Br. J. clin. Pharmac. (1989), **28**, 639–645

Codeine *O*-demethylation co-segregates with polymorphic debrisoquine hydroxylation

Q. Y. YUE, J.-O. SVENSSON, C. ALM, F. SJÖQVIST & J. SÄWE
Department of Clinical Pharmacology, Karolinska Institute, Huddinge University Hospital, Sweden

1 A single oral dose of codeine (25 mg) was given to 132 healthy Swedish Caucasians who had previously been phenotyped with respect to debrisoquine hydroxylation. The 'metabolic ratios' (MR) in urine of codeine *O*-demethylation (codeine/(morphine (M) + morphine-3- and 6-glucuronides (M3G and M6G) + normorphine)), *N*-demethylation (codeine/(norcodeine (NC) + norcodeine glucuronide + normorphine (NM))) and glucuronidation (codeine/codeine-6-glucuronide (C6G)) were calculated following h.p.l.c. analysis of urine samples collected over 8 h.

2 There was a significant correlation between the log MR for debrisoquine hydroxylation and the log MR for codeine *O*-demethylation ($r_s = 0.77$, $P < 0.001$). The poor debrisoquine hydroxylators had MRs of codeine *O*-demethylation between 8.3 and 55.1, while the values for extensive hydroxylators were between 0.4 and 5.5.

3 The poor debrisoquine hydroxylators excreted significantly less M, M3G, M6G and NM, while the urinary recovery of C6G and NC was significantly higher in these subjects compared to the extensive hydroxylators.

4 The MRs for glucuronidation and *N*-demethylation did not exhibit a bimodal distribution, and were not related to the MR of debrisoquine hydroxylation.

5 No associations were found between sex, body-weight, smoking habits, age, urine volume or urine pH and the *O*-demethylation of codeine.

6 The *O*-demethylation of codeine to form M appears to be under the same polymorphic genetic control as the 4-hydroxylation of debrisoquine.

Keywords codeine *O*-demethylation debrisoquine hydroxylation pharmacogenetics polymorphism man

Introduction

A number of isoenzymes in the cytochrome P-450 family are involved in the oxidative metabolism of many essential drugs (Boobis & Davies, 1984; Nebert & Gonzalez, 1985). There is compelling evidence that at least two of these enzymes are under monogenic control and that the population can be divided into extensive and poor hydroxylators using the probe drugs debrisoquine/sparteine and mephenytoin, respectively (Eichelbaum *et al.*, 1979, 1982; Küpfer & Preisig, 1984; Mahgoub *et al.*, 1977; Steiner *et al.*, 1985; Wedlund *et al.*, 1984).

Codeine is metabolized mainly by conjugation with glucuronic acid and, to a minor extent, by *O*-demethylation to morphine (M) and *N*-demethylation to norcodeine (NC) (Figure 1) (Adler *et al.*, 1955). This allows the study of oxidation (phase I) and conjugation (phase II) reactions with the same drug in the same individual. The structural similarities between codeine and dextromethorphan (Figure 1), and the fact that the *O*-demethylation of dextromethorphan co-segregates with the hydroxylation of debrisoquine (Pfaff *et al.*, 1983; Schmid *et al.*, 1985),

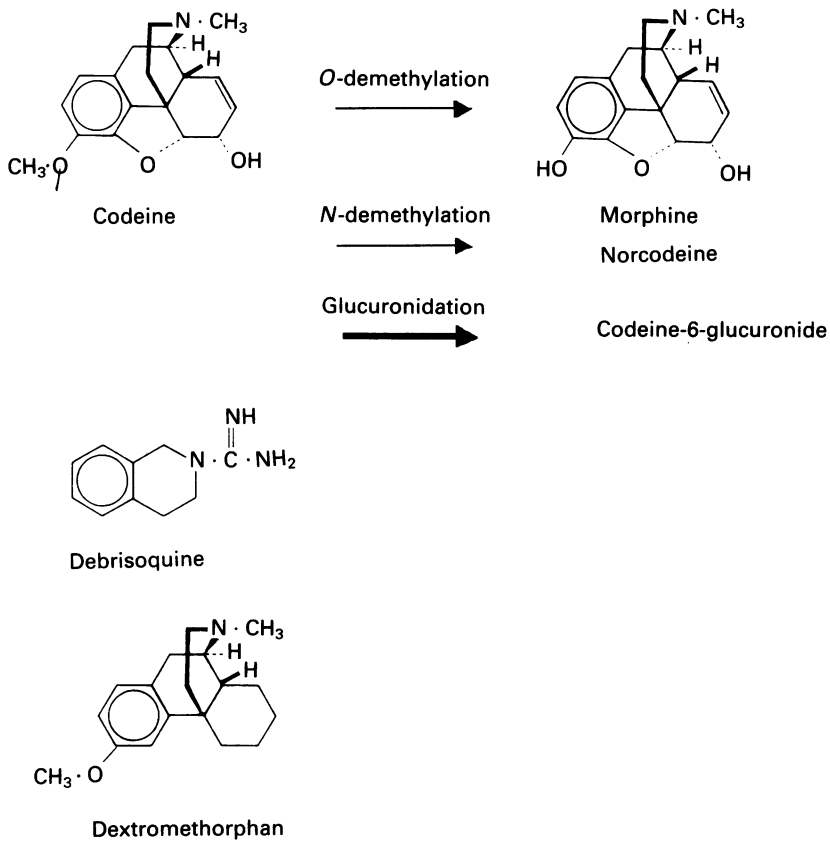

Figure 1 Structures of codeine, morphine, debrisoquine and dextromethorphan.

prompted us to investigate the *O*-demethylation of codeine in a large group of Swedish volunteers whose debrisoquine hydroxylation phenotype had been determined previously. Moreover, recent *in vitro* (Dayer *et al.*, 1988) and *in vivo* (Chen *et al.*, 1988) studies have indicated that the *O*-demethylation of codeine is related to that of debrisoquine-4-hydroxylation. Data on the *N*-demethylation and glucuronidation of codeine are also presented for comparison.

A preliminary account of this paper was presented in abstract form at the Swedish Annual Meeting in Medicine (Yue *et al.*, 1987) and at the 5th South East Asian and Western Pacific Regional Meeting of Pharmacologists (Yue *et al.*, 1988).

Methods

Subjects of the study were 132 healthy Swedish volunteers (50 males and 82 females) aged 15 to 74 years who had been phenotyped previously with respect to their ability to hydroxylate debrisoquine. Thirty-nine were smokers and 20 women took oral contraceptives. None of the subjects received any other medication. The ratio of debrisoquine/4-hydroxydebrisoquine in urine (6 h collection) was measured after ingestion of 10 mg oral debrisoquine sulphate (Declinax® tablets, Hoffman-La Roche). The parent drug and the metabolite were measured by gas chromatography (Lennard *et al.*, 1977). The debrisoquine/4-hydroxy-debrisoquine ratio in urine varied between 0.07 and 380. Eighteen subjects were classified as poor and 114 as extensive hydroxylators.

The study was approved by the Ethics Committee of Huddinge University Hospital.

The subjects received a single oral dose of 25 mg codeine phosphate (Kodein®, ACO) at bedtime after emptying the bladder. Urine was collected overnight for 8 h. The urine volume and pH were measured and an aliquot was stored frozen at −20° C until analysed.

Codeine and its seven known metabolites, M, M-3- (M3G) and M-6- (M6G) glucuronide, normorphine (NM), codeine-6-glucuronide (C6G), NC and NC-glucuronide (NCG) were measured using ion-pair high-performance liquid chromatography by a modification of the method of Svensson (1986) as described by Yue *et al.* (1989).

M6G was obtained from Ultrafine Chemicals, Manchester, England. C6G and NC were supplied by the National Institute of Drug Abuse, MD, USA. Codeine was obtained from Apoteksbolaget, Stockholm, Sweden. For the sources of all other chemicals see Svensson *et al.* (1982).

Because a reference sample of NCG was not available, concentrations of this metabolite were calculated using the standard curve for C6G. Codeine and NC have similar u.v.-response and standard curves (4% differences in the slope) so it can be assumed that their glucuronides should behave similarly. Moreover, the glucuronidation of codeine has very little influence on u.v.-response.

The metabolic ratios (MRs) of codeine were calculated for *O*-demethylation (as the ratio between codeine and the sum of M, M3G, M6G and NM), *N*-demethylation (the ratio between codeine and the sum of NC, NCG and NM) and glucuronidation (codeine/C6G).

Statistical analysis was by the unpaired Student's *t*-test, the Mann-Whitney U-test, linear regression and the Spearman rank correlation test. Frequency histograms were constructed for the values of log MR.

Results

The amounts of codeine and its seven metabolites excreted in urine (percentage of dose) are shown in Table 1. The 8 h urinary recovery was on average 75.5 ± 11.9% of the administered dose. There was no significant difference in total recovery between extensive and poor debrisoquine hydroxylators. Glucuronidation, the major metabolic pathway, accounted for (83 ± 4%) of total recovery. The corresponding values for *O*-demethylation and *N*-demethylation were 4.7% and 7.1%, respectively.

There was a significant correlation between the log MR for debrisoquine hydroxylation and the log MR for *O*-demethylation of codeine

Table 1 Percentage urinary recovery of codeine (Cod) and its metabolites (mean ± s.d.) 8 h after intake of 25 mg (61.5 nmol) codeine. (Abbreviations as described in materials and methods)

	Extensive hydroxylators	*Poor hydroxylators*	*All subjects*
n	114	18	132
C6G	62.2 ±3.2	64.7* ±3.8	62.5 ±3.3
Cod	4.7 ±2.0	5.2 ±2.3	4.7 ±2.1
NCG	2.3 ±0.7	2.5 ±0.7	2.3 ±0.7
NC	2.2 ±0.9	3.7** ±2.1	2.4 ±1.2
M3G	2.6 ±1.5	0.2** ±0.1	2.3 ±1.6
NM	0.8 ±0.4	0.03** ±0.02	0.7 ±0.4
M6G	0.5 ±0.2	0.03** ±0.02	0.4 ±0.3
M	0.1 ±0.08	0.02** ±0.03	0.1 ±0.08
Total	75.4 ±11.2	76.4 ±16.5	75.5 ±11.9

Student's *t*-test between rapid and slow debrisoquine hydroxylators, $*P < 0.05$, $**P < 0.001$

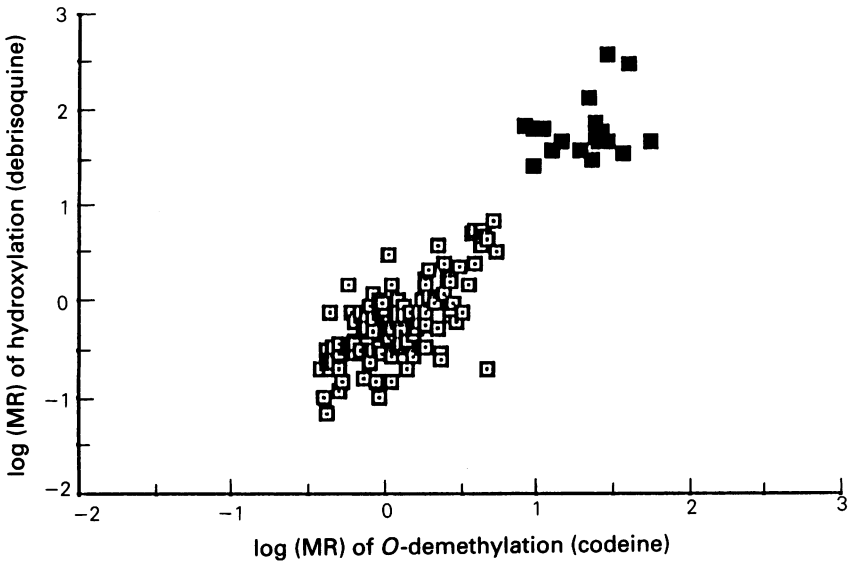

Figure 2 Correlation between log MR for debrisoquine hydroxylation and the log MR for codeine *O*-demethylation in 132 Caucasians ($r_s = 0.77$, $P < 0.001$). □ extensive metabolisers, ■ poor metabolisers.

Table 2 Ranges of the metabolic ratios for *O*-demethylation, *N*-demethylation and glucuronidation of codeine in 114 extensive and 18 poor debrisoquine (D) hydroxylators

Metabolic ratios	*Extensive*	*Poor*
n	114	18
D/4-OHD*	0.07–6.7	26.4–380**
O-demethylation*	0.4–5.5	8.3–55.1**
N-demethylation*	0.2–2.6	0.4–1.9
Glucuronidation*	0.02–0.23	0.04–0.22

*D/4-OH-D = debrisoquine/4-hydroxydebrisoquine, *O*-demethylation = codeine/(morphine + morphine-3- and 6-glucuronide + normorphine), *N*-demethylation = codeine/(norcodeine + norcodeine glucuronide + normorphine). Glucuronidation = codeine/codeine-6-glucuronide
**Mann-Whitney U-test between rapid and slow debrisoquine hydroxylators, $P < 0.0001$.

(Spearman r_s 0.77; $P < 0.001$) (Figure 2). The MRs for *O*-demethylation varied 145-fold (Table 2). The poor debrisoquine hydroxylators had significantly higher MRs for *O*-demethylation and no overlap was observed between the MRs of the poor hydroxylators with those of the extensive hydroxylators (Figure 3a). In the former group ($MR_{O\text{-}dem} > 8.3$) significantly less M, M3G, M6G and NM were excreted ($P < 0.001$), while the recovery of C6G and NC was significantly higher ($P < 0.05$, and $P < 0.001$ respectively) compared with that in extensive hydroxylators. The MRs for glucuronidation and *N*-demethylation varied 11- and 17-fold, respectively (Table 2). There was no significant correlation between the log MR for *N*-demethylation or glucuronidation and the log MR for debrisoquine hydroxylation (Figure 3).

No association was evident between sex, body-weight, smoking habits, age, urine volume or pH and *O*-demethylation.

Discussion

We have demonstrated that codeine *O*-demethylation co-segregates with debrisoquine hydroxylation and thus is likely to be genetically polymorphic in Swedish Caucasians. There was a 145-fold interindividual variation in the metabolic ratio for this reaction. The eighteen poor hydroxylators of debrisoquine constituted a separate group in the distribution of the *O*-demethylation MRs which ranged from 8.3 to 55.1. By contrast, MRs in the 114 extensive hydroxylators of debrisoquine varied from 0.4 to 5.5. The recovery of metabolites of codeine formed by *O*-demethylation was less than 0.4% of the dose in the poor hydroxylator group and more than 5% in the extensive hydroxylators.

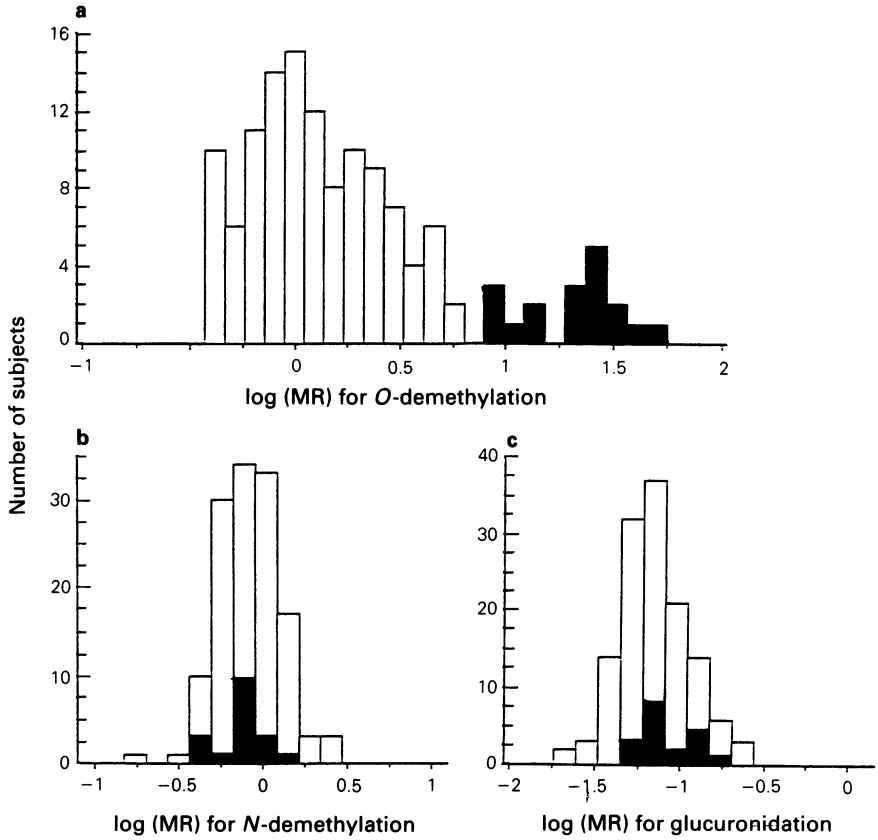

Figure 3 Frequency distributions of the log MRs for codeine *O*-demethylation, *N*-demethylation and glucuronidation in 114 extensive (□) and 18 poor (■) debrisoquine hydroxylators.

This finding is consistent with results reported recently in seven individuals (Chen *et al.*, 1988). Using our analytical method, however, the exact proportion of each compound was determined. The occurrence of individuals showing little formation of M from codeine was noted previously in a kinetic study (Rogers *et al.*, 1982). The genetic control of this phenomenon is clear from the results of our present study and the findings by Chen *et al.* (1988) that the *O*-demethylation index of codeine correlates strongly with the debrisoquine hydroxylation index. The two oxidations are probably catalyzed by the same cytochrome P-450 isozyme. Dayer *et al.* (1988) reported reduced *O*-demethylating activity *in vitro* in liver samples from a subject phenotyped as a poor metaboliser; they showed that the rates of *O*-demethylation of codeine and dextrometorphan were correlated in a small number of human livers (n = 6) and that the *O*-demethylation of codeine was inhibited by quinidine, a potent inhibitor of the debrisoquine hydroxylase (Otton *et al.*, 1984).

The recoveries of NM, M6G and M were 30, 13 and 6 times higher, respectively, in the extensive compared with the poor hydroxylators. This may have clinical implications because these compounds appear to have analgesic activity in both animals and man. In fact, it has been suggested that codeine exerts its analgesic effect by transformation to M (Sanfilippo, 1948) but other hypotheses exist (Quiding *et al.*, 1986). NM is also considered to be an active metabolite of codeine and M, being approximately one-fourth as potent as M in relieving postoperative pain (Lasagna & Kornfeld, 1958). More recently, M6G was tested as an analgesic in six cancer patients with pain and found to be active (Osborne *et al.*, 1988), confirming observations in animals (Abbott & Palmour, 1988). NC has also been shown to possess analgesic activity in animals (Miller & Anderson, 1954). The full

extent of the contribution of these active metabolites to the analgesic effect of codeine in man can be explored by using known inhibitors of the debrisoquine hydroxylase such as desmethylimipramine and quinidine (Spina *et al.*, 1984; Dayer *et al.*, 1988).

The present study has also demonstrated that poor hydroxylators of debrisoquine excrete more NC than extensive hydroxylators and that this phenomenon may be a consequence of a low expression of the *O*-demethylation pathway. The amounts of NC excreted differed by a factor of less than two between the two groups.

Thus far, debrisoquine has been the most commonly used pharmacogenetic probe in studies of drug oxidation (Mahgoub *et al.*, 1977; Steiner *et al.*, 1985). Debrisoquine is a sympatholytic agent previously used in a few countries for the treatment of hypertension (Jackson, 1972). The polymorphic control of debrisoquine hydroxylation has been shown unequivocally (Evans *et al.*, 1980; Steiner *et al.*, 1985). It was originally thought that the poor hydroxylator phenotype originates from a single mutation, but Gonzales *et al.* (1988) have shown recently that at least four different mutations can occur, to give rise to the poor hydroxylator phenotype. These mutations do not occur within the coding exons of the gene, but in the introns, giving rise to instability of the incorrectly spliced messenger RNAs and no immunodetectable protein. These defects are unusual, difficult to detect and will require sequencing of the complete normal human P-450dbl gene before the source of aberrant splicing of the pre-mRNA in poor hydroxylators can be clarified. These problems make it difficult to 'diagnose' poor hydroxylators without drug administration. Codeine may prove to be a suitable probe drug since it is safe and commonly available world-wide. In addition, its MRs are independent of sampling time and reproducible (unpublished data).

This work was supported by grants from the Swedish Medical Research Council (3902) and the Swedish Cancer Society (1910-B88-01V), associated with COST B1.

References

Abbott, F. V. & Palmour, R. M. (1988). Morphine-6-glucuronide: analgesic effects and receptor binding profile in rats. *Life Sci.*, **43**, 1685–1695.

Adler, T. K., Fujimoto, J. M., Way, E. L. & Baker, E. M. (1955). The metabolic fate of codeine in man. *J. Pharmac. exp. Ther.*, **114**, 251–262.

Boobis, A. R. & Davies, D. S. (1984). Human cytochromes P-450. *Xenobiotica*, **14**, 151–185.

Chen, Z. R., Somogyi, A. A. & Bochner, F. (1988). Polymorphic *O*-demethylation of codeine. *Lancet*, **ii**, 914–915.

Dayer, P., Desmeules, J., Leemann, & Striberni, R. (1988). Bioactivation of the narcotic drug codeine in human liver is mediated by the polymorphic monooxygenase catalyzing debrisoquine 4-hydroxylation (cytochrome P-450 dbl/bufl). *Biochem. Biophys. Res. Comm.*, **152**, 411–416.

Eichelbaum, M., Bertilsson, L., Säwe, J. & Zekorn, C. (1982). Polymorphic oxidation of sparteine and debrisoquine: related pharmacogenetic entities. *Clin. Pharmac. Ther.*, **31**, 184–185.

Eichelbaum, M., Spannbrucker, N., Steincke, B. & Dengler, H. J. (1979). Defective N-oxidation of sparteine in man: a new pharmacogenetic defect. *Eur. J. clin. Pharmac.*, **16**, 183–187.

Evans, D. A., Mahgoub, A., Sloan, T. P., Idle, J. R. & Smith, R. L. (1980). A family and population study of the genetic polymorphism of debrisoquine oxidation in a white British population. *J. med. Genet.*, **17**, 102–105.

Gonzalez, F. J., Skoda, R. C., Kimura, S., Umeno, M., Zanger, U. M., Nebert, D. W., Gelboin, H. V., Hardwick, J. P. & Meyer, U. A. (1988). Characterization of the common genetic defects in humans deficient in debrisoquine metabolism. *Nature*, **331**, 442–446.

Jackson, W. B. (1972). Tolerance and side effects during long term treatment of hypertension with debrisoquine. *Aust. N.Z.J. Med.*, **4**, 357–365.

Küpfer, A. & Preisig, R. (1984). Pharmacogenetics of mephenytoin: a new drug hydroxylation polymorphism in man. *Eur. J. clin. Pharmac.*, **26**, 753–759.

Lasagna, L. & De Kornfeld, T. J. (1958). Analgesic potency of normorphine in patients with postoperative pain. *J. Pharmac.*, **124**, 260–263.

Lennard, M. S., Silas, J. H., Smith, A. J. & Tucker, G. T. (1977). Determination of debrisoquine and its 4-hydroxymetabolite in biological fluids by gas chromatography with flame-ionization and nitrogen-selective detection. *J. Chromatogr.*, **133**, 161–166.

Mahgoub, A., Idle, J. R., Dring, L. G., Lancaster, R. & Smith, R. L. (1977). Polymorphic hydroxylation of debrisoquine in man. *Lancet*, **ii**, 584–586.

Miller, J. W. & Anderson, H. H. (1954). The effect of *N*-demethylation on certain pharmacologic actions of morphine, codeine and meperidine in the mouse. *J. Pharmac.*, **112**, 191–196.

Nebert, D. W. & Gonzalez, F. J. (1985). Cytochrome P-450 gene expression and regulation. *Trends pharmac. Sci.*, **6**, 160–164.

Osborne, R., Joel, S., Trew, D. & Slevin, M. (1988). Analgesic activity of morphine-6-glucuronide. *Lancet*, **i**, 828.

Otton, S. V., Inaba, T. & Kalow, W. (1984). Com-

petitive inhibition of sparteine oxidation in human liver by β-adrenoceptor antagonists and other cardiovascular drugs. *Life Sci.*, **34**, 73–80.

Pfaff, G., Briegel, P. & Lamprecht, I. (1983). Inter-individual variation in the metabolism of dextromethorphan. *Int. J. Pharm.*, **14**, 173–189.

Quiding, H., Anderson, P., Bondesson, U., Boréus, L. O. & Hynning, P. Å. (1986). Plasma concentrations of codeine and its metabolite, morphine, after single and repeated oral administration. *Eur. J. clin. Pharmac.*, **30**, 673–677.

Rogers, J. F., Findlay, J. W. A., Hull, J. H., Butz, R. F., Jones, E. C., Bustrack, J. A. & Welch, R. M. (1982). Codeine disposition in smokers and nonsmokers. *Clin. Pharmac. Ther.*, **32**, 218–227.

Sanfilippo, G. (1948). Contributo sperimenteale all'ipotesi della smetilazione della codeina nell'organismo. I. Influence della dose sull'assuefazione alla codeina. II. Assuefazione alla codeina attenuta con somministrazione prolungata di morfina. *Boll. Soc. Ital. Biol. Sper.*, **24**, 723–726.

Schmid, B., Bircher, J., Preisig, R. & Küpfer, A. (1985). Polymorphic dextromethorphan metabolism: Co-segregation of oxidative *O*-demethylation with debrisoquine hydroxylation. *Clin. Pharmac. Ther.*, **38**, 618–624.

Spina, E., Birgersson, C., von Bahr, C., Ericsson, O., Mellström, B., Steiner, E. & Sjöqvist, F. (1984). Phenotypic consistency in hydroxylation of desmethylimipramine and debrisoquine in healthy subjects and in human liver microsomes. *Clin. Pharmac. Ther.*, **36**, 6–000.

Steiner, E., Iselius, L., Alván, G., Lindsten, J. & Sjöqvist, F. (1985). A family study of genetic and environmental factors influencing polymorphic debrisoquine hydroxylation. *Clin. Pharmac. Ther.*, **38**, 394–492.

Svensson, J. O., Rane, A., Säwe, J. & Sjöqvist, F. (1982). Determination of morphine, morphine-3-glucuronide and (tentatively) morphine-6-glucuronide in plasma and urine using ion-pair high-performed liquid chromatography. *J. Chromatogr.*, **230**, 427–432.

Svensson, J. O. (1986). Determination of morphine, morphine-6-glucuronide and normorphine in plasma and urine with high-performance liquid chromatography and electrochemical detection. *J. Chromatogr.*, **375**, 174–178.

Wedlund, P. J., Aslanian, W. S., McAllister, C. B., Wilkinson, G. R. & Branch, R. A. (1984). Mephenytoin hydroxylation deficiency in Caucasians: frequency of a new oxidative drug metabolism polymorphism. *Clin. Pharmac. Ther.*, **36**, 773–780.

Yue, Q. Y., Säwe, J., Svensson, J. O. & Sjöqvist, F. (1988). Interindividual and interethnic differences in the *O*-demethylation and glucuronidation of codeine—Observations in Chinese and Swedish volunteers. *5th Southeast Asian and Western Pacific Regional Meeting of Pharmacologists*, Beijing, China, July.

Yue, Q. Y., Sjöqvist, F., Svensson, J. O. & Säwe, J. (1987). Stor interindividuell variation i konjugering och speciellt O-demethylering av kodein. *Medicinska Riksstämman*, Stockholm, December.

Yue, Q. Y., Svensson, J. O., Alm, C., Sjöqvist, F. & Säwe, J. (1989). Interindividual and inter-ethnic differences in the demethylation and glucuronidation of codeine. *Br. J. clin. Pharmac.*, **28**, 629–637.

(Received 2 May 1989, accepted 3 August 1989)