AN INVESTIGATION INTO THE INTER-RELATIONSHIPS OF SULPHUR XENO-BIOTRANSFORMATION PATHWAYS IN PARKINSON'S AND MOTOR NEURONE DISEASES

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SUMMARY

The role of defective 'sulphur xenobiotic' biotransformations in the aetiology of Parkinson's and motor neurone diseases has been in the literature for over a decade. Problems in the *S*-oxidation of aliphatic thioethers, sulphation of phenolic compounds and the *S*-methylation of aliphatic sulphydryl groups have all been reported. These reports have also been consistent in observing that only a 'significant minority' of patients express these problems in sulphur biotransformation pathways. However, no investigation has yet reported on the incidence of these three defective pathways in control invididuals and in patients with Parkinson's and motor neurone disease. This investigation has found that:

1. Forty percent of patients with Parkinson's and motor neurone disease have a defect in the *S*-oxidation of *S*-carboxymethyl-L-cysteine compared to 4% of controls.

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2. 35-40% of patients with Parkinson's and motor neurone disease have a defect in the sulphation of paracetamol compared to 4% of controls.
3. 60% of patients with motor neurone disease have a high capacity for the *S*-methylation of 2-mercaptoethanol compared to 4% of controls.
4. 38% of patients with Parkinson's disease have a low capacity for the *S*-methylation of 2-mercaptoethanol compared to 4% of controls.
5. There is no correlation between the *S*-oxidation phenotype, low paracetamol sulphation phenotype and low or high *S*-methylation phenotype in controls or patients with Parkinson's or motor neurone disease.
6. The number of controls that expressed one of the aberrant phenotypes was 4% compared to 38% of the patients with Parkinson's disease and 47% of the patients with motor neurone disease.
7. The number of controls that expressed two of the aberrant phenotypes was 0% compared to 18% of the patients with Parkinson's disease and 19% of those with motor neurone disease.
8. No controls or patients with Parkinson's disease or motor neurone disease expressed all three of the aberrant phenotypes.

The results indicate that the three xeno-biotransformation pathways are under separate genetic control in the three population groups studied and that patients with Parkinson's and motor neurone disease do not have a widespread defect in their sulphur xenobiochemistry capacity.

KEY WORDS

sulphur xenobiotic biotransformations, pharmacogenetics, Parkinson's disease, motor neurone disease

INTRODUCTION

The reports of defective 'sulphur xenobiotic' metabolism in patients with Parkinson's disease (PD) and motor neurone disease

(MND) over the last decade have suggested a generalised pattern of an inability to metabolise sulphur-containing compounds in those individuals /1-3/. These reports have hypothesised either environmental exposure to as yet unknown sulphur containing xenobiotic(s) or a failure to detoxify unidentified endogenously derived compound(s) as possible causative agents of these two neurodegenerative diseases /4,5/. To complicate the picture further for patients with PD were the reports of aberrant monoamine oxidase activity and nicotinamide metabolism /6,7/. The most commonly investigated of these biotransformation reactions was the *S*-oxidation of the cysteine-liked xenobiotic, *S*-carboxymethyl-L-cysteine (SCMC) /1,3/. The two conjugating biotransformation pathways of *S*-methylation and *O*-sulphation have been less extensively studied in patients with PD and MND /8,9/. The information about the enzyme systems responsible for these reactions ranges from almost nothing (SCMC *S*-oxidation) to extensive (sulphation of paracetamol). Although the metabolism of SCMC in man and experimental animals has been vigorously investigated at the whole body level, little information exists to date about the cellular location or biochemical and molecular mechanism of SCMC *S*-oxidation. What little information that exists was published in 1986. Waring *et al.* /10/ reported that the *S*-oxygenase had a cytosolic location in the liver of a number of mammalian species, and required molecular oxygen and an unknown divalent cation for activity. The presence of SCMC *S*-oxygenase in other tissues was not investigated /10/. The *S*-methylation of 2-mercaptoethanol (2-ME) is more fully understood but is still seen as a poor relation of its cytosolic counterpart, thiopurine methyltransferase (TPMT) /11/. This enzyme is present in hepatic, renal and CNS tissue and is localised in the endoplasmic reticulum. The enzyme requires *S*-adenosyl-L-methionine as a cofactor and metabolises aliphatic sulphydryl compounds. Finally, the enzymes involved in the *O*-sulphation of paracetamol, ST1A1, -2 and -3, have been more extensively investigated at the biochemical and molecular level /12/. They are present in the cytosol of numerous tissues, require PAPS (3′-phosphoadenosine-5′-phosphosulphate) as cofactor and have a wide and overlapping substrate specificity.

The observation that only 35-40% of patients with PD and MND /3/ showed abnormal SCMC *S*-oxidation, 2-ME *S*-methylation or paracetamol *O*-sulphation indicates that only a subpopulation of PD

and MND patients have problems with 'sulphur xenobiotic' metabolism. However, no-one to date has investigated the possible associations of abnormal *S*-oxidation, *S*-methylation and *O*-sulphation in control, PD and MND populations. This raises the possibility of patients with PD or MND having either individual or multiple defects in xenobiotic metabolism of sulphur-containing xenobiotics. This papers reports on the investigation of possible associations of *S*-oxidation, *S*-methylation and *O*-sulphation problems in patients with PD and MND.

MATERIALS AND METHODS

Materials

S-Carboxymethyl-L-cysteine, *S*-methyl-L-cysteine (SMC), paracetamol (*N*-acetyl-4-aminophenol), 2-mercaptoethanol, chloroplatinic acid, methanol, bovine β-glucuronidase type B1, aryl sulphatase (*Helix pomatia* type H-1), D-saccharic acid-1,4-lactone, acetone and glacial acetic acid were supplied by Sigma-Aldrich Chemical Company Ltd., Poole, Dorset, UK. Hplc grade methanol and ethyl acetate were supplied by BDH Laboratory Supplies, Poole, Dorset, UK.

Synthesis

The *S*-oxide metabolites of *S*-carboxymethyl-L-cysteine, *S*-methyl-L-cysteine, *N*-acetyl-*S*-carboxymethyl-L-cysteine, *N*-acetyl-*S*-methyl-L-cysteine and thiodiglycolic acid *S*-oxide were synthesised as described by Messe *et al.* /13/ and Staffeldt *et al.* /14/.

Volunteers

Control individuals (n = 53), hospital in-patients who were age-matched with PD and MND patients, patients with PD (n = 40) and patients with MND (n = 53) were selected as previously reported /3/.

Phenotyping investigation

On day 1, a 10 ml fasted venous blood sample was removed from each volunteer at 08.45 h and the erythrocyte membranes prepared by

the method of Weinshilboum *et al.* /15/. Erythrocyte membranes were stored at -20°C for no longer than 7 days prior to analysis. At 09.00 h each volunteer emptied his or her bladder. 750 mg of SCMC (2 x 325 mg capsules) was taken orally and the total urine output from 09.00 to 17.00 h was collected. The total urine volume was measured and 2 x 10 ml aliquots stored at -20°C until analysed. No sample was stored for longer than 12 weeks prior to analysis. On day 14 at 09.00 h, each volunteer emptied their bladder. 500 mg of paracetamol (1 x 500 mg tablet) was taken orally and the total urine output from 09.00 to 17.00 h was collected. The total urine volume was measured and 2 x 10 ml aliquots stored at -20°C until analysed.

Analysis

Urinary *S*-oxide analysis was carried out by descending paper chromatography as reported by Mitchell *et al.* /16/.

Urinary paracetamol, paracetamol sulphate and paracetamol glucuronide were analysed by hplc with UV detection as reported by Steventon *et al.* /9/.

Erythrocyte membrane thiol methyltransferase (TMT) activity was determined by a radioenzyme assay as reported by Keith *et al.* /17/. One unit of TMT activity is defined as the production of 1 nmole of *S*-methyl 2-ME/min/mg erythrocyte membrane protein.

Statistical analysis

Data analyses were performed with SPSS 10.0 using Student's t-test, χ^2-test with Yates' correction, Wilcoxon rank sum test and Spearman's correlation coefficient. A value of $p < 0.05$ was taken to indicate statistical significance.

RESULTS

The mean age (± SD) and sex of the control, PD and MND volunteers can be seen in Table 1. There was no significant difference in age between the three groups under investigation. Previous work has found no significant difference in urinary *S*-oxide, paracetamol sulphate recovery and erythrocyte TMT activity between the sexes in

TABLE 1

Population data

Population	n	Age (yr)	
		Mean ± SD	Range
Controls			
Males	25	65.6 ± 18.7	25-90
Females	28	61.2 ± 17.5	26-80
Total	53	62.3 ± 17.9	26-90
Parkinson's disease			
Males	20	60.1 ± 13.2	42-80
Females	20	66.2 ± 10.1	45-81
Total	40	62.1 ± 12.7	42-81
Motor neurone disease			
Males	27	64.5 ± 10.7	41-79
Females	26	60.2 ± 11.7	42-79
Total	53	62.3 ± 10.5	41-79

control, PD and MND patients /3,8/. These observations were also found in this investigation (data not shown).

The association of the patients with PD and MND with the *S*-oxidation poor metaboliser (PM) phenotype can be seen in Table 2a. Both the PD (40%) and MND (39.6%) populations were significantly over-represented in the PM phenotype (urinary *S*-oxide recovery <1.6%) compared to the controls (3.7%, $p < 0.001$, χ^2-test with Yates' correction for both PD and MND patients). These results were balanced by a significant reduction in the number of PD (30.0%) and MND (30.2%) patients phenotyped as extensive metabolisers (EM, urinary *S*-oxide recovery >14.3%) compared to the controls (64.2%, $p < 0.05$, χ^2-test with Yates' correction for both PD and MND patients). The number of individuals phenotyped as intermediate metabolisers (IM, urinary *S*-oxide recovery 1.6-14.3%) was not

TABLE 2

Phenotyping investigation

a. Urinary SCMC S-oxides recovery distribution

Population	n	<1.6%	1.6-14.3%	>14.3%
Controls	53	3.7%	33.9%	64.2%
PD	40	40.0%*	30.0%	30.0%$^{+}$
MND	53	39.6%*	30.2%	30.2%$^{+}$

* $p<0.001$, χ^2-test with Yates' correction.
$^{+}$ $p<0.05$, χ^2-test with Yates' correction.

b. Urinary paracetamol sulphate recovery distribution

Population	n	<3%	3-19%	>19%
Controls	53	3.7%	92.6%	3.7%
PD	40	35.0%*	65.0%$^{+}$	0.0%
MND	53	39.6%*	60.4%$^{+}$	0.0%

* $p<0.001$, χ^2-test.
$^{+}$ $p<0.05$, χ^2-test.

c. *In vitro* erythrocyte TMT activity distribution

Population	n	<303	303-1467	>1467
Controls	53	3.7%	92.6%	3.7%
PD	40	37.5%*	62.5%$^{+}$	0.0%
MND	53	0.0%	39.6%$^{+}$	60.4%*

* $p<0.001$, χ^2-test.
$^{+}$ $p<0.05$, χ^2-test.
PD = Parkinson's disease; MND = motor neurone disease;
SCMC = *S*-carboxymethyl-L-cysteine; TMT = thiol methyltransferase.

significantly different between the three populations (controls 33.9%, PD 30% and MND 30.2%, $p>0.05$, χ^2-test with Yates' correction for both PD and MND patients).

The association of paracetamol sulphation with PD and MND patients can be seen in Table 2b. The urinary recovery of paracetamol sulphate in the control population was normally distributed. Individuals were classified as having extensive sulphation capacity (urinary paracetamol sulphate recovery >19%), normal sulphation capacity (urinary paracetamol sulphate recovery 3-19%) and low sulphation capacity (urinary paracetamol sulphate recovery <3%). Both the PD and MND patients had significantly more individuals in the low sulphation capacity group (controls 3.7%, PD 35% and MND 39.6%, $p < 0.001$, χ^2-test for PD and MND patients). The results for the normal sulphation capacity group (controls 92.6%, PD 65.0% and MND 60.4%) showed that the PD and MND patients had a significantly decreased number of individuals in this grouping ($p < 0.05$, χ^2-test for PD and MND patients). There were no MND or PD patients in the extensive sulphation capacity group (controls 3.7%, PD and MND 0%).

The association of PD and MND patients with erythrocyte TMT activity can be seen in Table 2c. The erythrocyte TMT activity in the control population was normally distributed. Individuals were classified as having extensive TMT activity (erythrocyte TMT activity >1,467 units/mg protein), normal TMT activity (erythrocyte TMT activity 303-1467 units/mg protein) and low TMT activity (erythrocyte TMT activity <303 units/mg protein). The patients with PD showed a significant increase in the number of individuals classified with low TMT activity compared to the controls (37.5% versus 3.7%, $p < 0.001$, χ^2-test). The number of patients with PD classified as having normal TMT activity was significantly reduced compared to the controls (62.5% versus 92.6%, $p < 0.05$ χ^2-test). There were no patients with PD classified as having high TMT activity.

In contrast, the patients with MND showed a significant increase in the number of individuals classified as having high TMT activity compared to the controls (60.4% versus 3.7%, $p < 0.001$, χ^2-test). A significant reduction in the number of patients with MND classified as having normal TMT activity compared to the controls (39.6% versus 92.6%, $p < 0.05$, χ^2-test). No patients with MND were classified as having low TMT activity (Table 2c).

The association of the PM phenotype for *S*-oxidation, low sulphation or low TMT activity with the control and PD patients can be seen in Table 3a. None of the controls or PD patients was found to

TABLE 3

Statistical analyses of phenotypes

a. Expression of poor metaboliser phenotypes in control and Parkinson's disease (PD) groups

Phenotype	Controls	PD patients
3/3	0.0%	0.0%
2/3	0.0%	17.5%
1/3	3.7%	37.5%

3/3 = number of individuals phenotyped with a urinary recovery of S-oxides <1.6%, urinary paracetemol sulphate recovery <3.0%, and erythrocyte thiol methyltransferase (TMT) activity <303 units.
2/3 = number of individuals with two of the above three phenotypes.
1/3 = number of individuals with one of the above three phenotypes.

b. Expression of poor/extensive metaboliser phenotypes in control and motor neuron disease (MND) groups

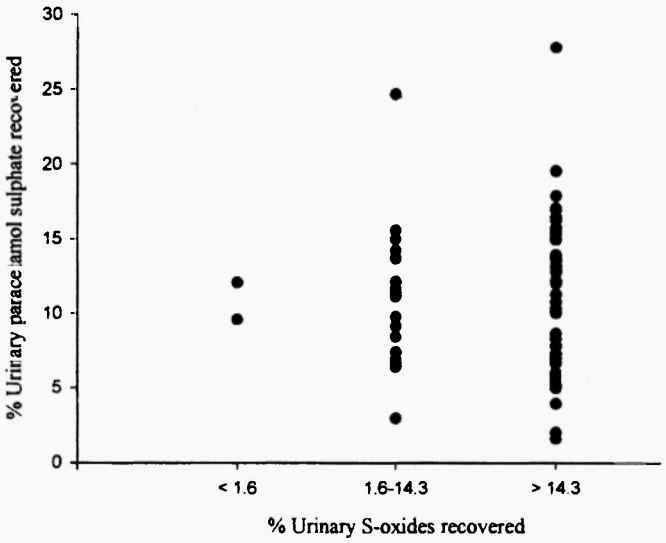
Phenotype	Controls	MND patients
3/3	0.0%	0.0%
2/3	0.0%	18.8%
1/3	3.7%	46.5%

3/3 = number of individuals phenotyped with a urinary recovery of S-oxides <1.6%, urinary paracetemol sulphate recovery <3.0%, and erythrocyte TMT activity >1,467 units.
2/3 = number of individuals with two of the above three phenotypes.
1/3 = number of individuals with one of the above three phenotypes.

express all three of these phenotypes (PM for SCMC *S*-oxidation, low sulphation capacity and low erythrocyte TMT activity). In contrast, 17.5% of the patients with PD expressed two of these three phenotypes compared to 0% of the control population. When the number

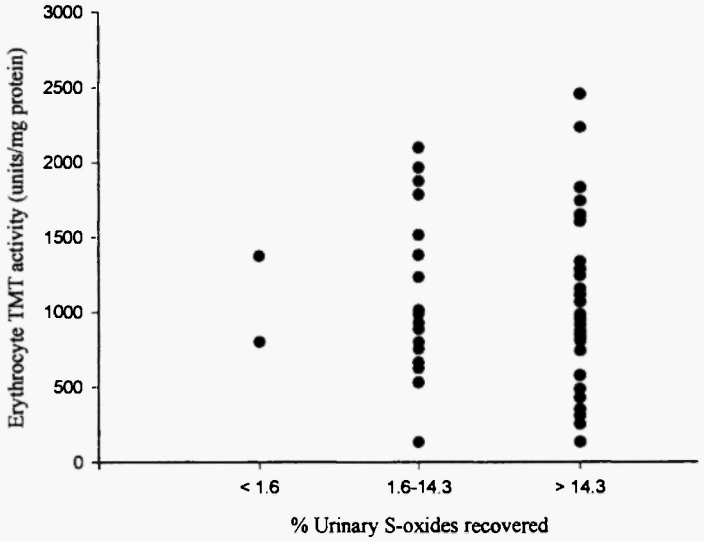
of individuals expressing just one of these three phenotypes was compared, only 3.7% of the control population was found in this category compared to 37.5% of the PD population. A similar set of results can be seen in Table 3b for the patients with MND. None of the patients with MND or controls expressed all three phenotypes; 18.8% of the patients with MND expressed two of these three phenotypes compared to 0% of the controls. Finally, 46.5% of the patients with MND expressed one of these three phenotypes compared to 3.7% of the control population.

The results in Tables 2 and 3 are supported by the results shown in Figures 1-3. There is no obvious relationship between the *S*-oxidation phenotype and paracetamol sulphation in the controls (Fig. 1a). No relationship was seen between control erythrocyte TMT activity and *S*-oxidation phenotype (Fig. 1b). Finally, there was also no linear relationship between control paracetamol sulphation and erythrocyte TMT activity (r_s = -0.14) (Fig. 1c). Similar results were seen for the patients with PD (Fig. 2) and MND (Fig. 3).

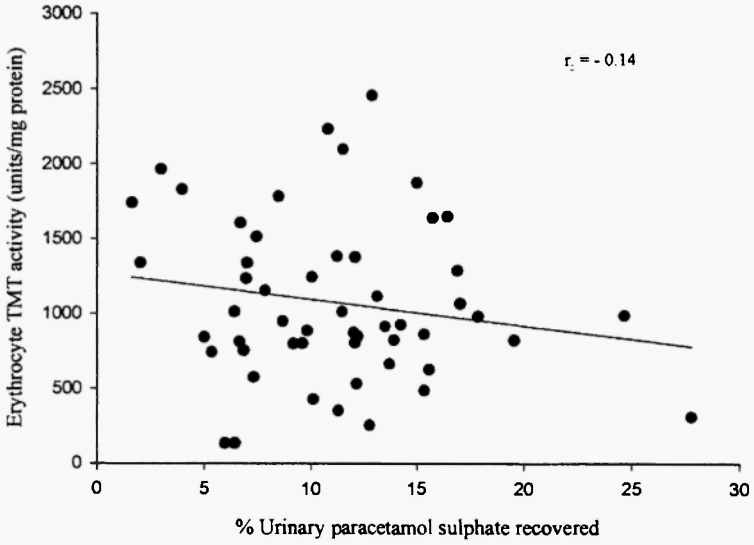


a. % urinary paracetamol sulphate recovered and S-oxidation phenotypes in controls

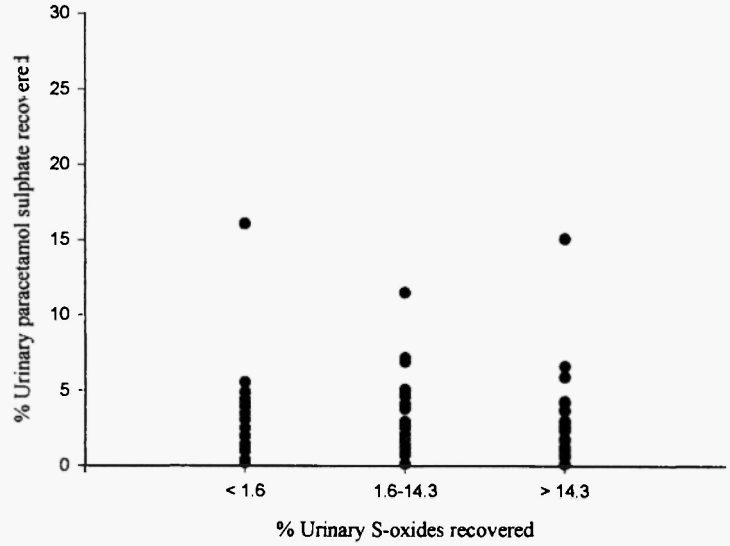
Fig. 1: Correlation studies in controls. Investigation into the urinary S-oxide and paracetamol sulphate recoveries and thiol methyltransferase (TMT) activities.



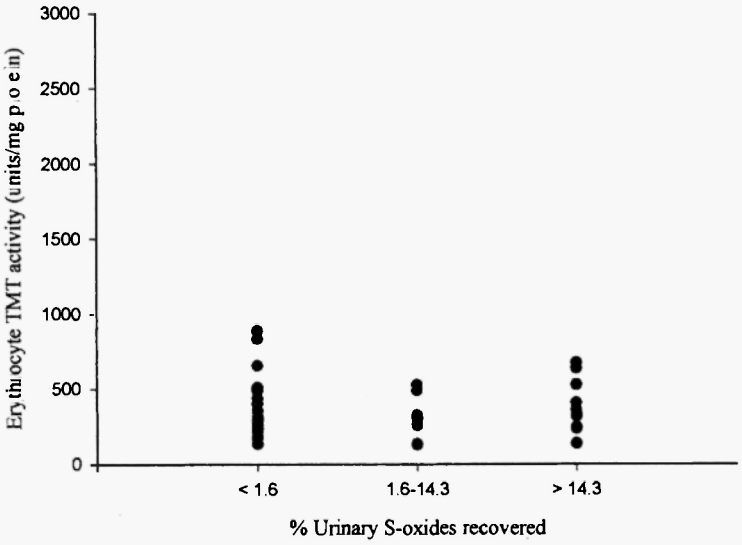
b. Erythrocyte TMT activity and S-oxidation phenotypes in controls



c. Correlation between paracetamol sulphation and TMT activity in controls

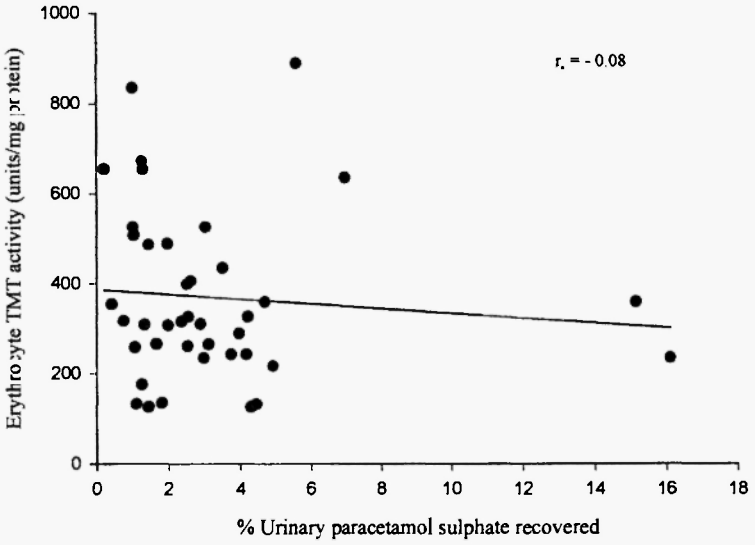


a. % urinary paracetamol sulphate recovered and S-oxidation phenotypes in PD



b. Erythrocyte TMT activity and S-oxidation phenotypes in PD

Fig. 2: Correlation studies in patients with Parkinson's disease (PD). Investigation into the urinary S-oxide, paracetamol sulphate recoveries and thiol methyltransferase (TMT) activities.



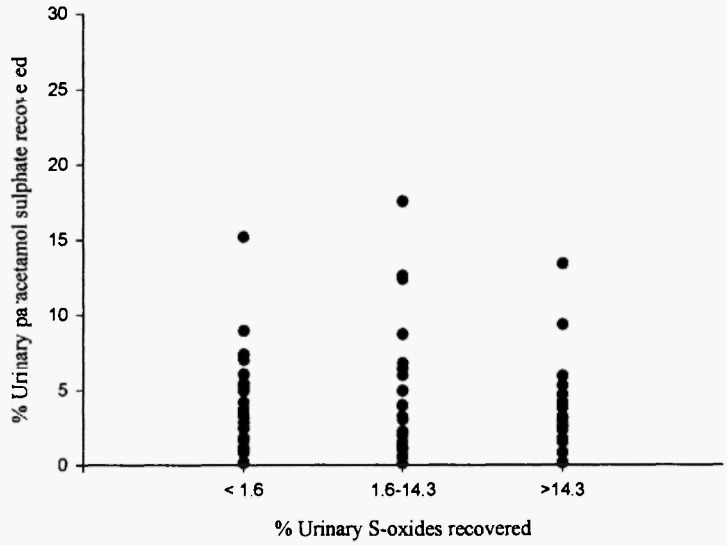
c. Correlation between paracetamol sulphation and TMT activity in PD

DISCUSSION

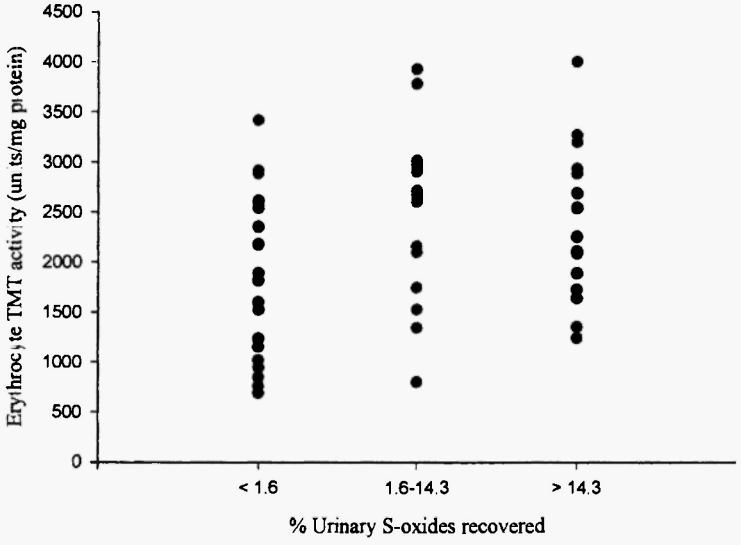
The results from Table 1 show no age differences between controls and patients with PD or MND ($p > 0.05$, Student's t-test). In addition, there were no age differences between male and female volunteers either within or between the controls and patients with PD or MND.

The results of the three phenotyping investigations shown in Table 2 confirm previous reports of defective sulphur metabolism in patients with PD and MND /8,9,18/. There was a significant over-representation of PD and MND patients with the PM phenotype for SCMC *S*-oxidation compared to the controls (Table 2a, $p < 0.001$, χ^2-test with Yates' correction). This over-representation of patients with the PM phenotype was balanced by the significant under-representation of PD and MND patients with the EM phenotype compared to the controls (Table 2a, $p < 0.05$, χ^2-test with Yates' correction). These results are consistent with a defect in the hepatic cytosolic enzyme 'SCMC *S*-oxygenase'.

The results of the paracetamol sulphation investigation (Table 2b) show that the patients with PD and MND are significantly over-represented in the 'low sulphation capacity' region of the normal

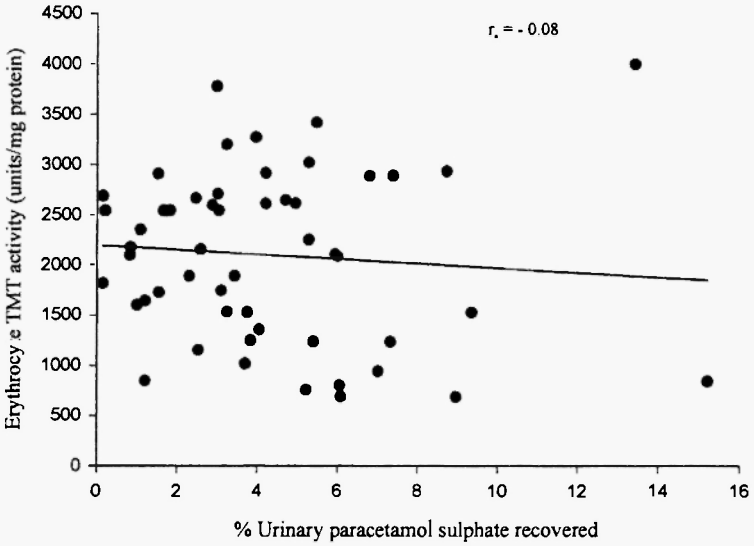


a. % urinary paracetamol sulphate recovered and S-oxidation phenotypes in MND



b. Erythrocyte TMT activity and S-oxidation phenotypes in MND

Fig. 3: Correlation studies in patients with motor neurone disease (MND). Investigation into the urinary S-oxide, paracetamol sulphate recoveries and thiol methyltransferase (TMT) activities.



c. Correlation between paracetamol sulphation and TMT activity in MND

distribution (controls 3.7%, PD 35.0%, MND 39.6%, p <0.001, χ^2-test). Similarly the PD and MND patients are under-represented in the 'normal and high sulphation capacity' regions of the normal distribution (controls 96.3%, PD 65.0%, MND 60.4%). Both the PD and MND patients showed a normal distribution in terms of their capacity to sulphate paracetamol but their respective distributions had lower mean values than the controls /9/.

The results for the erythrocyte TMT activity investigation (Table 2c) produced the first differences in terms of sulphur metabolism between the PD and MND patients. The control group showed a normal distribution of erythrocyte TMT activity /17/ as did the PD and MND patients. However, the number of patients with PD classified as having 'low TMT activity' was significantly higher than the controls and the patients with MND (controls 3.7%, PD 37.5%, MND 0.0%, p <0.001, χ^2-test). The patients with PD and MND both had a significantly reduced number of individuals classified as having 'normal TMT activity' (controls 92.6%, PD 62.5%, MND 39.6%, p <0.05, χ^2-test). The number of patients with MND classified as having a 'high TMT activity' was significantly higher than in the

controls or patients with PD (controls 3.7%, PD 0.0%, MND 60.4%, $p < 0.001$, χ^2-test). Thus the mean TMT activity in the patients with PD was significantly reduced compared to the controls and patients with MND, but the mean TMT activity in patients with MND was significantly higher than in the controls or PD patients /8/.

The possible association of the *S*-oxidation phenotypes, low paracetamol sulphation and low or high TMT activity in controls can be seen in Figure 1. There appears to be no obvious relationship between the *S*-oxidation phenotypes (EM, IM or PM) and paracetamol sulphation capacity (Fig. 1a). A similar conclusion can be drawn from Figure 1b between the *S*-oxidation phenotypes and erythrocyte TMT activity. Finally, there appears to be no linear relationship between paracetamol sulphation capacity and erythrocyte TMT activity ($r_s = -0.14$). A similar pattern of results can be seen for the patients with PD (Fig. 2) and MND (Fig. 3). Thus these three sulphur metabolism pathways would appear to be independently controlled and not linked in any way in the three populations under investigation. This argument is further strengthened by the results in Table 3. Here the frequency of the three PM/EM phenotypes (<1.6% urinary *S*-oxide recovery, <3% urinary paracetamol sulphate recovery and erythrocyte TMT activity <303 or >1,467 units) was investigated. None of the controls or patients with PD or MND possessed all three of these phenotypes (Table 3). None of the controls were found to express two of these three phenotypes, but 17.5% of the PD and 18.8% of the MND patients (Table 3) were found to have two out of the three phenotypes ($p < 0.05$, χ^2-test). Finally, 3.7% of the controls, 37.5% of the patients with PD and 46.5% of the patients with MND were found to express one of the three phenotypes under investigation (Table 3, $p < 0.01$, χ^2-test). These results provide further evidence of a disturbance in the sulphur xeno-biotransformation pathways in patients with PD or MND that are under independent genetic control. The exact mechanisms by which these three biotransformation pathways (aliphatic thioether *S*-oxidation, phenolic sulphation and aliphatic sulphydryl methylation) contribute to the aetiology of these diseases are unknown. However, all three are susceptibility factors for the diseases /3/ and a detailed investigation of the roles of these biotransformation pathways in PD and MND is warranted.

In conclusion, these initial observations indicate that patients with PD and MND do not have a generalised defect in sulphur biotrans-

formation. However, 38% of patients with PD and 47% of patients with MND have at least one defect in aliphatic thioether *S*-oxidation, phenolic sulphation or aliphatic sulphydryl methylation. These biotransformations are carried out by:

1. SCMC *S*-oxygenase, a yet to be identified hepatic cytosolic protein.
2. ST1A1, ST1A2 and ST1A3. However, the inability to sulphate paracetamol *in vivo* may be due to a defect in PAPS (3′-phospho-adenosine-5′-phosphosulphate) synthesis.
3. TMT. When using substrates other than 2-ME only the patients with MND showed altered *S*-methylation capacity /3,19/.

A drawback of the present observations is the limited number of patients involved. Thus a detailed investigation into sulphur xeno-biotransformation should be undertaken in a larger population of controls and PD and MND volunteers in order to give greater statistical power to the associations of poor SCMC *S*-oxidation/low paracetamol sulphation/low or high TMT activity in these neurological diseases.

REFERENCES

1. Steventon G, Williams AC, Waring RH, Pall HS, Adams D. Xenobiotic metabolism in motorneuron disease. Lancet 1988; ii: 644-647.
2. Steventon GB, Heafield MTE, Waring RH, Williams AC. Xenobiotic metabolism in Parkinson's disease. Neurology 1989; 39: 883-887.
3. Steventon GB, Sturman S, Waring RH, Williams AC. A review of xenobiotic metabolism enzymes in Parkinson's and motor neuron disease. Drug Metab Drug Int 2001; 18: 79-98.
4. Williams AC, Steventon GB, Sturman S, Waring RH. Hereditary variation of liver enzymes involved with detoxification and neurodegenerative disease. J Inherit Metab Dis 1991; 14: 431-435.
5. McFadden SA. Phenotypic variation in xenobiotic metabolism and adverse environmental response: focus on sulfur-dependent detoxification pathways. Toxicology 1996; 111: 43-65.
6. Humfrey CDN, Steventon GB, Sturman SG, Waring RH, Williams AC. Monoamine oxidase substrates in Parkinson's disease. Biochem Pharmacol 1990; 40: 2562-2564.
7. Green S, Buttrum S, Molloy H, Steventon G, Sturman S, Waring R, Pall H, Williams A. *N*-Methylation of pyridines in Parkinson's disease. Lancet 1991; 338: 120-121.

8. Waring RH, Steventon GB, Sturman SG, Heafield MTE, Smith MCG, Williams AC. *S*-Methylation in motorneuron disease and Parkinson's disease. Lancet 1989; ii: 356-357.
9. Steventon GB, Heafield MTE, Sturman S, Waring RH, Williams AC. Metabolism of low-dose paracetamol in patients with chronic neurological disease. Xenobiotica 1990; 20: 2562-2564.
10. Waring RH, Mitchell SC, O'Gorman J, Fraser M. Cytosolic sulphoxidation of *S*-carboxymethyl-L-cysteine in mammals. Biochem Pharmacol 1986; 35: 2999-3002.
11. Weinshilboum RM. Thiopurine pharmacogenetics: clinical and molecular studies of thiopurine methyltransferase. Drug Metab Disp 2001; 29: 601-605.
12. Hagata K, Yamazoe Y. Pharmacogenetics of sulfotransferases. Annu Rev Pharmacol Toxicol 2000; 40: 159-176.
13. Meese CO, Specht D, Hofmann U. Synthesis of metabolites of *S*-carboxymethyl-L-cysteine and *S*-methyl-L-cysteine and some isotopically labelled (^{2}H, ^{13}C) analogues. Arch Pharm (Weinheim) 1990; 323: 957-965.
14. Staffeldt B, Brockmoller J, Roots I. Determination of *S*-carboxymethyl-L-cysteine and some of its metabolites in urine and serum by high-performance liquid chromatography using fluorescent pre-column labelling. J Chromatogr 1991; 571: 133-147.
15. Weinshilboum RM, Sladek S, Klumpp S. Human erythrocyte thiol methyltransferase: radiochemical microassay and biochemical properties. Clin Chim Acta 1979; 97: 59-71.
16. Mitchell SC, Waring RH, Haley CS, Idle JR, Smith RL. Genetic aspects of the polymodally distributed sulphoxidation of *S*-carboxymethyl-L-cysteine in man. Br J Clin Pharmacol 1984; 18: 507-521.
17. Keith RA, Van Loon J, Wusson LF, Weinshilboum RM. Thiol methylation pharmacogenetics: heritability of human erythrocyte thiol methyltransferase activity. Clin Pharm Ther 1983; 34: 521-528.
18. Steventon G, Waring R, Sturman S, Heafield MTE, Williams AC. The metabolism of *S*-carboxymethyl-L-cysteine in man. A neurodegenerative disease study. Med Sci Res 1999; 27: 119-120.
19. Peters L, Steventon GB, Green S, Sturman S, Waring RH, Williams AC. D-Penicillamine metabolism in neurodegenerative disease: an in vivo/in vitro sulphydryl methylation study. Xenobiotica 1994; 24: 1013-1020.