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Mini Review

Prediction of Xenobiotic Metabolism by Non-Invasive Methods

Steffen Loft and Henrik Enghusen Poulsen*

Department of Pharmacology, University of Copenhagen, Juliane Mariesvej 20, DK-2100 Copenhagen Ø, and Department of Medicine A, Rigshospitalet, Blegdamsvej, DK-2100 Copenhagen Ø, Denmark

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The elimination of many drugs and environmental xenobiotics requires biotransformation which can result in bioactivation. The capacity of the biotransformation enzymes may vary extensively between individuals due to genetic control and/or environmental factors. The regulation of these enzymes is of key importance for drug therapy as well as for toxicology and environmental medicine.

The purpose of the present paper is to give an outline of the methods available for the non-invasive study of biotransformation enzymes *in vivo*. In this context the administration of a test compound is not regarded as an invasive procedure. The term "non-invasive" refers exclusively to the collection of relevant biological samples.

Kinetic parameters.

In vivo, the activity of biotransformation enzymes is preferably expressed by the clearance from plasma or other relevant body fluids of a specific substrate; in the present context termed "probe" (Wilkinson 1987). Usually, this will mainly reflect the enzyme activities of the liver (Park 1982). A probe may be a specific substrate of a particular enzyme or one of its metabolites may be an enzyme specific product. The clearance of a substrate through a particular pathway can be determined if the resulting metabolite is excreted unchanged or in quantifiable form into the urine. If the elimination of a single dose can be described by a one-compartment model and the volume of distribution can be estimated, e.g. from simple demographic parameters, the clearance can be determined from a single sample without loss of precision compared to conventional multiple sample measurements (Poulsen & Loft 1988; Loft *et al.* 1988). This method was originally described for antipyrine in man, but it is applicable in the rat as well and has been extended to a range of other compounds (Døssing *et al.* 1982; Poulsen & Pilsgaard 1985; Bachmann *et al.* 1998). It is, however, crucial that the one sample is collected later than 1.4 half-lives after dosing and preferably before three half-lives have passed (Døssing *et al.* 1983; Poulsen & Loft 1988; Loft *et al.* 1988).

The elimination and metabolite formation rate constants are dependent on the volume of distribution. Thus, they are less ideal than the clearance for expression of enzymatic activity *in vivo* (Sultatos *et al.* 1980). Ratios of metabolite and mother compound in urine are even less attractive from a kinetic point of view. The interpretation rests on the assumptions that mother compound and metabolite are excreted at the same rate and that the elimination rate through other pathways is constant (Jackson *et al.* 1986). Accordingly, urinary metabolic ratios are particularly suited for phenotyping subjects according to distinct metabolic polymorphisms. In fact, many of the probes subjected to polymorphic metabolism have high hepatic extraction ratios with resulting first pass effects upon oral administration and the clearance is difficult to interpret (Jackson *et al.* 1986). However, if the substrate concentration or the dose cannot be determined, e.g. in case of endogenous or dietary compounds, or complete urine collection during the excretion of metabolites from a probe cannot be accomplished, metabolic ratios may be preferred. Nevertheless, the relation between the ratio and the clearance for metabolite formation should be ascertained.

Non-invasive sampling for kinetic estimations.

Biological samples are necessary for the determination of the *in vivo* kinetics of a probe. Trained personnel are required for venous blood sampling whereas subjects instructed, e.g. in writing, can collect saliva and urine, allowing large scale test series with minimum cost (Døssing 1983). Moreover, venous blood sampling is unpleasant to many subjects and contain an, albeit remote, risk of blood born infection. It has even been suggested that blood sampling for determination of antipyrine clearance in healthy volunteers is unethical (Svensson 1988). In experimental animals collection of blood may imply stressful handling and/or indwelling catheters inserted under general anaesthesia. This may have considerable inducing and/or inhibiting effects on the enzymes metabolizing foreign compounds (Capel *et al.* 1980; Van Dyke *et al.* 1987; Chindavijac *et al.* 1988). Moreover, with non-invasive sampling the animal will be available for repeated studies, possibly circumventing interanimal variation and reducing animal expenditure.

Saliva. The most important difference between plasma and saliva is that the concentration of protein for binding of xenobiotics is negligible in the latter (Ritschel & Thompson 1983). Thus, for compounds not bound to protein, saliva can usually replace plasma samples. Moreover, for protein

*To whom correspondence should be directed

bound drugs the unbound clearance or at least the elimination rate constant can probably be estimated from saliva samples. Ideally, the *in vivo* protein binding can be estimated as 1-(saliva concentration × plasma concentration^{-1}) with proper correction using pH and pKa values for acidic or alkaline compounds (Ritschel & Thompson 1983). If large saliva samples are required, the secretion may be stimulated by a variety of methods, such as sucking pebbles, chewing Parafilm®, or application of citric acid on the tongue. In experimental animals, injection of pilocarpine can be applied (Wilson *et al.* 1982; Ritschel & Thompson 1983). For a particular probe the saliva to plasma concentration ratio should be investigated over the expected range to ascertain the interchangeability of the two fluids.

Breath. Exhaled CO_2 from dealkylation of a (radio)labelled probe can be collected by bubbling expiratory air through a trapping agent. Experimental animals, such as rats, may be placed in glass tubes with one-way air flow for collection of CO_2 (Rhodes & Houston 1983). The elimination rate constant can be determined from the rate of radiolabel excretion over time. Ideally, the rate of radiolabel excretion reflects the dealkylation rate of the labelled probe. The method was originally described for the use of aminopyrine in rats but has been extended to man and a number of other compounds, including antipyrine, caffeine, diazepam, erythromycin, and diazepam (Lauterburg & Bircher 1973; table 1). In man the test has been simplified to sampling once and the cumulated exhalation of CO_2 is estimated assuming a fixed endogenous output of CO_2 which should be ascertained during bed rest (Hepner & Vesell 1974). There are, however, several problems in the interpretation of the cumulated exhalation as a measure of the probe clearance. Thus, only 50% of the labelled carbon generated by demethylation of aminopyrine is eventually oxidized to bicarbonate according to highly variable kinetics (Irving *et al.* 1982; Brüch *et al.* 1987). The use of radioactive material in man may raise ethical problems. Stable isotopes are preferred but they are demanding with regard to equipment, such as mass spectrometry. From a practical point of view, the breath tests are not easily self-administered by layman subjects.

Urine. The main application of urine samples relates to the excretion of metabolites. The major problem with urine sampling is the completeness of the collection. Unfortunately, the urinary excretion of creatinine shows large interindividual variation and is therefore not of much value as a check on the completeness (Bingham & Cummings 1985). Another application is the determination of the elimination rate constant of a drug provided a fraction is excreted unchanged at a rate independent of urine production (Atiba *et al.* 1987).

In the following a collection of probes for the non-invasive estimation of biotransformation capacity will be assessed particularly in relation to their metabolism by specific enzymes and possible predictive value. The probes are summarized in table 1.

Table 1.

Tests for non-invasive assessment of the capacity of xenobiotic metabolism.

Probe	Sampling procedure			Cytochrome P450 activity	Other reactions	Selected references
	Breath	Saliva	Urine			
Aminopyrine	+	+	+	Unknown		Lauterberg & Bircher (1973) Vesell *et al.* (1975)
Antipyrine	+	+	+	Unknown		Rhodes & Houston (1983); Poulsen & Loft (1988)
Caffeine	+	+	+	P450IA2	Acetylation	Campbell *et al.* (1987b); Wietholtz *et al.* (1981)
					Xanthine oxidase	Grant *et al.* (1983); Knutti *et al.* (1981)
Carboxymethylcysteine			+		Sulfoxidation	Mitchell *et al.* (1984)
Codeine			+	P450IID6	Glucorunidation	Yue *et al.* (1989a & b)
Debrisoquine			+	P450IID6		Mahgoub *et al.* (1977)
Dextromethorphan			+	P450IID6		Schmid *et al.* (1985)
Diazepam	+			Unknown		Hepner *et al.* (1977)
Erythromycin	+			P450IIIA		Watkins *et al.* (1989)
Ethosuximide		+		Unknown		Bachmann *et al.* (1986)
6β-Hydroxycortisol			+	P450IIIA		Ohnhaus *et al.* (1989)
Isoniazid		+	+		Acetylation	Inaba & Arias (1987); Hutchings *et al.* (1988)
Mephenytoin			+	P450IIC9		Wilkinson *et al.* (1989) (review)
Metoprolol			+	P450IID6		McGourty *et al.* (1985)
Metronidazole		+	+	Unknown	Glucuronidation	Loft *et al.* (1988)
Paracetamol		+	+	P450IA2?	Glucuronidation	Bock *et al.* (1987); Raucy *et al.* (1989)
				P450IIE1?	Sulfation	Critchley *et al.* (1986)
Phenacetin	+	+	+	P450IA2		Schoeller *et al.* (1985); Vesell *et al.* (1975)
Phenytoin		+	+	Unknown		Bachmann *et al.* (1985)
Scoparone	+			Unknown		Legrum *et al.* (1984)
Sparteine			+	P450IID6		Eichelbaum *et al.* (1979)
Sulfadimidine			+		Acetylation	Clark (1985) (review)
Theophylline		+	+	P450IA2?		Trnavska *et al.* (1987)

Probes for assessment of particular cytochrome P450 activities.

Specific probes are available for non-invasive study of the activity of some of the cytochrome P450's which have been characterized up to now (Gonzales 1988; Nebert *et al.* 1989).

Cytochrome P450IA2. This enzyme is induced by polyaromatic hydrocarbons and thought to be one of the major enzymes responsible for the activation of carcinogens (Shimada *et al.* 1989). Measurement of cytochrome P450IA2 activity *in vivo* is thus of toxicological interest. In preparations of human liver microsomes, the successive demethylation of caffeine, the O-deethylation of phenacetin and possibly the demethylation of theophylline are catalysed by cytochrome P450IA2 (Campbell *et al.* 1987a; Sesardic *et al.* 1988). The elimination rate of caffeine and theophylline can be determined from saliva samples (Knutti *et al.* 1981; Trnavska *et al.* 1987). However, the determination of the caffeine clearance requires abstinence from caffeine containing beverages for 24 hrs or longer and from all methylxanthine containing beverages and food items, if each individual elimination pathway is to be investigated. The latter requirement relates to the theophylline metabolism as well. The elimination rate of radiolabelled caffeine may be determined by a breath test (Wietholtz *et al.* 1981). Recently, a much simpler approach has been suggested and appears very attractive for large scale epidemiologic studies. Thus, the ratio between the two step demethylated metabolites of caffeine (1-methylxanthine, 1-methyluric acid and 5-acetyl-6-formulamino-3-methyluracil) and the one step demethylated metabolite (1,7-dimethyluric acid) in urine correlates closely with the clearance of caffeine (Campbell *et al.* 1987b). Moreover, a number of factors known to influence the clearance of caffeine, i.e. smoking, use of oral contraceptives, and age, affects this metabolic ratio of caffeine similarly (Campbell *et al.* 1987b). Knowledge of the exact dose of caffeine is not required and the content of a single cup of coffee is sufficient for determination of the ratio.

Radiolabelled phenacetin may be used for a breath test but the hepatic extraction ratio is so high that the result mainly depends on liver blood flow and the excretion of labelled CO_2 may not reflect the deethylation rate (Schoeller *et al.* 1985). The same applies to the determination of phenacetin kinetics from saliva and/or urine samples (Vesell *et al.* 1975).

Cytochrome P450IID6. This cytochrome P450 is controlled by an autosomal recessive gene resulting in its absence in approximately 7% of the Caucasian population (Brøsen & Gram 1989). The molecular biology of the enzyme has been studied in detail, including DNA sequencing and identification of some of the alterations responsible for its absence (Gonzalez 1988; Zanger *et al.* 1988). Cytochrome P450IID6 catalyses the metabolism of a large number of commonly prescribed drugs, including tricyclic antidepressants and some neuroleptics and antiarrhythmics (Brøsen & Gram 1989). The consequence of a poor metabolizer phenotype for the kinetics of a drug subjected to polymorphic metabolism depends on the existence of alternative pathways (Jackson *et al.* 1986). If the elimination of a drug mainly depends on oxidation by cytochrome P450IID6 the phenotype will predict the elimination rate of that drug (Brøsen & Gram 1989). So far, this enzyme is probably the only example of the value of predicting the kinetics of one drug from the kinetics of another. The phenotype regarding the enzyme is determined non-invasively from the ratio between a substrate and its metabolite(s) excreted into the urine collected for 8–12 hrs after oral administration. The substrates are usually debrisoquine, sparteine, dextromethorphan, metoprolol and codeine (table 1). The discriminative efficacy of these probes are probably not very different although the polymorphism affects the pharmacokinetics of each differently. Thus, e.g. debrisoquine and sparteine have high and low hepatic extraction ratios and the bioavailability and half-life are mainly affected by the polymorphism, respectively (Kalow 1987).

Cytochrome P450IIC9. This cytochrome is controlled by an autosomal recessive gene resulting in its absence in approximately 4% of the Caucasian population (Küpfer & Preisig 1984; Wedlund *et al.* 1984; Wilkinson *et al.* 1989). Briefly, this enzyme catalyses the hydroxylation of S-mephenytoin and poor metabolizers experience side effects from conventional doses of this drug. So far, the clinical consequences of the polymorphism of mephenytoin hydroxylation are much less explored than those of the debrisoquine/sparteine oxidation. The capacity to metabolize mephobarbital and possibly hexobarbital and- diazepam co-segregates with the mephenytoin hydroxylator phenotype (Jacqz *et al.* 1986a; Knodell *et al.* 1988; Bertilsson *et al.* 1989). Cytochrome P450IID6 and P450IIC9 catalyse different pathways of propranolol metabolism and subjects with poor metabolizer phenotype for both enzymes are at risk of accumulation of this drug (Ward *et al.* 1989). For determination of the mephenytoin hydroxylator phenotype, several non-invasive methods have been suggested, including various metabolic ratios in the urine. The simplest approach appears to be the ratio between the R- and S-enantiomer excreted in urine collected for 8 hrs after oral administration of the racemate (Wilkinson *et al.* 1989). Since the rate of hydroxylation of S-mephenytoin is very high and reflects first pass metabolism in extensive metabolizers, the exact clearance is of limited interest.

Cytochrome P450IIIA. Recent studies in human liver microsomes attributed the bioactivation of a number of procarcinogens, e.g. mycotoxins, to cytochrome P450IIIA (Shimada *et al.* 1989). This enzyme also catalyses the oxidation of a number of important drugs, including macrolide antibiotics, nifedipine, cyclosporin, and endogenous steroids, e.g. cortisol (Kronbach *et al.* 1988; Gonzalez 1988; Waxman *et al.* 1988). The urinary excretion of 6β-hydroxycortisol appears to reflect the activity of this enzyme (Ohnhaus *et al.* 1989).

Thus, rifampicin, an inducer of cytochrome P450IIIA, dramatically increased this index as well as the enzyme activity and content of cytochrome P45OIIIA in liver samples (Ged *et al.* 1989). For correction of variation in the production of cortisol, the ratio of 6β-hydroxycortisol to 17-hydroxycorticosteroids or to free cortisol, even in spot urine samples, may be employed (Saenger 1983; Ohnhaus *et al.* 1989). However, it remains to be demonstrated that the metabolic ratios of cortisol in urine predict the metabolism of other substrates of cytochrome P45OIIIA, e.g. cyclosporin. A breath test involving radiolabelled erythromycin may reflect the activity of cytochrome P45OIIIA in man and in the rat (Watkins *et al.* 1989). Recently, this test was used in a study of cyclosporin toxicity (Lucey *et al.* 1990).

Probes for assessment of unidentified cytochrome P45O activities.

A large number of oxidatively metabolized compounds have been suggested as probes for the non-invasive study of cytochrome P45O activities. The oxidation of many of these cannot be attributed to a particular cytochrome P45O and the predictive value for the metabolism of other compounds may be limited. Nevertheless, these probes have been valuable tools for the study of the factors affecting foreign compound metabolism.

Aminopyrine. This compound is demethylated by several cytochrome P45O's (Slusher *et al.* 1987). Although aminopyrine kinetics may be studied by means of saliva samples and urine, the necessary dose houses a significant risk of toxicity (Vesell *et al.* 1975). Instead, a small dose of radiolabelled aminopyrine may be used for a breath test with the reservations considered above (Lauterburg & Bircher 1973; Hepner & Vesell 1974). Moreover, dual isotope studies have demonstrated that a major metabolic pathway of the aminopyrine metabolite monomethylaminopyrine does not involve demethylation and the score of the breath test is subjected to other factors than hepatic demethylation, i.e. aminopyrine absorption, metabolism of monomethylaminoantipyrine, intermediate carbon metabolism and bicarbonate kinetics (Irving *et al.* 1982; Brüch *et al.* 1987). The aminopyrine breath test has been used to measure liver function similarly to the antipyrine test and to study interactions with phenobarbital and disulfiram, (Hepner & Vesell 1974; Villeneuve *et al.* 1986). The aminopyrine test has not been shown to predict the kinetics of other drugs.

Antipyrine. This is one of the most extensively studied compounds regarding hepatic drug metabolism (Vesell 1979; Poulsen & Loft 1988). The excretion into urine of the three major metabolites of antipyrine reflects their formation by possibly different relatively product specific P45O enzymes at first order kinetics (Boobis *et al.* 1981; Teunissen *et al.* 1985a; Poulsen & Loft 1988). The formation of each metabolite may be under genetic control which, however, usually is obscured by environmental factors (Penno & Vesell 1983). In man and in rat antipyrine can be administered orally and the clearance estimated from a single saliva sample (Døssing *et al.* 1982; Poulsen & Pilsgaard 1985). In the rat N-methyl-^{14}C-antipyrine and exhalation of $^{14}CO_2$ can be used to determine the elimination rate constant (Rhodes & Houston 1983).

The major drawback of antipyrine is that the particular cytochrome P45O's involved in its metabolism have yet to be identified. It may be argued that a number of P45O's including the phenobarbital and 3-methylcholanthrene inducible types, are capable of forming all three metabolites of antipyrine but with differing rate, indicating that the selectivity is only relative (Slusher *et al.* 1987; Loft & Poulsen 1989). Moreover, so far the prediction of the kinetics of other drugs from the formation rates of antipyrine metabolites has been confined to theophylline and hexobarbital (Teunissen *et al.* 1985b; Schellens *et al.* 1988).

The low intraindividual variation in the rate of elimination and metabolite formation allows a high sensitivity to enzyme inducing and inhibiting effects when the classic antipyrine test is applied with measurements before and after an environmental change (Vesell 1979; Poulsen & Loft 1988). Moreover, the antipyrine metabolite profile offers the possibility of detecting differential effects of the environmental change on various cytochrome P45O's (Toverud *et al.* 1981). The other main application of the antipyrine test is as a measurement of quantitative hepatic function in liver patients (Andreasen *et al.* 1974).

Metronidazole. In man this antimicrobial is mainly eliminated by hydroxylation by undefined cytochrome P45O('s), apparently different from those metabolizing antipyrine, whereas glucuronidation is a minor pathway (Loft *et al.* 1987; 1988). Metronidazole has a kinetic profile much like antipyrine with complete oral bioavailability, a saliva/plasma concentration ratio close to unity and the possibility of determining clearance from a single sample (Loft *et al.* 1988). The two drugs have been suggested as a probe cocktail for non-invasive assessment of drug metabolism capacity (Loft *et al.* 1988; Loft & Poulsen 1989). The drawbacks are the same as for antipyrine, i.e. the requirement of complete urine collection for 48 hrs for assessment of the individual metabolic pathways in man and that the predictive value regarding the metabolism of other compounds is unknown.

Phenytoin. The hydroxylation of phenytoin appears to be under specific genetic control and show large interethnic differences (Hvidberg 1986; Steiner *et al.* 1987). Determination of the clearance from a single saliva sample may allow large population studies of this possible genetic polymorphism (Bachmann 1985).

Various. The kinetics of a number of compounds, such as diazepam, ethosuximide, scoparone metabolized by some cytochrome P45O's may be studied by means of non-invasive samples (table 1). These compounds do not offer any apparent advantages as model substrates over those described above.

Other oxidative reactions.

Sulfoxidation. The capacity for the cytosolic sulfoxidation of S-carboxy-L-methylcysteine as assessed by its urinary metabolic ratio was bimodally distributed in the population suggesting genetic control (Mitchell *et al.* 1984; Waring *et al.* 1986). Moreover, poor sulfoxidizers of this probe have been reported to be more prone to penicillamine toxicity and to various central nervous system diseases and allergies, possibly attributed to exposure to foreign compounds (Emery *et al.* 1984; Steventon *et al.* 1988; Scadding *et al.* 1988; Olomu *et al.* 1988).

Xanthine oxidase. The activity of this enzyme may be assessed from the ratio between 1-methylxanthine and 1-methyluric acid from dietary caffeine in the urine (Grant *et al.* 1983). The expected effect of the xanthine oxidase inhibitor, allopurinol, on this ratio has been demonstrated (Grant *et al.* 1986).

Phase II reactions

The knowledge of the regulation, particularly regarding possible genetic control, of the phase II enzymes is much less than that of the cytochrome P45O's. Moreover, the capacity of the phase II reactions may rely not only on the enzyme activity but also on the availability of the cofactors, e.g. activated sulfate, uridine 5'-diphosphoglucuronic acid and glutathione (Slattery *et al.* 1987; Price & Jollow 1988). Furthermore, there is a significant extrahepatic capacity for some conjugation reactions (Jacqz *et al.* 1986b). So far, only a few probes are available for the non-invasive study of phase II reactions.

Glucuronidation and sulfation. The major elimination pathways for paracetamol are glucuronidation and sulfation (Prescott 1980). Paracetamol has complete oral bioavailability and the pharmacokinetics may be determined from saliva samples (Kamali *et al.* 1987). The clearance representing each elimination pathway of paracetamol may then be determined from the excretion of the metabolites in urine collected for 24 hrs subsequent to administration to ensure complete recovery (Prescott 1980). For the sake of simplicity, the ratio of glucuronide and sulfate to parent compound in urine collected for 8 hrs has been suggested as a non-invasive procedure to monitor conjugation capacities (Bock *et al.* 1987). With this approach the expected inducing effects of phenytoin, rifampicin and smoking were demonstrated (Bock *et al.* 1987). However, it remains to be demonstrated that the metabolic ratios of sulfate and glucuronide to paracetamol reflect the respective clearance rates. Moreover, the rate of conjugation of paracetamol has not yet been shown to predict the rate of conjugation of other compounds. A small fraction of paracetamol is oxidatively metabolized, probably by cytochrome P45OIA2 and the ethanol-inducible P45OIIE1, to a reactive compound which subsequently is conjugated to glutathione (Raucy *et al.* 1989). It appears likely that the rate limiting step in the excretion of glutathione derived conjugates of paracetamol is dependent on the rate of oxidative metabolism and availability of the cofactors at low and high dose, respectively (Slattery *et al.* 1987).

The ratio of codeine-6-glucuronide to mother compound in urine after oral administration of codeine has been used to study interethnic differences in glucuronidation capacity (Yue *et al.* 1989a). However, it remains to be demonstrated that this ratio reflects the clearance of codeine by glucuronidation.

Acetylation. The capacity for N-acetylation of amine drugs and other foreign compounds is under autosomal recessive genetic control dividing the population into slow and fast acetylators with approximately equal frequency in Caucasians (Clark 1985). The fast acetylators may be further characterized as heterozygotes and homozygotes according to their acetylation capacity. The acetylation phenotype predicts the rate of elimination of various drugs, e.g. hydralazine, isoniazid and sulfasalazine, with potential for toxic reactions at therapeutic doses (Clark 1985). The capacity for O-acetylation of hydroxylamines is under the same genetic control as the N-acetylation and the two reactions are important for the metabolic activation and deactivation of environmental carcinogens, such as arylamines (Hein 1988). Indeed, the slow and fast acetylator phenotype is overrepresented among patients with bladder and colorectal cancer, respectively (Hein 1988).

The acetylator phenotype may be determined non-invasively with caffeine, isoniazid and sulfadimidine as probes and measurement of urinary metabolic ratios (Clark 1985; table 1). The rate of isoniazid acetylation can also be determined by means of saliva samples (Hutchings *et al.* 1988). The determination of phenotype by means of dietary caffeine relies on that the intermediary after two consecutive demethylations may be acetylated (Grant *et al.* 1983). The ratios between the acetylated metabolite, 5-acetyl-6-formylamino-3-methyluracil (AFMU), and the alternative metabolite(s), 1-methylxanthine or 1-methylxanthine plus 1-methyluric acid plus 1,7-dimethyluric acid, discriminate between the two phenotypes in complete accordance with other indices of acetylation capacity (Grant *et al.* 1983, 1984). The problem of AFMU instability may be solved by either immediate acidification of urine samples and storage at lowest possible temperature or by deliberately converting AFMU to 5-acetyl-6-amino-3-methyluracil by alkalinization and assaying this compound (Grant *et al.* 1983; Tang *et al.* 1987).

Conclusions.

As demonstrated in the present review, a number of non-invasive tests are available for the assessment of the capacity for foreign compound metabolism. Unfortunately, the number of biotransformation enzymes, particularly the cytochrome P45O's, is large with variable and partly overlapping substrate specificity. Thus, for a number of important catalytic reactions non-invasive methods are not yet available. Moreover, except for the genetic polymorphisms, the rate

of metabolism of one compound will rarely be an accurate predictor of that of another compound. Consequently, for dose prediction or adjustment of a drug with a narrow therapeutic range, monitoring concentrations in plasma or similar cannot be replaced, but occasionally supplemented, by the kinetics of a probe drug. In our opinion, the main applications of the probes are the study of the factors, genetic and environmental, regulating foreign compound metabolism. One line of research in this area may be prospective studies of the possible relations between the capacity of particular foreign compound metabolizing enzymes and disease, particularly cancer, suggested from retrospective studies (Kellerman *et al.* 1980; Caporaso *et al.* 1989; reviews: Guengerich 1988; Hein 1988). Indeed, *in vitro* studies of human liver microsomes suggest that the enzymes activating the known mutagens are the ones assessable *in vivo* by the metabolic ratios of dietary caffeine and the excretion of 6β-hydroxycortisol (Shimada *et al.* 1989). Moreover, the metabolic ratios of dietary caffeine will also allow determination of the acetylator phenotype, important for the activation and deactivation of amine carcinogens (Hein 1988).

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