The Role of Human Glutathione Transferases and Epoxide Hydrolases in the Metabolism of Xenobiotics

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Human glutathione transferases (GSTs) are a multigene family of enzymes that are involved in the metabolism of a wide range of electrophilic compounds of both exogenous and endogenous origin. GSTs are generally recognized as detoxifying enzymes by catalyzing the conjugation of these compounds with glutathione, but they may also be involved in activation of some carcinogens. The mammalian GSTs can be differentiated into four classes of cytosolic enzymes and two membrane bound enzymes. Human epoxide hydrolases (EHs) catalyze the addition of water to epoxides to form the corresponding dihydriodiol. The enzymatic hydration is essentially irreversible and produces mainly metabolites of lower reactivity that can be conjugated and excreted. The reaction of EHs is therefore generally regarded as detoxifying. The mammalian EHs can be distinguished by their physical and enzymatic properties. Microsomal EH (mEH) exhibits a broad substrate specificity, while the soluble EH (sEH) is an enzyme with a "complementary" substrate specificity to mEH. Cholesterol EH and leukotriene A₄ hydrolase are two EHs with very limited substrate specificity. The activities of either GSTs or EHs expressed in vivo exhibit a relatively large interindividual variation, which might be explained by induction, inhibition, or genetic factors. These variations in levels or activities of individual isoenzymes are of importance with respect to an individual's susceptibility to genotoxic effects. This article gives a general overview of GSTs and EHs, discussing the modulation of activities, determination of these enzymes ex vivo, and the polymorphic expression of some isoenzymes. — Environ Health Perspect 105(Suppl 4):791–799 (1997)

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Introduction

Epidemiological evidence indicates that most of our human cancers are originally caused from environmental exposures to genotoxic agents. It is estimated that as much as two-thirds of all human cancers are caused from exposures to tobacco smoke and dietary components (1). To be able to protect cells from damage by the exposure of various forms of reactive substances, cellular systems for detoxification are essential. Two major systems that strongly regulate the susceptibility to, e.g., cancer have been identified, namely the enzymes involved in drug metabolism and DNA repair. Since most carcinogens or mutagens need to be altered chemically by drug-metabolizing enzymes (2) before they can exert their genotoxic effect, the importance of these enzymes in regulating the levels of induced genotoxic effect in cells is obvious. The cytochrome P450 system is able to metabolize a various number of xenobiotics to highly reactive intermediates such as epoxides, which can bind covalently to cellular components. The enzymatic conversion of these epoxides to less reactive intermediates is protective and occurs by spontaneous conjugation with glutathione (GSH) or enzymatic conjugation by glutathione transferases (GSTs), as well as by hydration to the corresponding trans-dihydriodiol catalyzed by epoxide hydrolases (EHs) (3,4).

Glutathione Transferases and Glutathione

Glutathione transferases (GSTs) are a complex multigene family of enzymes that possess many biological functions such as the important step in detoxification of a large number of electrophiles. Such conjugation reactions result in the synthesis of mercapturic acids and represent an important excretory route for many xenobiotics (5,6). For some classes of substrates, the initial conjugates are broken down by a second reaction with glutathione (GSH). One example is organic hydroperoxides (7) and ozonides (8), in which in addition to glutathione, disulfide (GSSG), an alcohol, is formed. Another example is chloroalcohol ketones (9), in which halogen is replaced by hydrogen in addition to the formation GSSG. These examples are shown in Figure 1.

Although GSTs are generally recognized as detoxifying enzymes, they may also be involved in activation processes of some carcinogens such as haloalkanes and halogenated compounds (10,11). It has been shown that GSTs can catalyze the formation of an epoxiranium ion via ethylene dibromide and GSH. If this reactive intermediate is formed, it will react with nucleophiles such as water, GSH, or guanine in DNA (Figure 2).

Cytosolic GSTs are dimeric proteins with an approximate molecular weight of 25,000 Da for each subunit. The largest amount of GST protein is present in the liver, representing 3 to 5% of total soluble proteins (12). The mammalian GST can be separated into four classes, based on substrate specificity, immunological identity, protein, and DNA sequence. The different classes comprise alpha, mu, pi, and theta. The structural similarity of the amino acid sequence identity among members within each class is > 50%, with

1. R-X + GSH → R-SG + XH
2. R-OH + GSH → R-OSG + H₂O
3. R-CO₂-X + GSH → R-CO₂-SG + XH

Figure 1. GST reaction pathways for the conjugation of xenobiotics with GSH.
less than 25% identity between members of different classes.

Structurally unrelated to the cytosolic GSTs are two exclusively membrane-bound proteins, microsomal GST and leukotriene C4 synthase, which may have arisen by convergent evolution (13).

**Nomenclature and Classification of Glutathione Transferase**

A variety of nomenclature systems for GSTs have been proposed by several investigators over a number of years. Recently, a unifying nomenclature for human GSTs has been proposed (Table 1) (14).

**The Alpha Class Glutathione Transferase**

Among the alpha gene family, five human GST isoenzymes have been identified (15–17) that are present on chromosome 6p12 (may include some pseudogenes). Of these, at least GSTA1 and GSTA2 are widely expressed in human tissues but their expressions are subject to a marked variation. Thus, levels of GSTA1 and GSTA2 may vary considerably between different tissues and individuals (18). The alpha class isoenzymes are strongly expressed in, e.g., liver, kidney, and adrenal tissue. However, it has been reported that this alpha class of isoenzymes is not expressed in these organs in some individuals (19). This genetically determined deficiency is believed to be extremely rare. An apparently common restriction fragment length polymorphism (RFLP) of GSTA2 has been reported (20). The significance of this polymorphism is still unknown.

Characteristic properties of the alpha class GSTs include, besides conjugation of various agents with GST, isomerization of different ketosteroids and the non-selenium-dependent GST peroxidase activity towards organic hydroperoxides (21). A different form, other than the liver isoenzymes of alpha class GST, has been identified in human skin (16,17) which exhibits a considerably high peroxidase activity. Since ultraviolet light is known to cause lipid peroxidation in liposomal membranes, it is likely that the expression of this “skin” GST can contribute to the protection of these cells against the oxidative stress. Compared with other GSTs, GSTA2 has been shown to exhibit the highest activity towards the recently discovered 9,10-methyl-linolate ozonide, which is postulated to be the intermediate in the toxicity of ozone (8). The alpha class GSTs appear also to be the major GST isoenzyme responsible for the hepatic binding of bilirubin and other organic anions (22).

**The Mu Class Glutathione Transferase**

Five genes of the mu class GST, numbered M1 to M5, have been identified in humans (23,24). These genes occur as a cluster on chromosome 1p13 (23,24). The expression of these genes exhibits a comparatively large between-tissue variation. The most commonly expressed gene is GSTM1, which has a similar expression pattern to that of the alpha class isoenzymes. GSTM2 is more restricted to skeletal muscle, as is GSTM3, which also has been found in brain, lung, and testis. GSTM4 has been found in a human lymphoblastoid cell line and GSTM5 is expressed in the brain (25,26).

The most interesting aspect of the mu gene family may be the genetic polymorphism noticed for GSTM1. This phenomenon was first recognized in human liver, where the isoenzyme GST μ (previous designation) was expressed in only a part of the population of the samples analyzed (27). The expression of GSTM1 has been shown to be autosomal dominant inherited, and between 40 and 60% of most populations express GSTM1 (28). Two allelic variants have been described for GSTM1, GSTM1α and GSTM1β, with similar enzyme activity towards substrates for GSTM1. It has been shown that the cDNAs coding for these variants only differs in one amino acid, at the position 172 (29,30). The genetic polymorphism exhibited by GSTM1 may be a factor in determining an individual’s susceptibility to the toxic effects of various xenobiotics. For example, the high activity of GSTM1 (a and b) towards many epoxide metabolites from polycyclic aromatic hydrocarbons (PAHs), which are generally thought to possess considerable mutagenic and carcinogenic potential, may be of particular significance (31). It has been suggested, for example, that these isoenzymes could serve as genetic markers in the susceptibility for certain forms of cancer (32). The deficiency of GSTM1 has been shown to increase the susceptibility to DNA-adduct formation (33) and cytogenetic damage (34).

Polymorphism among other GSTM genes has not yet been investigated, even though a minority (about 10%) has been shown to fail to express GSTM3 in brain in one study (25).

**The Pi Class Glutathione Transferase**

Among the pi genes, GSTP1 has been identified; this gene has been mapped on chromosome 11q13 (35). This isoenzyme appears to be the most widely distributed enzyme of all GSTs and it is the most abundant form in many tissues (36) except in the liver, where the alpha and mu gene families are highly expressed. However, in the fetal liver the major GST form is a pi class isoenzyme. Several cDNA clones have been isolated (37) with only some minor

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**Figure 2.** Reaction of 1,2-dibromoethane with GSH to form episulfonium ion.

| Enzyme | Gene |
|--------|------|
| GST A1-1 | GSTA 1 |
| GST A2-2 | GSTA 2 |
| GST M1a-1a | GSTM1 |
| GST M1b-1b | GSTM2 |
| GST M2-2 | GSTM3 |
| GST M3-3 | GSTM4 |
| GST M5-5 | GSTM5 |
| GST P1-1 | GSTP1 |
| GST T1-1 | GSTT1 |
| GST T2-2 | GSTT2 |
| Microsomal leukotriene C4 synthase | |
differences, which probably represent allelic variants of a single locus, rather than transcripts of different genetic loci (38). It remains to be determined whether these differences contribute to individual variations in activity.

The enzyme activity exhibits a large interindividual variation that may be of clinical significance. Several reports have suggested a possible role for GSTP1 as a general tumor marker, determined in plasma (39,40). However, the appearance of GSTP1 in, e.g., human hepatic tumors is not specific only to the preneoplastic state, since this expression can also be seen in cirrhotic liver patients (41).

The Theta Class Glutathione Transferase

This gene family is the most recently identified GST gene. Two genes, GSTT1 and GSTT2, have been isolated from human liver (42, 43). Their expression seems to be widespread in humans, and it might have a fundamental role that has not been fully investigated.

The human GSTT1 isoenzyme has attracted interest since Ketterer and colleagues (44) identified a null allele at this locus. Since the enzyme catalyzes the detoxification of monohalomethanes and ethylene oxide in vitro, lymphocytes from expressers of the gene appear to be protected against the sister chromatid exchange (SCE) induced by these compounds. It is possible that, like individuals with GSTM1 null genotype, homozygous individuals for the GSTT1 null allele will have altered cancer risk. The frequency of the null allele has been reported to be 30 to 40% in one study from Germany (45), while in a Swedish population only 10% expressed this null allele (46).

Since some of the lesions in DNA may be peroxides, e.g., irradiation of free thymidine, which gives a substantial yield of thymidine hydroperoxides (47,48) and since it has been shown that GSTT has a considerably high activity towards these DNA hydroperoxides, it is likely that GSTT plays a protective role in this manner. On the other hand, it can also be assumed that the positive phenotype may be more sensitive to exposure to halogenated solvents such as methylene chloride. This is converted into formaldehyde, which is considered to be more toxic than methylene chloride itself (49).

Membrane-bound Glutathione Transferases

The microsomal GST appears to have an evolutionary origin totally distinct from that of the cytosolic GSTs. It is present in the endoplasmic reticulum and in the outer mitochondrial membrane and consists of three subunits, each with a molecular weight of 17,300 Da (50). It is most abundant in the liver but some studies have also shown the expression of this enzyme in the lung. The cDNA for this enzyme has been cloned and it seems to be encoded as a single copy gene on chromosome 12 (51). Of special interest for the microsomal GST is the ability to be activated in vitro by different sulfydryl agents such as N-ethylmaleimide (NEM), diethylmaleate, dithiopyridol, cystine, and glutathione disulfide. The activation of this enzyme can be up to 20-fold by NEM towards the substrate 1-chloro-2,4-dinitrobenzene (CDNB). The more pronounced the activation process seems to be the more reactive the substrates are. No activation in vivo has yet been seen in humans but it has been demonstrated in the rat by CC14 and phorone, both of which produce reactive oxygen radicals. Acrolein, which itself is a product of oxidative stress, has also been shown to increase the microsomal GST activity in vivo (52,53).

Leukotriene C4 synthase, an enzyme that is quite specific for the conjugation of leukotriene A4 to leukotriene C4, and some other closely related lipid epoxides may not take part in the cellular detoxification system. A summary of some selected substrates for different GSTs is shown in Table 2.

### Glutathione

The reaction of xenobiotics with GSH may occur spontaneously or enzymatically by GSTs as described above. If the spontaneous conjugation of a specific electrophile with GSH is the only means whereby a particular electrophile can be detoxified, the concentration of GSH will be of critical importance for this detoxification process. Nearly all cells contain GSH with varying concentrations, depending on the origin of the cell. In certain cells, e.g., hepatocytes, the GSH concentrations (5–10 mM) may vary according to the circumstances. For example, GSH may be depleted during starvation or after heavy electrophile load, or the cells may have levels higher than normal as the result of a rebound effect following a period of depletion (54,55).

### Epoxide Hydrolases

Epoxide hydrolases catalyze the addition of water to an epoxide to form the vicinal dihydrodriol, which almost always has the trans-configuration. The enzymatic hydration is essentially irreversible and produces metabolites of lower reactivity that can be readily conjugated and excreted. Therefore the action of EHs is generally regarded as detoxifying. A number of EHs, which are characterized by unique immunological properties, molecular weights, and substrate specificities have been identified.

### Microsomal Epoxide Hydrolases

Microsomal EH (mEH) has been purified to homogeneity from a number of species and tissues, including human liver. High specific activity of mEH has also been found in human brain. The presence of several drug-metabolizing enzyme activities in human brain microvessels, and particularly the high activity of EH, suggests a participation of these enzymes in the metabolic blood–brain barrier (56). Relatively high levels of mEH have also been found in the human adrenal gland, suggesting that the enzyme may be of particular importance in this tissue (57). However, very little cytochrome P450-catalyzed metabolism of xenobiotics has been demonstrated in the human adrenal (58). Researchers have detected mEH in various cell populations, for example in human

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Table 2. Specific activities (μmol/min/mg) of human GSTs with some selected substrates.

| Substrate                  | GST          |
|----------------------------|--------------|
|                            | A1-1         | A2-2 | M1-1 | M2-2 | M3-3 | M4-4 | P1-1 | T2-2 | Microsomal |
| CDNB, 1-chloro-2,4-dinitrobenzene | 80           | 80   | 180  | 220  | 7    | 1.4  | 105  | ND   | 1.9 (24)* |
| Ethydraic acid             | 0.2          | 0.1  | 0.08 | 0.2  | 0.2  | 0.1  | 0.9  | ND   |           |
| Leukotriene A4             | 0.009        | 0.04 |      |      |      |      |      |      | 0.002     |
| Styrene, 7,8-oxide         | 0.02         |      | 2.6  |      |      |      |      |      | 0.14      |
| trans-Stilbene oxide       | 0.002        |      | 0.0003| 0.0004| 0.003| 0.002|      |      |           |
| BP,4,5-oxide               | 0.05         |      | 0.9  |      |      |      |      |      | 0.13      |
| BP,7,8-diol,9,10-oxide     | 0.04         |      | 0.6  |      |      |      |      |      | 0.8       |
| Cumene hydroperoxide       | 10           | 100  | 0.6  |      |      |      |      | 0.03 | 7         | 0.043*     |

*Values in parentheses are for activated enzyme.

CDNB, 1-chloro-2,4-dinitrobenzene; BP, benz(a)pyrene; ND, no detectable activity.

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lymphocytes (59), human blood resting mononuclear leukocytes (60) and adult human hepatocytes (61). It is also notable that the level of mEH activity in human liver is approximately 50% higher in men than in women, while in extrahepatic organs, such as lung and adrenal, mEH activity is the same in both sexes. In kidney, the activity is slightly higher in women than in men (62).

The human mEH protein contains 455 amino acids. The mEH protein and nucleic acid sequences in humans, rats, and rabbits are evolutionarily conserved, with similarities between the relevant sequences exceeding 83 and 75%, respectively. Several cDNA clones have been isolated (63) with variation at only two residues, amino acids 113 and 139. The amino acid variation, however, does not exert a primary influence on catalytic function, but may affect the stability of the mEH protein. The human gene (EPHX1) has been localized to the long arm of chromosome 1 (64). The existence of a single functional mEH gene per haploid genome in humans and rats has been proposed (65). In contrast to the GSTs and cytochrome P450s, an absence of genetic complexity has been observed for mEH.

The mEH exhibits a broad substrate specificity, ranging from simple aliphatics, e.g., octane oxide, to large polycyclic aromatic hydrocarbons, e.g., different benzo[a]pyrene epoxides.

In addition, it has recently been suggested that mEH might mediate the transport of bile acids, such as taurocholate, into hepatocyte smooth endoplasmic reticulum vesicles (66) and that the protein can exist in two orientations in the membrane.

**Soluble Epoxide Hydrolases**

Soluble EH (sEH) is an enzyme with a complementary substrate specificity to mEH and it seems to be present in all species investigated. The specific substrate is trans-stilbene oxide. Compared with mEH, sEH appears to be more substrate selective, unable to metabolize bulky steroids and polycyclic aromatic hydrocarbons. There seems to be a large interindividual variation in the expression of sEH that is much larger than for mEH. It has been shown that sEH in human lymphocytes decreases the induction of SCE caused by trans-2-ethyl styrene, which is an in vitro substrate for sEH (67).

The cloning and expression of a cDNA encoding sEH predicts a protein of 554 amino acids (62,640 Da). The cloned sEH hydrolized cis-stilbene oxide faster than it hydrolized trans-stilbene oxide, a reverse of the activity profile seen in sEH of the mouse (68). The enzymatic activity was inhibited by phenylglycidos. The gene (EPHX2) has been localized to chromosomal region 8p21-p12 (69).

Under certain conditions, mEH seems also to be present in the cytosol, where it has been detected in rat and human liver by using catalytic and immunochemical assays. This enzyme, sEPHNSO, has been purified from human liver cytosol (70).

**Cholesterol Epoxide Hydrolases**

This enzyme is expressed in the microsomes as mEH but has a very limited substrate specificity for certain steroids only. It is present in all species so far investigated. The specific substrate is cholesterol 5,6-oxide, which, it has been suggested, is formed during lipid peroxidation or ultraviolet irradiation of the skin (71). The level of cholesterol 5,6-oxide seems to increase under certain pathological conditions, such as ulcerative colitis, colon cancer, and hypercholesterolemia (72).

**Leukotriene A4 Hydrolase**

This cytosolic enzyme has been purified and characterized from various types of blood cells. The enzyme has a very narrow substrate specificity. The product of the hydration of leukotriene A4 (LTA4) is not vicinal but rather a 5,12-diol (73). There appears to be no immunologocial relationship between LTA4 hydrolase and any other EH characterized so far. A summary of the different substrate specificities for EHs is shown in (Table 3).

**Modulation of Glutathione Transferase and Epoxide Hydrolase Activities**

The activities of either GSTs or EHs expressed in vivo exhibit a relatively large interindividual variation, which might be explained by induction, inhibition, or genetic factors.

**Induction**

Several agents have long been known to induce GST and EH. Most studies have, however, been performed in rat and mouse by using inducers such as phenobarbital, carcinogens (3-methylcholanthrene, 2-acetylaminofluorene) or antioxidants (BHA, BHT), etc. The induction process in humans is not fully understood in detail, but the regulation of GST expression in humans and in rodents has been reviewed by Rushmore and Pickart (74). Phenobarbital and phenytoin have been shown to be inducers of the mEH activity in vivo (75).

Soluble epoxide hydrolase is induced by a diverse group of compounds known as peroxisome proliferators, including clofibrate, as well as by common environmental and dietary contaminants, such as phthalate ester (76). At present, no in vivo inducers have been found, either for cholesterol EH or for LTA4 hydrolase.

**Inhibition**

A relatively large number of inhibitors of GST are known. Some are very selective to specific GSTs and have been used in that manner to characterize individual isoenzymes in vitro (49). Whether the inhibition found in vitro with cytosolic fractions or by purified isoenzymes has any relevance in vivo requires further studies. However, a number of quinones have been shown to be capable of inhibiting GST activity in a cellular system, indicating that inhibition by these compounds is possible under physiological conditions and might occur in vivo. Misonidazole, a radiosensitizing drug, has been shown to inhibit GST activity in mouse liver in vivo (77). In humans, ethacrynic acid and its analogs interfere with hepatic GST, and a number of antibiotics (penicillins and cephalosporins) inhibit human GSTP1 with an irreversible mechanism (78).

Competitive inhibitors of mEH include oxiranes with a 1-aryl or 1-alkyl substituent, juvenile hormone epoxides, and the antiepileptic drug valpromide. Mercury
and zinc are noncompetitive inhibitors and cadmium iodide is a potent inhibitor, competitively inhibiting styrene 7,8-oxide activity (62). Several drugs besides valproate (cinomide, probabile, valnocamide, and valproic acid) were recently found to be effective inhibitors of human mEH in vivo (79,80).

Various chloracne oxide derivatives have been shown to be selective and potent inhibitors of mouse liver sEH (81). The inhibition of sEH by tobacco smoke may reduce the inactivation of carcinogenic epoxides in human lung tissues and thus may increase a person’s susceptibility to lung cancer (82). The sterol imine 5,6α-imino-5α-cholestan-3β-ol completely inhibits cholesterol EH, while having no effect on either mEH or sEH activities. It has therefore been used diagnostically in the identification of cholesterol EH (83).

**Determination of Glutathione Transferases and Epoxide Hydrolases ex Vivo**

**Enzymatic Activity**

In *in vitro* assays, most of the work done with GST involves substrates chosen not because of their biological significance but because their GSH conjugation results in an optical density change that can be used in a convenient spectrophotometric assay. Much of our current understanding of GSTs relates to these substrates (Table 2). The most commonly used substrate is CDNB, since it is well utilized by most of the human GSTs, except GSTs included in the theta class. Other substrates are chosen because they are relatively specific for a particular isoenzyme(s) and may be used to detect or quantify it, e.g., trans-stilbene oxide which seems to be very specific for the determination of GSTM1 (28).

*In vitro* assays used for the EHs often involve the use of radiolabeled substrates. Because of the ease and speed with which this method is carried out, it is mainly used for routine assays. Examples of substrates used are styrene 7,8-oxide, benzo[a]pyrene 4,5-oxide, estroside, and trans-ethyl styrene oxide. The advantages of using spectrophotometric assays are that no radiolabel is necessary and that the measurement is continuous and therefore useful for kinetic work. Substrates used are trans-stilbene oxide and p-nitrooxyrene oxide. To measure enzyme concentrations in complex mixtures, i.e., cell fractions, immunological methods are useful due to their high sensitivity. They have also proved to be very useful in the detection of EH in human serum.

**Determination of Glutathione Transferases in Plasma**

Some problems are associated with activity measurements of GST in plasma. First, normal plasma levels of GST are comparatively low and a precise measurement of activity is difficult to achieve. Second, several GSTs bind to a number of anions such as bile salts and bilirubin, which will inhibit the enzymatic activity (24). In liver disease, when high plasma concentrations of these anions occur, the activity of the GST released from the hepatocytes into plasma is inhibited, which will affect the sensitivity of such tests. Because of these problems, assays of plasma or serum GST based on activity measurements have proved to be of little clinical value (84,85).

Immunoperoxidase has been described and may allow the precise and specific determination of each of the cytosolic GST classes (86,87). For example, it is possible to determine a specific GST subunit within a certain class such as GSTA1 and GSTA2 (87,88). Immunoassay measurements also determine the GST concentrations even when they are enzymatically inactive, due to the presence of inhibitors such as bilirubin or bile salts. It has become apparent through the use of these immunomodassays that the determination of GST may have a clinical role in the diagnosis and monitoring of patients with different diseases (89).

A number of reports have appeared on the possible use of the occurrence of GSTP in plasma as a tumor marker. Although a number of other human tumors express increased levels of pi, this seems not to be a general phenomenon. Therefore, its use as a lung and renal tumor marker is questionable (90).

**Determination of Epoxide Hydrolases in Vivo**

Carbamazepine 10,11-epoxide undergoes almost complete hydrolysis to the 10,11-transdihydrodiol metabolite in humans. This reaction is catalyzed by mEH. Measurement of *in vivo* mEH activity in Caucasian subjects after administration of carbamazepine 10,11-epoxide did not show outliers indicating enzyme-deficient phenotypes, and the frequency distribution was unimodal normal (75). The log metabolic ratio (transdihydrodiol/epoxide) detected indicated pronounced inhibition of EH by valproate and induction by phenobarbital or phenytoin. A tight range of *in vivo* enzyme activities (1.6-fold) was observed in the healthy Caucasian population (75), whereas *in vitro* mEH activity has been shown to have an individual variation ranging from 2.5- to 63-fold, depending on substrate and the source and handling of liver tissue (91).

**Distribution of Glutathione Transferase in Human Tissues**

Although the determination of subunit and/or isoenzyme composition has thus far been mainly performed with the aid of (polyclonal) antibodies, a high performance liquid chromatography (HPLC) technique has recently been developed (92) that shows great promise and in any event provides a demonstration of the subunit composition of human tissues.

**Polymorphism**

Some of the GSTs are polymorphically expressed (above) with the expression of a null allele, GSTM1 (Table 4) and GSTTI. This makes it possible to detect their expressions in different individuals either by phenotyping or genotyping. The phenotyping of individuals expressing GSTM1 can easily be performed in whole blood, or other tissues available, by using the specific substrate trans-stilbene oxide for GSTM1. In the same manner, GSTT1 can be detected in whole blood by using methyl chloride as substrate. The phenotyping procedure enables us to distinguish between individuals who express the null allele and those who express the enzyme. In general, using the phenotyping technique as a measuring system may involve some problems such as an inhibitory effect on the enzyme from endogenous agents or inappropriate storage of the sample. To get around these problems, an ELISA technique might be useful if antibodies to the specific enzyme studied are available.

Restriction fragment length polymorphism (RFLP) or polymerase chain reaction (PCR) techniques have recently become very useful tools for genotyping different enzymes. Following the isolation of the cDNA for GSTM1 and GSTTI, the PCR technique has been used in many studies to determine the different genotypes. This technique may, however, not allow any evaluation of the level of the activity expressed *in vivo*, e.g., between heterozygous and homozygous individuals’ expression of the enzyme. On the other hand, it may be possible to determine a mutation of an enzyme that results in a
defect in enzymatic activity or expression of an unstable protein.

Several full-length human mEH DNA sequences have been cloned (63). The respective protein sequences deduced from these DNAs demonstrated less than 2% variation in human mEH amino acid content. Despite the similarities, there are striking interindividual differences in mEH activity measured in human populations (91,100). The PCR technique has been used to identify variation in the amino acid composition in humans. The variation detected at residues 113 and 139 did not exert a primary influence on catalytic function but may affect the stability of mEH protein (63). The entire EPHXI gene has been cloned and sequenced (101). Results from expression experiments in COS-1 and HepG2 cells suggested that the human mEH gene contained a weak core promoter and that inclusion of DNA sequences 5' of the minimal promoter region negatively regulated constitutive transcription (101).

### Table 4. Frequency of the GSTM1 null allele in different ethnic groups.

| Ethnic or national group | No. | Gene frequency | Reference |
|--------------------------|-----|----------------|-----------|
| Swedish                  | 248 | 0.732          | Seidegård et al. (34) |
| Finnish                  | 142 | 0.661          | Hirvonen et al. (95) |
| German                   | 145 | 0.743          | Brockmiller et al. (33) |
| French                   | 45  | 0.683          | Gruppi et al. (94) |
| Scottish                 | 42  | 0.673          | Hussey et al. (86) |
| English                  | 49  | 0.640          | Strange et al. (18) |
| Russian                  | 100 | 0.697          | Afanasjeva and Spitsyn (36) |
| Saudi Arabian            | 804 | 0.749          | Evans et al. (97) |
| Chinese                  | 96  | 0.765          | Board et al. (98) |
| Indian                   | 43  | 0.580          | Board et al. (98) |
| Japanese                 | 168 | 0.691          | Harada (59) |
| Filipino                 | 80  | 0.814          | Evans et al. (97) |
| Micronesian               | 37  | 1.000          | Board et al. (30) |
| Melanesian               | 49  | 0.795          | Board et al. (38) |
| Polynesian               | 49  | 0.904          | Board et al. (38) |

Genetic factors that regulate sEH have been studied in normal human lymphocytes (102). The study included mono- and dizygotic twins and 100 unrelated male subjects. The variability of the activity was markedly less within monozygotic than dizygotic twins. The extent of the interindividual variation was as much as 11-fold in the 100 subjects. The variation of sEH activity in human lymphocytes may be explained with either monogenic or polygenic control of this mode.

### Concluding Remarks

GSTs and EHs are involved in the metabolism of a wide range of drugs and other xenobiotics. The enzymes are the products of several multigene families that are differently regulated by a variety of factors. These genetic and regulatory factors are therefore likely to play a significant role in the response of individuals to many exogenous as well as endogenous compounds. Deficiencies of particular enzymes may alter the susceptibility of certain individuals to particular compounds. Improved diagnostic tests that can rapidly identify enzyme deficiencies are therefore required to allow their clinical significance to be fully investigated.

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