect and influence on drug oxidations, albeit usually inhibitory, have been previously demonstrated with steroids (Homeida *et al.*, 1978; O'Malley *et al.*, 1972; Rudofsky & Crawford, 1966). It is, of course, possible that the difference observed between these two groups is not due to a direct effect of the contraceptive pill but to self-selection of those taking the pill if poor sulphoxidation in some way predisposes individuals to pill side-effects.

Interindividual variation

A marked variation was seen to exist between individuals in the amount of sulphoxide metabolites excreted, ranging from 0.6% to 59.1% of the recovery, virtually a 100-fold difference. Such a large dissimilarity was immediately apparent from the chromatograms produced (Figure 4).

For further analysis the data was normalised by conversion to a mean of 0 and a variance of 1, leaving the skewness (0.244) and kurtosis (2.100) of the original distribution unchanged. The normalised data was then plotted as a cumulative distribution, the sulphoxidation scores being an empirical plot of $[r(x) - \frac{1}{2}]/n$ (ordinate) against x (abscissa), where x is the measurement of % recovery as sulphoxides in

Table 2 Reproducibility of sulphoxide metabolite production in four individuals given 750 mg S-carboxymethyl-L-cysteine (p.o.) on five separate occasions

Volunteer 1			Volunteer 2			Volunteer 3			Volunteer 4		
A	B	C	A	B	C	A	B	C	A	B	C
0	20.8	3.8	0	41.7	1.4	0	0.9	113	0	52.6	0.9
5	22.2	3.5	3	40.0	1.5	3	0.8	120	14	58.8	0.7
8	22.7	3.4	6	38.5	1.6	10	1.5	64	16	55.6	0.8
17	22.7	3.4	20	40.0	1.5	13	2.0	49	17	50.0	1.0
20	24.4	3.1	29	35.7	1.8	15	1.1	90	25	55.6	0.8
Mean	22.6	3.4		39.2	1.6		1.3	87.2		54.5	0.8
s.d.	1.3	0.3		2.3	0.2		0.5	30.6		3.4	0.1

A = months since first test.

B = percentage recovery present as sulphoxide metabolites.

C = Sulphoxidation index (S.I.).

Figure 4 Photograph of a chromatogram obtained in solvent b showing a 'poor metaboliser' (a) and an 'extensive metaboliser' (b). Only the non-acetylated metabolites can be resolved in this solvent system.

Figure 5 Cumulative plot of the sulphoxidation scores obtained for the 200 subjects. A theoretical cumulative normal distribution is included for comparison (solid curve).

one individual and $r(x)$ is the rank of x amongst n (200) measurements. The solid curve is a cumulative normal distribution included for comparison (Figure 5). This method of plotting the data avoids the problems encountered when the choice of class interval directly influences the overall picture obtained. The experimental curve deviates quite extensively from the theoretical normal plot and contains evidence of discontinuities. This suggests that the experimental distribution may be a mixture of two or more distributions as has previously been found for blood clotting Factor X in a human population (Lester *et al.*, 1972).

Maximum likelihood analysis also provides evidence for this assumption. When fitting a single mode, the likelihood was significantly increased by the inclusion of a power transform ($X_1^2 = 8.55$). The likelihood was further increased by allowing a second mode ($X_2^2 = 28.19$) but it was not possible to significantly improve the bimodal model by including a third mode ($X_2^2 = 4.79$) and omitting the power transform from the model significantly decreased its likelihood ($X_1^2 = 19.66$). Attempts to fit three modes in Hardy-Weinberg equilibrium proportions failed to produce any improvement over the bimodal model. The parameters of this best model are given in Table 3 (model 1). However, the maximum likelihood analyses reported so far were conducted under the assumption that, when the distribution has more than a single mode, the within mode variance is the same for each mode. This may not be the case when the multimodality is caused by other than genetic reasons, and when genetically caused, Murphy & Bolling (1967) have stated that it is a matter of experience that the variance of the heterozygote class is greater than that of either homozygote. Relaxing the restriction of a common variance and employing a two mode model without transformation gives a significant improvement over a one mode model ($X_3^2 = 34.48$). Including transformation or a third mode does not improve this

model significantly. The estimated parameters for this bimodal model without transformation are given in Table 3 (model 2), and the distribution is shown in Figure 6. The mean values of the two modes which comprise this model correspond to 5.5% and 28.1% recovery as sulfoxides (equivalent to S.I. values of 17.2 and 2.6 respectively) with the antimode at 14.3% (S.I. of 6.0). The proportion of the population within the smaller mode is 22.7% (equivalent to 45 subjects) and in the larger mode 77.3% (155 subjects).

It was felt unjustifiable to remove those females who were taking oral contraceptives from the initial study as it was not certain whether or not the pill had directly influenced sulfoxide output or if they were a self-selected group within the population, as previously mentioned. Nevertheless, when the differences between groups were removed by standardisation to a common mean of zero, a bimodal model without power transform was again the most probable and had parameters very similar to those previously cited (model 2), with 72.6% of the subjects falling into the larger mode and 27.4% in the smaller mode. Thus, the mean differences between the groups either taking or not taking oral contraceptives had contributed little to the observed bimodal distribution.

The data may also be expressed in terms of the sulfoxidation index (Figure 7) which considerably skews the data giving relatively more importance to those individuals who produce smaller amounts of urinary sulfoxides, a phenomenon which may be of importance in certain clinical situations (Panayi *et al.*, 1983). As a consequence, the probit transformation of the data deviates significantly from linearity ($X^2 = 168$; $P < 0.0005$) (Finney, 1953) and a small break is present at around an S.I. of 6.0, the suggested antimode value from the maximum likelihood studies. However, although the majority of the individuals (131; lay within the major population mode (S.I. 0.7 to 5.9), it is apparent from this histogram that

Table 3 Estimated parameters and standard deviations obtained when a mixture of two normal distributions are fitted to the sample data

		<i>Model 1</i>		<i>Model 2</i>	
		<i>s.d.</i>		<i>s.d.</i>	
Mean of first mode	g_1	-1.417	0.147	-1.207	0.245
Mean of second mode	g_2	0.460	0.086	0.354	0.160
Variance of the first mode	v_1				
Variance of the second mode	v_2	0.354	0.043	0.718	0.150
Proportion in the first mode	b_1	0.358	0.049	0.227	0.070
Proportion in the second mode	b_2	0.642	0.049	0.773	0.070
Power of transform	p	0.010	0.130	not applicable

Figure 6 Maximum likelihood computer-generated distribution (solid line) for sulphoxidation capacity (normalised percentage recovery as sulphoxides), showing the two theoretically generated modes which comprise this distribution (faint lines). The original data, in the form of a histogram, is superimposed for comparison (dotted line).

there is not a discrete sub-group of 'impaired' sulphoxidisers isolated from this majority but rather an uninterrupted spread of sulphoxidation capacities.

Family studies

To investigate further whether or not the phenomenon of impaired sulphoxidation was under genetic influence twelve families were selected for study and their pedigrees together with the percentage of the recovery present as sulphoxides are given in Figure 8. It is possible to observe the magnitude of the correlation between relatives and these may be useful despite the families being non-random.

Marital correlation $r = 0.498$ $n = 12$

Parent-offspring correlation $r = 0.728$ $n = 44$

Sib-sib correlation $r = 0.920$ $n = 12$

These values cannot be tested as normal correlations as individuals contribute more than once to each. However, their magnitude is large and in decreasing order from sib-sib to marital, suggesting both a genetic effect and perhaps a family environmental effect, although the latter could be due to non-random sampling, the families being ascertained via extreme probands.

Those individuals excreting 14.3% or less of the recovery as sulphoxides (S.I. ≥ 6.0) were designated as impaired sulphoxidisers (one phenotype). However, it must be realised that this assignment was made from the position of the antimode as derived from the maximum

Figure 7 Frequency distribution of 'sulphoxidation index' in 200 unrelated volunteers.

Figure 8 The pedigrees of 12 families in whom the percentage of 0–8 h drug recovery excreted as sulphoxide metabolites has been determined (indicated by the values shown).

□ = male

○ = female

■ = homozygous impaired metaboliser (by definition, see text)

◐ = individuals who can be identified as heterozygotes with respect to S-carboxymethyl-L-cysteine sulphoxidation (autosomal recessive inheritance, see text).

likelihood studies and that taking this as the cut off point will lead to a certain amount of misclassification, with 1.3% of the impaired being misclassified as extensive and 12.3% of the extensives being misclassified as impaired. Nevertheless, the pedigree information shows families in which all the members are affected (impaired) or totally unaffected, that poor metabolisers of both sexes can occur in the same sibship and that affected parents can father non-affected sons and daughters.

These results are compatible with either a model of the impaired sulphoxidation capacity being controlled by a gene which is dominant but shows incomplete penetrance or by a gene which is incompletely recessive in character, both overlying a background of polygenic effects which mediate their expression. We cannot, from this data, distinguish quantitatively the relative contributions of heritance and environment within each phenotype, since the width of the modes of the distribution are dictated by both environmental and genetic factors.

If we assume that the mode of inheritance is autosomal and incompletely recessive in nature, as has been advocated for the impaired oxidation of debrisoquine and sparteine (Eichelbaum

et al., 1979; Evans *et al.*, 1980, 1983), then those individuals of impaired sulphoxidation phenotype will be of a homozygous recessive genotype. Consequently, assuming that this character is controlled by two alleles at one autosomal locus and that the distributions for each phenotype are normal (see Murphy & Bolling, 1967) then an estimate of the degree of dominance may be calculated from the extent of displacement of the mean observed $\log_{10}$ % recovery as sulphoxides for heterozygotes (1.417; positively identified from the family studies) from the mid-point between the $\log_{10}$ % recovery as sulphoxides for the homozygous recessive (0.739; from population; note the similarity with the computed model 2, 0.74) and homozygous dominant (1.636; from population and Hardy-Weinberg equation) states. Such a calculation, which has been described in detail by Evans *et al.* (1980, 1983), gives a displacement from the mid-homozygous point (zero dominance; co-dominance) towards the homozygous dominant (extensive sulphoxidiser) of 51.2%. Employing the same calculation without using logarithmic values gives a dominance value of 20.7%.

Using this approach it can be seen that applying the Hardy-Weinberg quadratic to the

population data permits the frequency of the allele controlling the recessive character, q , to be calculated as 0.59 and that controlling the dominant character, p , as 0.41. The population can also be divided into its constituent genotypes with the heterozygotes comprising the largest group (97 subjects; 48.5%; $2pq$) followed by the homozygous recessives (69 subjects; 34.5%, q^2) with the homozygous dominants forming the smallest section (34 subjects; 17.0%; p^2).

Although earlier preliminary work using the phenotyped panel approach (Idle *et al.*, 1979b) had suggested that the sulphoxidation of S-carboxymethyl-L-cysteine and the hydroxylation of debrisoquine were closely linked (Waring *et al.*, 1981), further studies, still in progress, with a larger number of volunteers have not confirmed this and it now appears that the impaired carbon and sulphur oxidation traits may be independent phenomena.

Discussion

It can be seen from the results presented in this paper that a substantial difference exists between individuals with respect to their ability to produce the urinary sulphoxide metabolites of S-carboxymethyl-L-cysteine and this phenomenon was reproducible in the 40 subjects reinvestigated. Exploration of the likelihood surface of the data for uni- or multimodality indicated that the model with the best fit had two modes and raised the possibility that this phenomenon may be under the control of two alleles of major effect at a single autosomal

locus, the variation within the modes being due to other genes with comparatively small effects and to other environmental influences (Vesell & Penno, 1983). However, a multimodal distribution only provides very preliminary evidence for a segregating major gene and although the data obtained from the family studies can be interpreted to support the assumption of a genetic mode of inheritance of the impaired sulphoxidation trait, final proof of its existence must await the isolation and characterisation of the specific gene products.

It is, as yet, not known whether or not this phenomenon is true for the metabolic handling of other sulphur containing compounds but an association has already been observed amongst a population of rheumatoid arthritics where a higher incidence of poor sulphoxidisers was found in those patients displaying adverse reactions to penicillamine therapy (Panayi *et al.*, 1983). In this study the expression of the sulphoxidation capacity as the S.I. was most useful. Penicillamine is a structurally similar drug where, apart from disulphide formation, a large proportion of the dose is probably degraded by way of sulphur oxidation pathways thereby removing the offending agent (Planas-Bohne, 1981). Consequently, the ability to oxidise S-carboxymethyl-L-cysteine may reflect the ability to remove penicillamine from the body and hence reduce its toxicity. This concept is a complicated issue which is still under investigation.

The authors are grateful to Dr R. R. Shah for help with parts of this study. S.C.M. was financed by a Wellcome Trust interdisciplinary grant and J.R.I. is a Wellcome Trust Senior Lecturer.

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(Received February 6, 1984,
accepted June 2, 1984)