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The Glutathione *S*-Transferase Supergene Family: Regulation of GST* and the Contribution of the Isoenzymes to Cancer Chemoprotection and Drug Resistance

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ABSTRACT: The glutathione *S*-transferases (GST) represent a major group of detoxification enzymes. All eukaryotic species possess multiple cytosolic and membrane-bound GST isoenzymes, each of which displays distinct catalytic as well as noncatalytic binding properties: the cytosolic enzymes are encoded by at least five distantly related gene families (designated class alpha, mu, pi, sigma, and theta GST), whereas the membrane-bound enzymes, microsomal GST and leukotriene C_4 synthetase, are encoded by single genes and both have arisen separately from the soluble GST. Evidence suggests that the level of expression of GST is a crucial factor in determining the sensitivity of cells to a broad spectrum of toxic chemicals. In this article the biochemical functions of GST are described to show how individual isoenzymes contribute to resistance to carcinogens, antitumor drugs, environmental pollutants, and products of oxidative stress.

A description of the mechanisms of transcriptional and posttranscriptional regulation of GST isoenzymes is provided to allow identification of factors that may modulate resistance to specific noxious chemicals. The most abundant mammalian GST are the class alpha, mu, and pi enzymes and their regulation has been studied in detail. The biological control of these families is complex as they exhibit sex-, age-, tissue-, species-, and tumor-specific patterns of expression. In addition, GST are regulated by a structurally diverse range of xenobiotics and, to date, at least 100 chemicals have been identified that induce GST; a significant number of these chemical inducers occur naturally and, as they are found as nonnutrient components in vegetables and citrus fruits, it is apparent that humans are likely to be exposed regularly to such compounds. Many inducers, but not all, effect transcriptional activation of GST genes through either the antioxidant-responsive element (ARE), the xenobiotic-responsive element (XRE), the GST P enhancer 1(GPE), or the glucocorticoid-responsive element (GRE). Barbiturates may transcriptionally activate GST through a Barbie box element. The involvement of the Ah-receptor, Maf, Nrl, Jun, Fos, and NF-κB in GST induction is discussed. Many of the compounds that induce GST are themselves substrates for these enzymes, or are metabolized (by cytochrome P-450 monooxygenases) to compounds that can serve as GST substrates, suggesting that GST

* The numbering of the amino acids in class alpha glutathione *S*-transferase (GST) includes the initiator methionine, whereas none of the numbering of other GST includes this residue. The standard single-letter abbreviations are used to denote amino acids.

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induction represents part of an adaptive response mechanism to chemical stress caused by electrophiles. It also appears probable that GST are regulated *in vivo* by reactive oxygen species (ROS), because not only are some of the most potent inducers capable of generating free radicals by redox-cycling, but H_2O_2 has been shown to induce GST in plant and mammalian cells: induction of GST by ROS would appear to represent an adaptive response as these enzymes detoxify some of the toxic carbonyl-, peroxide-, and epoxide-containing metabolites produced within the cell by oxidative stress.

Class alpha, mu, and pi GST isoenzymes are overexpressed in rat hepatic preneoplastic nodules and the increased levels of these enzymes are believed to contribute to the multidrug-resistant phenotype observed in these lesions. The majority of human tumors and human tumor cell lines express significant amounts of class pi GST. Cell lines selected *in vitro* for resistance to anticancer drugs frequently overexpress class pi GST, although overexpression of class alpha and mu isoenzymes is also often observed. The mechanisms responsible for overexpression of GST include transcriptional activation, stabilization of either mRNA or protein, and gene amplification.

In humans, marked interindividual differences exist in the expression of class alpha, mu, and theta GST. The molecular basis for the variation in class alpha GST is not known. Absence of certain class mu and theta GST can be attributed to deletion of the *GSTM1* gene in 50% of the population and deletion of the *GSTT1* gene in 16% of the population. The biological consequences of failure to express hGSTM1 or hGSTT1 protein can include susceptibility to bladder, colon, skin, and possibly lung cancer.

KEY WORDS: glutathione *S*-transferases, chemoprotection, enzyme induction, adaptive response, antioxidants, drug resistance, population polymorphisms, carcinogenesis.

I. INTRODUCTION

All organisms are exposed continuously to toxic chemicals. The threat provided by such compounds is not a recent problem caused by the activities of the chemical industry, but has existed since life began. Many of the toxic chemicals we encounter are found naturally in the environment. Humans

Abbreviations used: **ACTH,** adrenocorticotropic hormone; **Ah,** aryl hydrocarbon; **ARE**, antioxidant-responsive element; **ARNT**, Ah-receptor nuclear translocator; **BCNU**, 1,3-*bis*(2-chloroethyl)-1-nitrosourea; **BHA**, butylated hydroxyanisole; **bHLH**, basic helix-loop-helix; **β-NF**, β-naphthoflavone; **tBHQ**, *tert*-butylhydroquinone; **bp**, base pairs; **CAT**, chloramphenicol acetyl transferase; **CDNB**, 1-chloro-2,4-dinitrobenzene; **CYP**, cytochrome P450; **DDT**, dichlorodiphenyltrichloroethane; **DNP-SG**, dinitrophenol *S*-glutathione; **DCNB**, 1,2-dichloro-4-nitrobenzene; **EPNP**, 1,2-epoxy-3-(*p*-nitrophenoxy)propane; **EPN**, *O*-ethyl-*O*-(4′-nitrophenyl)phenylphosphonothioate; **EpRE**, electrophile-responsive element; **EPTC**, *S*-ethyl-*N*,*N*-dipropylthiocarbamate; **GH**, growth hormone; **FLAP**, 5-lipoxygenase-activating protein; **GPE1**, glutathione transferase P enhancer 1; **GSH**, reduced glutathione; **GST**, glutathione *S*-transferase; **GRE**, glucocorticoid-responsive element; **HNF**, hepatic nuclear factor; **HPLC**, high-pressure liquid chromatography; **LTC_4S**, leukotriene C_4 synthase; **MAP**, mitogen-activated protein; **MOAT**, multispecific organic anion transporter; **3-MC**, 3-methylcholanthrene; **MIF**, macrophage-migration inhibitory factor; **MRP**, multidrug resistance-associated protein; **4-NBC**, 4-nitrobenzyl chloride; **NF**, nuclear factor; **NQO**, NAD(P)H:quinone oxidoreductase; **PAH**, polycyclic aromatic hydrocarbon; **PB**, phenobarbital; **PGDS**, glutathione-dependent prostaglandin D synthetase; **PhIP**, 2-amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine; **pI**, isoelectric point; **RA**, retinoic acid; **ROS**, reactive oxygen species; **SDM**, site-directed mutagenesis; **SDS/PAGE**, sodium dodecyl sulfate/polyacrylamide-gel electrophoresis; **SF-A**, silence factor A; **TCBOP**, 1,4-*bis*[2-(3,5-dichloropyridyloxy)]benzene; **TPA**, 12-*O*-tetradecanoylphorbol-13-acetate; **TRE**, TPA-responsive element; **tPBO**, *trans*-4-phenyl-3-buten-2-one; **XRE**, xenobiotic-responsive element.

may consume as much as 1.5 g of natural pesticide each day in the form of plant phenols, flavonoids, glucosinolates, and saponins.[1] Significant numbers of these naturally occurring pesticides have been found to be rodent carcinogens: allyl isothiocyanate, benzyl acetate, caffeic acid, ethyl acrylate, D-limonene, and 5-methoxypsoralen, all of which are widespread in food and drink and yield positive results in mutagenicity and clastogenicity tests.[1] Certain natural toxins, including mold aflatoxins, phorbol esters, and pyrrolizidine toxins, are among the most potent carcinogenic and clastogenic compounds known.[2] In addition to these exogenous chemicals, reactive oxygen species (ROS), such as the superoxide radical, hydrogen peroxide, and the hydroxyl radical, which arise as a consequence of aerobic respiration, ionizing irradiation, and inflammation can generate a wide spectrum of harmful carbonyl-containing compounds through interaction with membrane lipids and DNA.[3] The phytoalexins, a group of plant stress metabolites, are a further source of oxidizing agents that we encounter daily.[4]

To ensure survival in the face of a wide spectrum of harmful chemicals, various defense mechanisms have evolved to protect cells against noxious compounds. Such protective mechanisms include drug efflux pumps,[5] drug sequestration,[6,7] drug metabolism,[8,9] and repair of drug-target sites.[10–12] Although these processes each provide protection against a different spectrum of chemicals, drug metabolism represents a particularly versatile protective mechanism. The metabolism of foreign compounds usually involves two distinct stages, commonly referred to as phases I and II. Phase I metabolism involves an initial oxidation of the xenobiotic by cytochrome P450 (CYP) monooxygenases.[13–15] This step is followed by phase II metabolism, which frequently involves conjugation reactions catalyzed by glutathione *S*-transferases (GST),[16] UDP-glucuronosyl transferases,[17] and sulfotransferases,[18] or reduction reactions catalyzed by epoxide hydrolase[19] and quinone reductase.[20] By contrast, protection against ROS and the breakdown products of peroxidized lipid and oxidized DNA is provided by superoxide dismutases,[21,22] catalase,[21,22] glutathione peroxidases,[23,24] GST,[25,26] aldo-keto reductases,[27] and DNA-repair enzymes.[28]

Many types of experimental models such as cofactor depletion, cDNA transfection, null mutants, acquired resistance in tumor cell lines, as well as selective toxicity and epidemiological studies, indicate that the sensitivity of cells to chemical stress is determined by the levels of expression of the various chemical defenses mentioned above. The relative importance of different mechanisms depends on the nature of the chemical insult. For example, resistance to toxic chemicals that do not have a defined target site is often achieved by increases in drug efflux, drug sequestration, drug metabolism, or DNA repair, whereas resistance to chemicals with a unique site of action usually involves amplification of the gene encoding the target protein, mutation and, modification of the target site, or bypass of target function.[29]

Among the detoxification systems, the GST (EC 2.5.1.18) play critical roles in providing protection against electrophiles and products of oxidative stress. GST isoenzymes display a remarkably broad substrate specificity[16] and are unusual in exhibiting several catalytic activities as well as possessing the ability to sequester nonsubstrate drugs and hormones.[30] In common with certain other drug-metabolizing enzymes, the levels of expression of GST in many species can be increased significantly by exposure to foreign compounds,[31] suggesting that they form part of an adaptive response to chemical stress. However, whereas the induction of GST is not in itself unusual,

it should be noted that GST appear to make a key contribution to this adaptive response mechanism as many of the inducing agents are themselves either GST substrates or are metabolized by CYP to become GST substrates.[32–34] It is, therefore, likely that GST modulate the induction of other enzymes, such as quinone reductase,[35] aflatoxin B_1-aldehyde reductase,[36,37] UDP-glucuronosyl transferase,[17] γ-glutamyl transferase,[38,39] and γ-glutamylcysteine synthetase,[40,41] through their ability to metabolize inducing agents. Both GST substrates[33] and glutathione conjugates[40] appear to possess the ability to induce a variety of proteins: it is probable that a number of phase II drug-metabolizing enzymes are regulated by GST substrates, whereas certain glutathione-dependent proteins and enzymes involved in glutathione homeostasis may be regulated by glutathione conjugates. The probability that GST can modulate the expression of other drug-metabolizing enzymes suggests that GST population polymorphisms in humans,[42–44] strain variations in animals,[45] and species differences[29] will influence other chemical defense mechanisms.

In this article, an overview is provided of the GST supergene family and the contribution of individual isoenzymes to protection against toxic chemicals, including anticancer agents. Emphasis is placed on the induction of GST by drugs, and on the involvement of specific isoenzymes in the adaptive response(s) to electrophiles and to oxidative stress. Recent advances in our understanding of the molecular mechanisms involved in the regulation of GST, and their possible contribution to cancer chemoprevention, will be discussed. Finally, the literature suggesting that null polymorphisms in GST expression represent risk factors in susceptibility to cancer will be reviewed.

II. FUNCTIONS OF GST

A. Catalytic Activities

The GST are a family of enzymes that catalyze a number of distinct glutathione-dependent reactions: in addition to their ability to catalyze the formation of conjugates, GST can also serve as peroxidases and isomerases.[16] The fundamental basis for all the various catalytic activities of GST is the ability of the enzyme to lower the pK_a of the sulfhydryl group of reduced glutathione (GSH) from 9.0 in aqueous solution to about 6.5 when bound in the active site.[46] Evidence suggests that glutathione exists as the thiolate (GS^-) anion at neutral pH when complexed with GST.[47–50] X-ray crystallographic studies have also shown that a conserved tyrosine (in classes alpha, mu, pi, and sigma) or serine (class theta), found at the *N*-terminus of most cytosolic GST, is involved in stabilizing GS^- through hydrogen bonding.[51–55] It is proposed that once GS^- is formed in the active site of GST, it becomes capable of reacting spontaneously, by nucleophilic attack, with electrophilic xenobiotics that are situated in close proximity.[56] Thus, catalysis by GST occurs through the combined ability of the enzyme to promote the formation of GS^- and to bind hydrophobic electrophilic compounds at a closely adjacent site. The glutathione-binding site exhibits a high specificity,[57] whereas, by contrast, the second substrate-binding site displays a broad specificity toward hydrophobic compounds. The GSH-binding site and the hydrophobic substrate-binding site have been called the G- and H-sites, respectively.[58] Evidence for the existence of such sites has been provided by X-ray crystallography, which has revealed that cytosolic GST subunits are folded into two separate domains of different structure. Domain

I, the *N*-terminal domain, contains much of the G-site, whereas domain II contains essentially all of the H-site.

B. Glutathione Conjugation and Detoxification

All GST possess the ability to conjugate GSH with compounds containing an electrophilic center. The electrophilic functional group for conjugation reactions can be provided by a carbon, a nitrogen, or a sulfur atom. Such groups are present in arene oxides, aliphatic and aromatic halides, α,β-unsaturated carbonyls, organic nitrate esters, and organic thiocyanates.[56,58,59] The range of compounds that contain electrophilic centers is extremely large and includes the parent chemical or metabolite of the carcinogens aflatoxin B_1, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), benzo[*a*]pyrene, 7,12-dimethylbenz[*a*]anthracene, 5-methylchrysene, and 4-nitroquinoline-*N*-oxide (Figure 1). GST also detoxify the pesticides alachlor, atrazine, dichlorodiphenyltrichloroethane (DDT), lindane, and methyl parathion (Figure 2), the oxidative-damage products acrolein, base propenals, cholesterol α-oxide, fatty acid hydroperoxides, and 4-hydroxynonenal (Figure 3), the anticancer drugs 1,3-*bis*(2-chloroethyl)-1-nitrosourea (BCNU), chlorambucil, cyclophosphamide, melphalan, and thiotepa, as well as the antibiotic fosfomycin (Figure 4).

To our knowledge, the majority of GST substrates are either xenobiotics or products of oxidative stress. However, it should be remembered that a small number of endogenous compounds, such as leukotriene A_4[60] and prostagladin H_2,[61,62] are also metabolized by GST as part of their normal biosynthetic pathways and therefore should not be classed as detoxification reactions.

The most widely used substrate to study GST is 1-chloro-2,4-dinitrobenzene (CDNB). When conjugated with GSH it gives *S*-(2,4-dinitrophenyl)glutathione, a compound possessing an absorbance spectrum sufficiently different from that of CDNB to allow a simple spectrophotometric assay at 340 nm.[63,64]

The formation of a thioether bond between electrophiles and GSH almost always yields a conjugate that is less reactive than the parental compound,[59] and therefore the actions of GST generally result in detoxification. From a teleological viewpoint, GSH conjugation is thought to be of value not only because it removes harmful electrophilic moieties from the cell but also because it increases the solubility of hydrophobic xenobiotics and, by preventing their partitioning into membrane lipid, decreases their half-life in the body. Although this is undoubtedly true, it is important to recognize that, once formed, the conjugates can be transported from the cell by ATP-dependent glutathione *S*-conjugate efflux pumps. It is more probable that the major biological value of GSH conjugation lies in its ability to provide a molecular "flag", which signals export of the conjugate from the cell, rather than the fact that it increases the solubility of lipophilic compounds. Several glutathione *S*-conjugate pumps have been described in mammalian[65–67] and plant cells.[68] Although it can be assumed that they are widely distributed in nature, it is unclear how many transporters exist and whether they display specificity solely for glutathione *S*-conjugates or can transport other classes of compounds in addition to drug conjugates. Ishikawa[65,69] has characterized an ATP-dependent glutathione *S*-conjugate pump, now called the GS-X pump, from rat heart and demonstrated that it can transport oxidized glutathione (GSSG), leukotriene C_4 (LTC_4), and S-(2,4-dinitrophenyl)glutathione. The

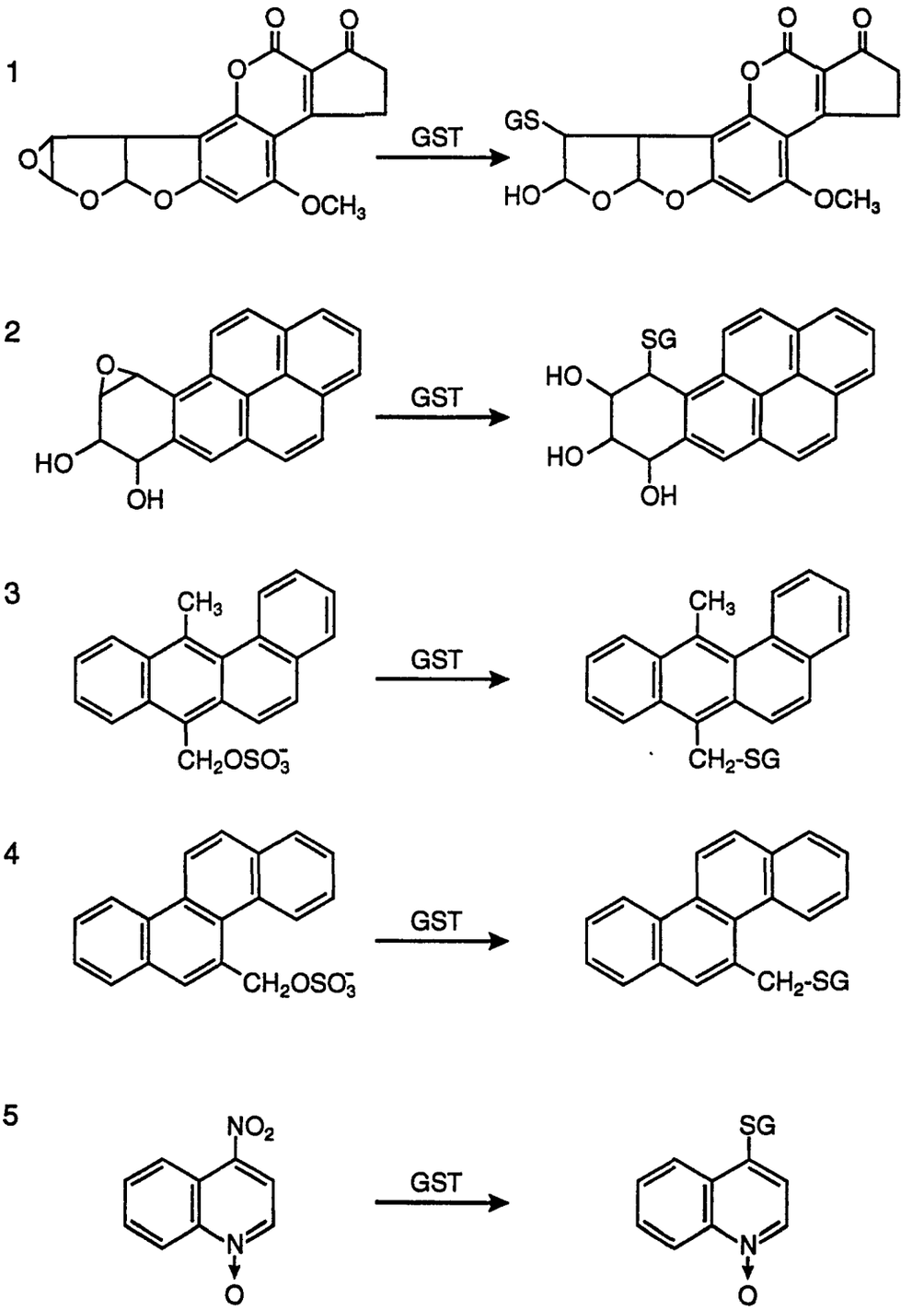


FIGURE 1. Detoxification of carcinogens by GST. The following reactions are catalyzed by GST: (1) aflatoxin B_1-8,9-epoxide; (2) benzo[*a*]pyrene-7,8-diol-9,10-oxide; (3) 7-hydroxymethylbenz[*a*]anthracene sulfate; (4) 5-hydroxymethylchrysene sulfate; (5) 4-nitroquinoline 1-oxide.

GS-X pump may also transport cisplatin-glutathione complexes as it is functionally overexpressed in cisplatin-resistant human leukemia HL-60 cells.[70,71] Ishikawa et al.[71] have identified three membrane proteins of 70, 100, and 200 kDa that are overexpressed

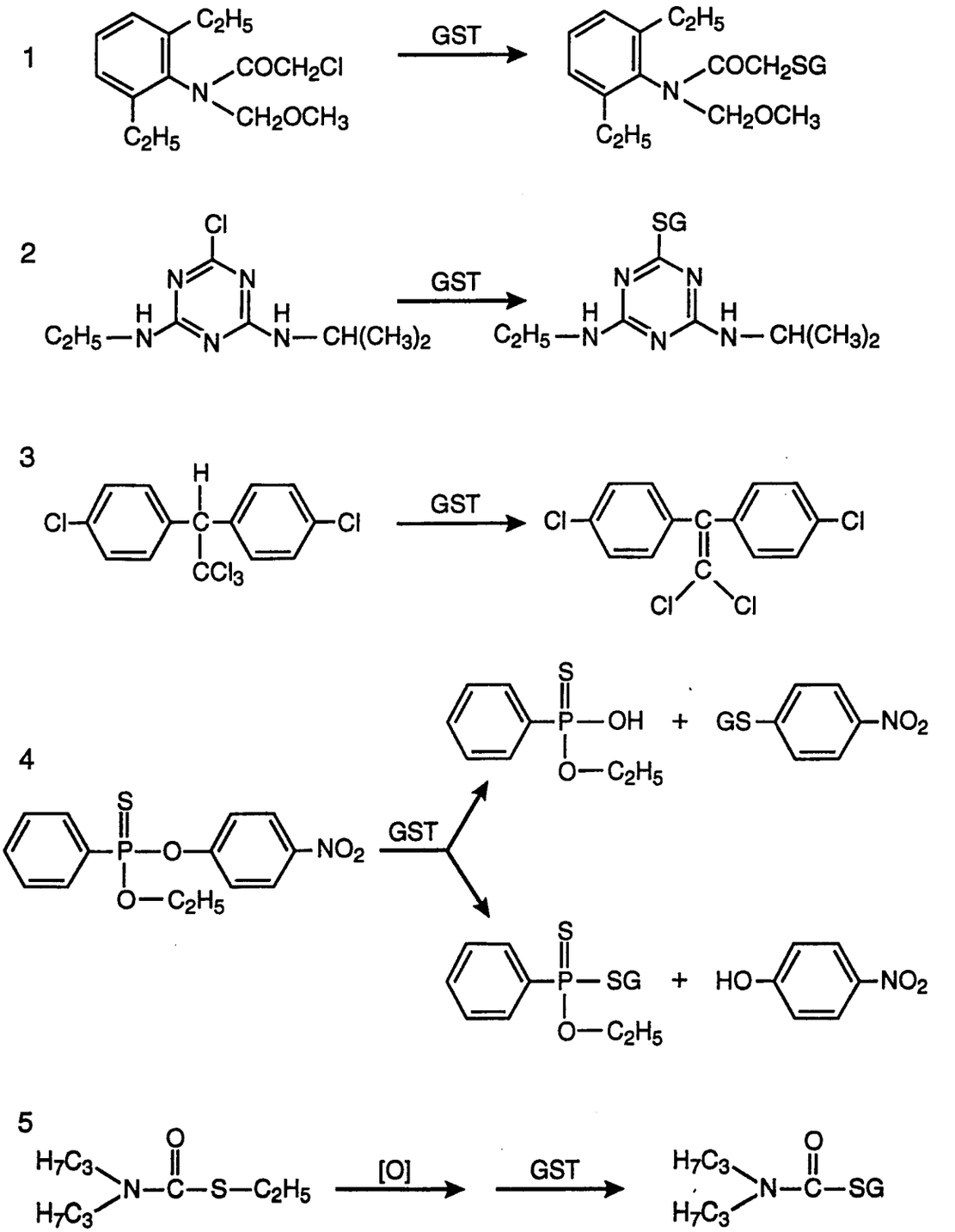


FIGURE 2. Metabolism of pesticides and environmental pollutants by GST: (1) alachlor; (2) atrazine; (3) DDT; (4) EPN; (5) EPTC; (6) fluorodifen; (7) lindane; (8) methyl parathion.

in cisplatin-resistant HL-60 cells. It is presumed that one of these polypeptides represents the GS-X pump. Independent studies of mutant Wistar TR$^-$ rats, with impaired biliary transport of bilirubin,[72] have led to the identification of a multispecific organic

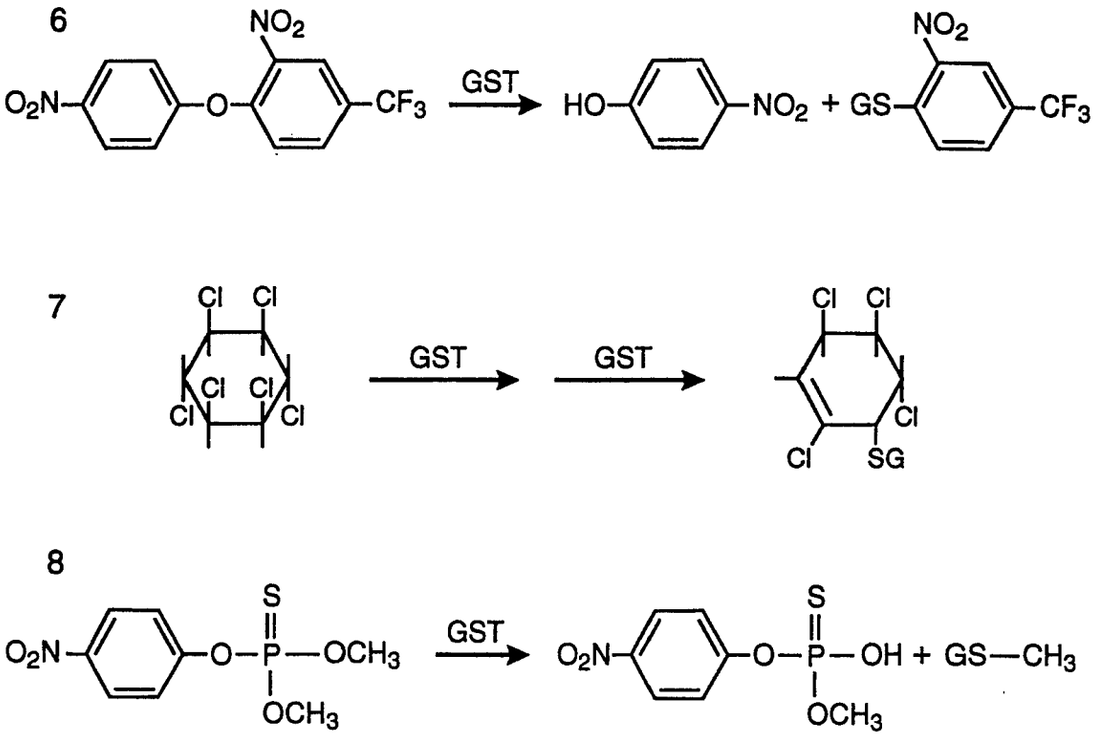


FIGURE 2 (continued)

anion transporter (MOAT) that is associated with transport of bromosulfophthalein, bilirubin-glucuronide conjugates, glutathione *S*-conjugates, GSSG, and cysteinyl leukotrienes.[73] It appears likely that GS-X and MOAT are either closely related or identical. By contrast, Awasthi and his co-workers[74,75] have purified a broad-specificity anion transporter of dinitrophenol *S*-glutathione (DNP-SG ATPase) that comprises a 38-kDa polypeptide. These workers[76] have provided evidence that, in addition to glutathione *S*-conjugates, DNP-SG ATPase can also transport doxorubicin, daunomycin, and vinblastine. Although it has been emphasized that DNP-SG ATPase is distinct from P-glycoprotein, the 170-kDa multidrug resistance pump,[5] it would also appear to be separate from GS-X and MOAT . Following the biochemical characterization of GS-X, MOAT, and DNP-SG ATPase, it has been found that the multidrug resistance-associated protein (MRP)[77–79] is also capable of transporting glutathione *S*-conjugates.[80,81] The MRP transporter is a 190-kDa glycoprotein and, although distinct from P-glycoprotein, is a member of the ABC superfamily of transporter proteins.[77] Transfection experiments have also shown that the MRP cDNA encodes a pump for LTC_4, and the fact that this activity can be inhibited by GSSG and the GSH conjugate of 4-hydroxynonenal suggests that MRP is a transporter of a range of glutathione *S*-conjugates.[81] However, although functional relationships exist among the GS-X pump, MOAT, and MRP, it is not certain that these transporters are all identical. Significantly, it is clear that multiple transport mechanisms exist for glutathione *S*-conjugates in rat liver.[82,83] The conjugates and dyes transported by the GS-X pump and MOAT are all dianionic com-

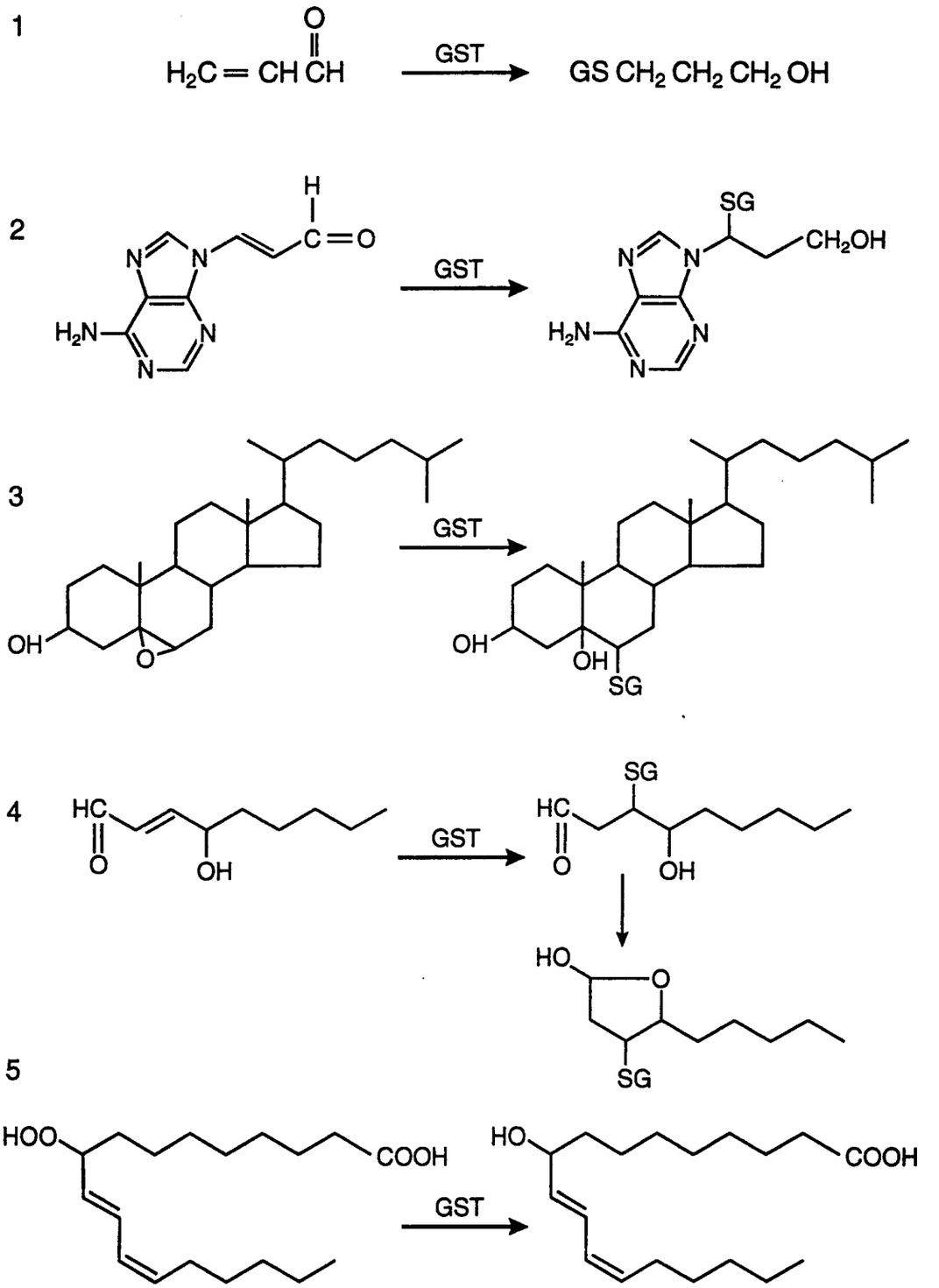


FIGURE 3. Examples of GST substrates that are produced by oxidative stress: (1) acrolein; (2) adenine propenal; (3) cholesterol-5,6-oxide; (4) 4-hydroxynon-2-enal; (5) 9-hydroperoxy-linoleic acid.

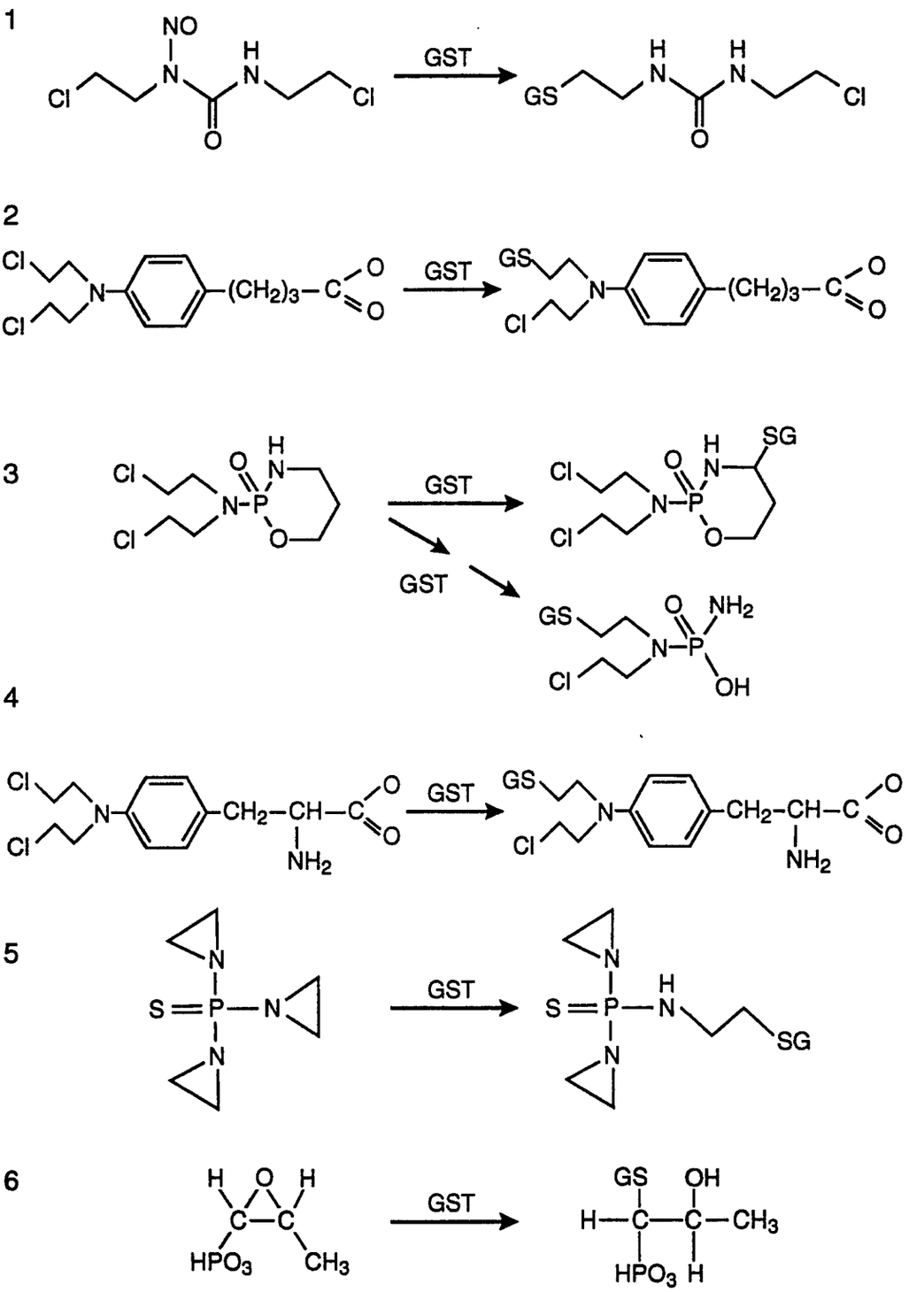


FIGURE 4. Examples of chemotherapeutic agents that are GST substrates: (1) BCNU; (2) chlorambucil; (3) cyclophosphamide; (4) melphalan; (5) thiotepa; (6) fosfomycin.

pounds. Neither doxorubicin nor vincristine, which are substrates for MRP, are known to form dianionic metabolites and it would therefore not be expected that doxorubicin and vincristine would be transported by GS-X or MOAT. It is possible that the GS-X

pump and MOAs have broader specificities than currently recognized, and can transport uncharged natural product drugs as well as dianionic compounds. It is, however, clear that the DNP-SG ATPase of 38 kDa is distinct from GS-X, MOAT, and MRP and that, in turn, these proteins are separate from P-glycoprotein.

The conjugation reaction between GSH and xenobiotics represents the first step in the synthesis of mercapturic acids, an important group of excretion products that were first identified more than 100 years ago in the urine of animals treated with bromobenzene. Following conjugation with GSH, the subsequent steps in mercapturic acid biosynthesis require the sequential actions of γ-glutamyl transpeptidase, cysteinyl glycinase, and *N*-acetyl transferase;[84] for further details about the measurement of mercapturic acids, see Vermeulen[85] and Alary et al.[86]

C. Glutathione Conjugation and Toxification

Although the vast majority of GSH conjugates represent detoxification products, several instances exist where GST activity does not result in the detoxification of xenobiotics. For example, a few GSH conjugates are relatively unstable and the reaction product is either cleaved to liberate an unconjugated metabolite that requires further detoxification, or the reaction is reversible allowing regeneration of the original electrophile. A potentially more serious situation can arise with a small number of GST substrates that yield a GSH conjugate, or a metabolite of the conjugate, that is more reactive than the parental compound; these two groups of compounds have been referred to as directly acting toxic GSH conjugates and indirectly acting toxic GSH conjugates. Although toxification by GST is undesirable in normal circumstances, it can be exploited in cancer chemotherapy to treat tumors that overexpress GST. For example, drugs that either yield directly acting toxic GSH conjugates or are cleaved by GST to produce toxic metabolites may be of value in targeting certain cancers. Nitrogen mustards have been synthesized that, when cleaved by GST, liberate a cytotoxic phosphate moiety.[87]

Incomplete detoxification by GST occurs with certain esters, ethers, and organic phosphates when conjugation leads to cleavage of the substrate with only one of the two products being conjugated. This process has been called thiolysis[88] and, in the case of *p*-nitrophenol acetate, the herbicide fluorodifen, and the insecticide EPN, it results in the release of *p*-nitrophenol (Figure 2); presumably, the *p*-nitrophenol is metabolized by UDP-glucuronosyl transferase and phenol sulfotransferase. Thiolysis represents incomplete detoxification because the unconjugated cleavage product still provides a chemical threat to the cell.

Reversible conjugation by GST can occur with certain cytotoxic isothiocyanates.[89,90] Following reaction of either benzyl, allyl, phenethyl isothiocyanate, or sulforaphane with GSH, their respective conjugates (dithiocarbamates) are not stable and readily yield the parental thiocyanate in mildly acidic media.[91] In the case of benzyl and phenethyl isothiocyanate, GST can catalyze both the forward and reverse reactions (Figure 5) but, at high enzyme concentrations, the equilibrium is shifted in favor of formation of the GSH conjugates.[92] The reversibility of this reaction means that the conjugates may not represent detoxification products but, rather, temporary storage or transport forms of the isothiocyanates. It has been speculated that accumulation and release of isothiocyanates at peripheral sites in the body, where GST is expressed in low

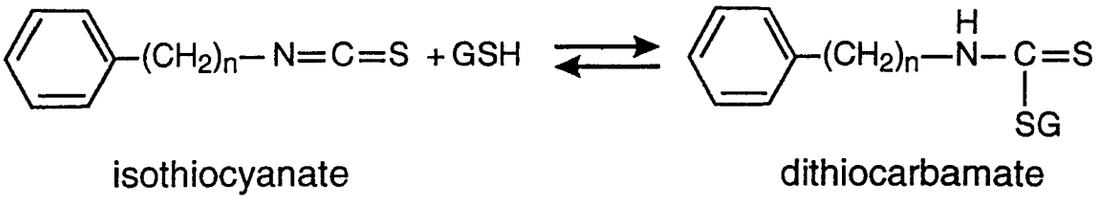


FIGURE 5. Reversible conjugation between GSH and an organic isothiocyanate catalyzed by GST.

amounts, can cause toxicity. Possibly the best known example of this type of glutathione-mediated transport of toxic substances is provided by the tragic industrial accident in 1984 involving leakage of methyl isocyanate from the Union Carbide chemical plant in Bhopal, India, which resulted in the deaths of about 3500 people. A surprising feature of the patients who survived the toxic methyl isocyanate gas was the diversity of their clinical symptoms and the large number of tissues affected; methyl isocyanate caused damage to eyes, heart, bones, muscle, gastrointestinal tract, and reproductive organs. It has been proposed that the damage to nonpulmonary tissue was caused by the formation of a labile conjugate between methyl isocyanate and GSH that was transported from the fluid lining the lung to sites around the body.[93,94] This conjugate, *S*-(*N*-methylcarbamoyl)glutathione, will release methyl isocyanate under alkaline conditions and has been found to be highly toxic to explanted mouse embryos.[95]

Directly acting toxic GSH conjugates are formed from a number of alkyl dihalides.[96,97] Attention has focused primarily on GST-mediated toxification of dihaloethanes and dihalomethanes. The conjugates formed from dihaloethanes may rearrange spontaneously prior to interaction with DNA, whereas those formed from dihalomethanes probably do not rearrange prior to interaction with DNA (Figure 6). GST-catalyzed reactions between 1,2-dihaloalkanes and GSH may yield *S*-(2-haloalkyl)glutathiones, which can form episulfonium ions by the internal displacement of the halogen atom; the episulfonium ion intermediates are potent electrophiles and act as alkylating agents.[96,97] In the case of dihalomethanes, the *S*-halomethylglutathione conjugate appears to be the ultimate mutagen. Analysis of a series of vicinal dihaloethanes has revealed that the better the leaving group ability of the halogen substituent the greater its activity as a GST substrate (i.e., $ICH_2CH_2I > BrCH_2CH_2Br > ClCH_2CH_2Cl$).[98] In mutagenicity tests, ethylene dibromide and ethylene dichloride are mutagenic, but ethylene diiodide is not effective:[98] it is uncertain whether the negative result obtained with this latter compound is due to methodological factors such as activation of the alkyl halide outwith the *Salmonella typhimurium* tester strain. The activation of methylene chloride (dichloromethane), a widely used industrial solvent, has been studied extensively because it produces liver and lung cancer in the mouse. Methylene chloride does not, however, cause liver cancer in the hamster or the rat, nor does it induce DNA damage in normal human hepatocytes, suggesting that species-specific differences exist in the GST isoenzyme(s) responsible for the formation of *S*-chloromethylglutathione.[99–101] In humans, different liver cytosol specimens or blood samples display marked inter-individual variations in ability to conjugate methylene chloride with GSH, indicating that the enzyme responsible is subject to

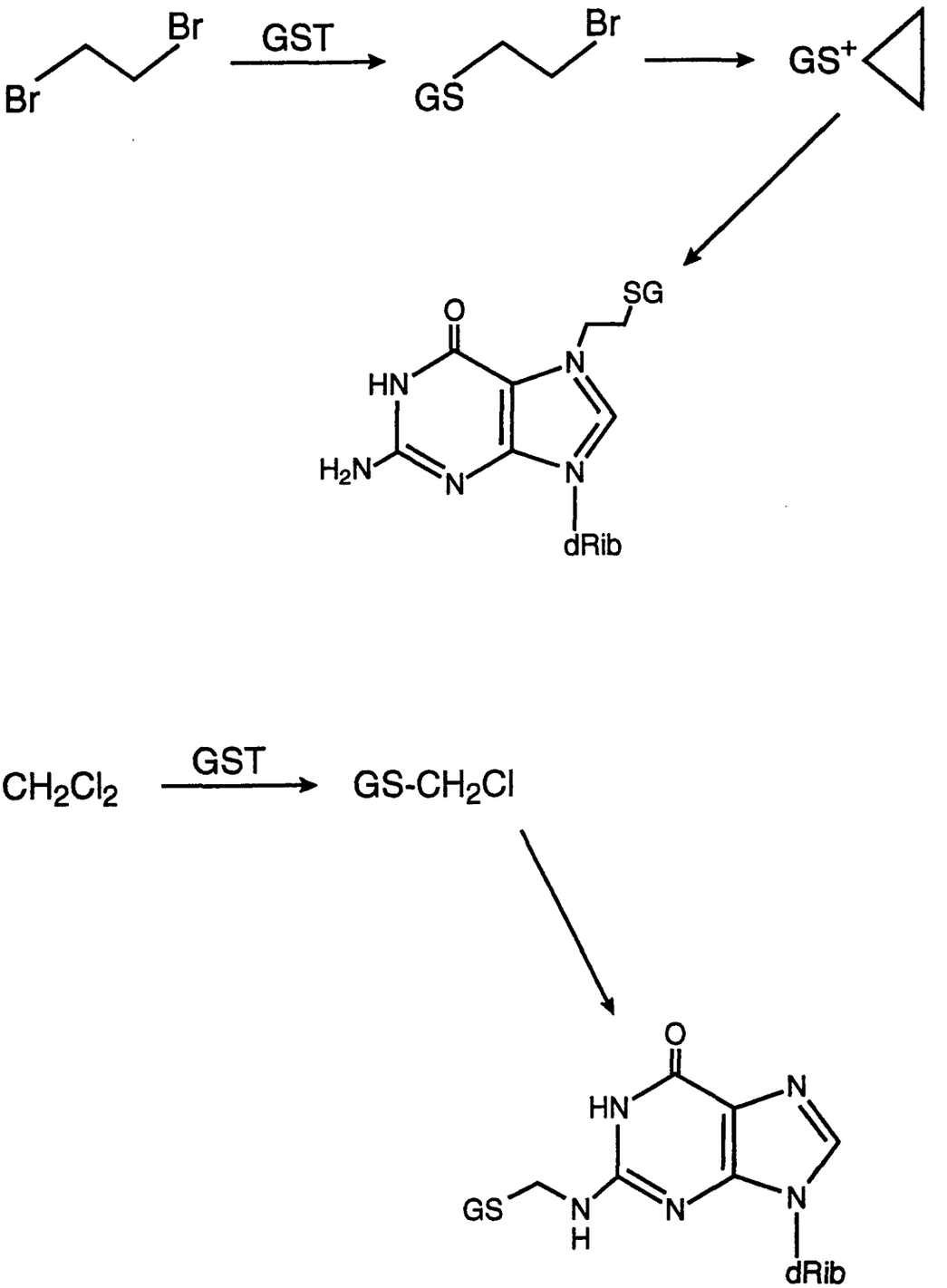


FIGURE 6. Reaction between GSH conjugates formed between (1) ethylene dibromide and DNA and (2) methylene chloride and DNA.

polymorphic expression.[44,102-104] It has been demonstrated that a rat transferase (rGSTT1-1), expressed within the Ames *S. typhimurium* TA1535 strain, can activate ethylene dibromide, dibromomethane, and to a small extent methylene chloride.[105] More

recently, the *S. typhimurium* tester strain has been used to demonstrate that in addition to alkyl dihalides, rGSTT1-1 can activate epoxide-containing bifunctional agents such as 1,2,3,4-diepoxybutane (butadiene diepoxide), 1,2-epoxy-4-bromobutane, 1,4-dibromo-2,3-epoxybutane, and 1,2-epoxy-3-bromopropane.[106] This experimental approach is powerful and will allow species- and izoenzyme-specific differences in the GST-mediated activation of a range of bifunctional alkylating agents to be examined.

Indirectly acting toxic GSH conjugates are formed from various halogenated alkenes and alkynes. They include hexachlorobutadiene, tetrachloroethene, trichlorotrifluoropropene, and dichloroacetylene (Figure 7), all of which are nephrotoxic and also possibly nephrocarcinogenic.[107,108] These compounds are all preferentially metabolized by membrane-bound GST, rather than by the soluble GST.[109] The renal damage caused by these halogenated compounds is not caused by the GSH conjugates formed in the liver, but by metabolism of the cysteine conjugate, formed by cysteinyl glycinase. During mercapturic acid biosynthesis, the cysteine conjugate is acetylated by *N*-acetyl transferase, but the nephrotoxicity of cysteine conjugates of halogenated alkenes arises from their metabolism by renal cysteine conjugate β-lyase, which produces unstable thiols (in addition to ammonia and pyruvate) that yield electrophilic acylating agents. Cysteine *S*-conjugates derived from chloroalkenes are mutagenic in the Ames test, and their mutagenicity depends on the presence of β-lyase. The GSH conjugates formed from 2-bromobenzoquinone are also nephrotoxic; although they require further metabolic activation, possibly oxidation, the mechanism of toxicity is uncertain.[110]

D. Peroxidase Activity of GST

Besides being able to catalyze the formation of a thioether bond between GSH

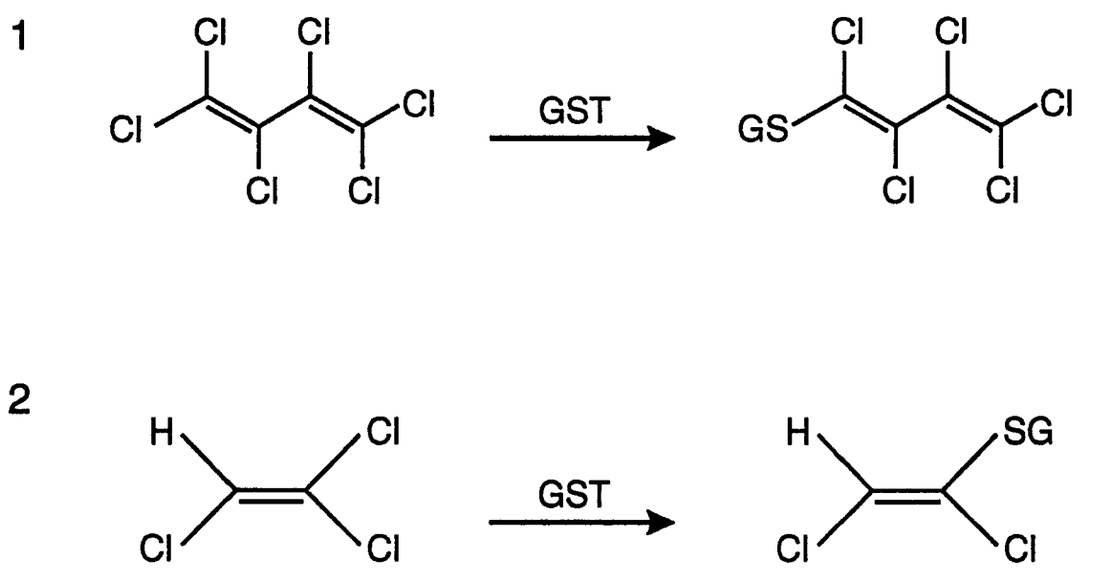


FIGURE 7. Examples of compounds that form indirectly acting toxic GSH conjugates: (1) hexachloro-1,3-butadiene; (2) trichloroethene.

and electrophilic xenobiotics, a significant number of the GST isoenzymes also exhibit glutathione peroxidase activity and catalyze the reduction of organic hydroperoxides to their corresponding alcohols. This type of reaction is thought to represent nucleophilic attack by GSH on electrophilic oxygen.[111] It is believed to involve two steps, only one of which is catalytic, and to proceed via formation of the sulfenic acid of glutathione as follows:

i. ROOH + GSH → ROH + [GSOH].....enzymatic
ii. [GSOH] + GSH → GSSG + H_2O.....spontaneous: to give the overall reaction,
iii. ROOH + 2 GSH → ROH + GSSG + H_2O.

The substrates that GST reduce include fatty acid, phospholipid, and DNA hydroperoxides. As these compounds are generated by lipid peroxidation and oxidative damage to DNA, it has been proposed that GST, as well as other GSH-dependent enzymes, help combat oxidative stress.[112] An important difference exists between the membrane-bound (microsomal) GST and cytosolic GST in their respective roles in protection against reactive oxygen species.[113] Detoxification of lipid hydroperoxides by microsomal GST can occur *in situ*, whereas detoxification of lipid hydroperoxides by cytosolic GST requires prior release of fatty acid hydroperoxides by phopholipase A_2.[114,115]

E. Isomerase Activity of GST

Several GST can catalyze the *cis-trans* isomerization of maleylacetone to fumarylacetone and maleylacetoacetic acid to fumarylacetoacetic acid. An even smaller number of GST isoenzymes possess ketosteroid isomerase activity and catalyze the conversion of Δ^5-3-ketosteroids to Δ^4-3-ketosteroids. The physiological significance of these isomerization reactions is unclear, but the isomerization of maleylacetoacetic acid occurs in the pathway of tyrosine degradation in mammalian liver (Figure 8).[88]

F. GST is not a Fatty Acid Ethyl Ester Synthetase

Despite a report in the literature indicating that GST possess fatty acid ethyl ester synthetase activity,[116] this claim has not been substantiated.[117,118]

G. Relationship between GST and Macrophage-Migration Inhibitory Factor (MIF)

MIF, the lymphokine that was isolated through its ability to inhibit the migration of macrophages from capillary tubes, has been reported by Blocki et al.[119] to possess GST activity toward 1,2-epoxy-3-(*p*-nitrophenoxy)propane (EPNP). More recently, these workers described preparations of MIF that can conjugate dichloromethane with GSH.[120] These results await confirmation in other laboratories, but it is clear that MIF can bind glutathione as well as glutathione *S*-conjugates.[121,122] Sequence alignments have shown MIF to lack the *N*-terminal tyrosine or serine residues that are responsible for the generation of the thiolate anion in most cytosolic GST, indicating that the hypothesis that MIF is a GST should be treated with caution. However, it is intriguing that MIF contains threonine at residue 8 (including the initiator methionine) which might conceivably function, like the *N*-terminal tyrosine or serine of cytosolic GST, in formation of GS^-.

H. Noncatalytic-Binding Activities

It has been known for many years that GST are able to bind, both covalently and

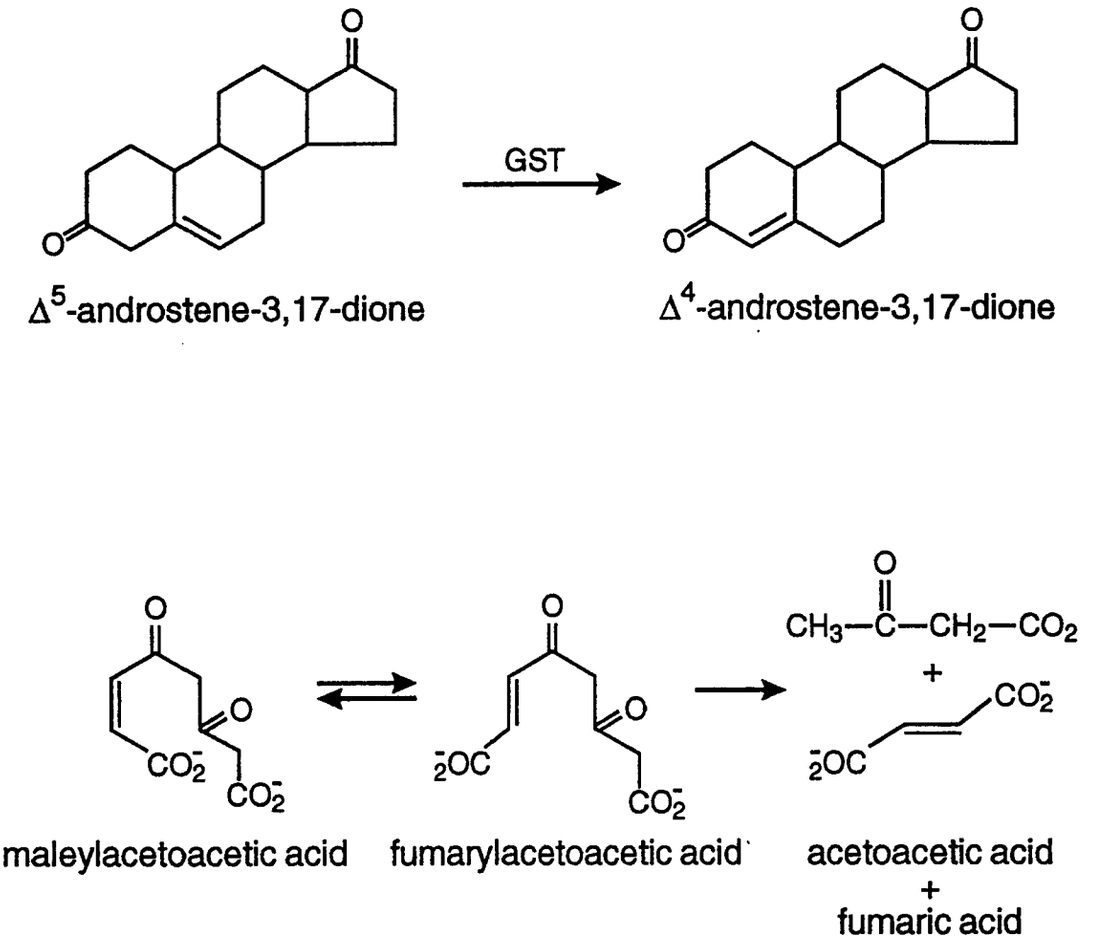


FIGURE 8. Isomerization of Δ^5-androstene-3,17-dione and maleylacetoacetic acid, both of which are catalyzed by GST.

noncovalently, a wide spectrum of chemicals. Compounds that have been shown to be bound covalently by GST are reactive metabolites formed from carcinogens such as dimethylaminoazobenzene and 3-methylcholanthrene (3-MC).[123] It is thought that the covalent binding of these compounds represents a "suicide" reaction by GST, serving to prevent genotoxic electrophiles from interacting with DNA.[124] The GST possessing this type of activity have historically been called "ligandin".[125] However, not all compounds that are bound covalently by GST are carcinogens. The diuretic ethacrynic acid can also be bound covalently by GST isoenzymes other than ligandin,[126,127] although the physiological significance, if any, of this is uncertain.

All GST bind noncovalently a range of neutral or anionic lipophilic chemicals that are not substrates, including steroid and thyroid hormones, bile acids, bilirubin, "heme", fatty acids, and penicillin.[128–133] Binding of these nonsubstrate compounds is usually of moderate affinity with K_d values of between 10^{-8} and 10^{-5} *M*. The biological significance of this noncovalent binding-activity has been the subject of much debate, but it is worth

pointing out that, because of the large amount of GST in most tissues (i.e., between 5 and 100 μ*M*), these proteins do provide a substantial intracellular-binding capacity for lipophilic ligands. It was first proposed many years ago that the binding of steroid hormones, bilirubin, and the bile acid lithocholate may contribute to the transport of these compounds across the liver and facilitate their elimination into bile. Similarly, GST in the kidney and small intestine may be involved in the transport of lipophilic compounds. Listowsky[131] has suggested that as GST constitute a high-capacity intracellular-binding pool for hormones, they might function as a binding reserve in target organs, possibly serving a "buffering" role to minimize the effects of transient fluxes in extracellular hormone levels. In support of this putative role for GST, it has been pointed out that GST are the predominant cytosolic proteins labeled by steroid hormone and thyroxine photoaffinity probes.[132,133]

GST have a significant affinity for glutathione *S*-conjugates. The surprising fact that the major cytosolic GST can bind certain glutathione *S*-conjugates more avidly *in vitro* than either the respective second substrate or GSH indicates that product inhibition may occur *in vivo*; for example, the K_i values of the major human cytosolic GST for the GSH conjugate of CDNB lie between 15 and 90 μ*M*, whereas the K_m values of the same enzymes for CDNB lie between 450 and 910 μ*M* and for GSH between 80 and 160 μ*M*. These data suggest that the sequestration of glutathione *S*-conjugates by GST represents a physiologically important function.[134] Such an activity may be advantageous to the cell as it could prevent toxic compounds from interacting with target molecules or, alternatively, it might stabilize reactive or unstable conjugates. This proposal is supported by the fact that the monoglutathionyl conjugate of the bifunctional alkylating agent chlorambucil, which is used in cancer chemotherapy, is bound by GST.[135] Furthermore, GST has been found to shift the equilibrium of the conjugation reaction between GSH and organoisothiocyanates in a dose-dependent fashion.[92] The various different GST isoenzymes may act collectively to bind GSH conjugates and thereby help minimize inhibition of the specific isoenzyme responsible for catalyzing the formation of the particular conjugate.

III. GLUTATHIONE *S*-TRANSFERASE GENES

A. Gene Families Encoding Cytosolic GST

GST are widely distributed in nature, being found in bacteria,[136–142] yeast,[143,144] molds,[145] fungi,[146] molluscs,[147,148] crustacea[149], worm parasites,[150–153] frogs,[154] insects,[155–159] plants,[160–167] fish,[168–170] birds,[171–173] and mammals.[174,175] Essentially all eukaryotic species appear to possess multiple isoenzymes. GST in rats and humans have been studied in greatest detail and, therefore, the classifications used to describe these enzymes have had a major impact on the study of GST in other organisms.

A large number of cytosolic GST isoenzymes have been purified from rat and human organs and, on the basis of their primary structures, these have been assigned to five separate families designated class alpha, mu, pi, sigma, and theta GST.[174–177] There are no clearly established criteria concerning the extent of sequence similarity required to place a GST in a particular class. It is generally accepted that GST that share greater than 40% identity are included in the same class, and those that possess less than 30% identity are assigned to separate classes. However, it should be emphasized

that this type of criterion is arbitrary and is fraught with difficulty when attempting to classify GST that possess limited (e.g., 30 to 40%) homology with recognized alpha, mu, pi, sigma,or theta enzymes. In particular, the definition of class sigma and theta GST is imprecise[176,177] and should be used with discretion when dealing with enzymes from nonmammalian species. Although no formal rules exist for classification, emphasis tends to be placed on the primary structure of the *N*-terminus because, within the classes, this region tends to be better conserved than others. It is important to point out that the "class" terminology is deliberately global in its attempt to encompass as many GST as possible. This masks the fact that within each class clearly defined subfamilies of rodent and human GST can be identified. Hence, a particular class of GST may be composed of two or three different subfamilies, each representing a unique subunit type, which may include as many as five separate highly homologous polypeptides that possibly share greater than 90% identity.

The hypothesis that these classes represent separate families of GST is supported by the distinct structures of their genes and their chromosomal localizations. The class alpha, mu, pi, and theta GST genes, which have been isolated to date, differ markedly in size and in their intron-exon structures (class sigma GST genes have yet to be characterized). All the class alpha genes isolated from the rats,[178,179] mice,[180] and humans[181–183] are 11 to 12 kb in length and comprise seven exons. The class mu genes isolated from rats,[184,185] mice,[186] and humans[187,188] are all about 5 kb and are composed of eight exons; by contrast, a hamster mu class GST comprises nine exons.[189] Class pi GST genes from rats,[190] mice[191,192] and humans[193,194] are about 3 kb and contain seven exons. A rat class theta gene has been cloned, which is 4 kb in length and contains five exons.[195] In humans, the class alpha, mu, pi, and theta GST genes are located on chromosomes 6, 1, 11, and 22, respectively.[196–200]

In the rat, only one functional class pi GST gene appears to exist.[190] Although humans possess a single functional class pi GST gene encoded on chromosome 11,[199] recent data suggest that allelic variation may occur at this locus.[201] By contrast, rat and human alpha, mu, and theta families contain multiple genes.[174,175] The number of class sigma GST genes in rats and humans remains to be established. The class alpha GST all share at least 55% identity, whereas the class mu GST share at least 65% identity and the theta class GST about 50% identity (a mitochondrial GST is described below that has been classed as a theta enzyme but shares only about 30% identity with other enzymes in this family). The rat, mouse, and human class alpha GST contain three subfamilies encoding distinct subunit types, whereas the class mu and theta both contain two subfamilies encoding different subunit types. Comparison among the five classes reveals that alpha, mu, pi, and sigma are more closely related to each other than to theta; specifically, the class alpha, mu, pi, and sigma GST all share at least 20% identity but possess only 5 to 15% identity with theta. One of the class theta GST possesses significant amino acid homology with a dichloromethane-dehalogenase enzyme from the prokaryote *Methylobacterium.*[137] On the basis of these sequence comparisons, it has been proposed that the ancestral progenitor gene for mammalian GST was possibly a class theta GST.[202] These sequence data have also suggested that the class sigma GST diverged from the progenitor GST first. Such sequence compari-

sons also suggest that class mu GST diverged before the class alpha and pi GST.

It is clear that the sigma and theta classes of GST are abundant in nonvertebrate species.[174,203] Molecular characterization of GST in nonvertebrates represents a rapidly expanding research area and doubtless a significant number of additional families will be identified in the future. The first evidence for a separate class of sigma GST was provided by Buetler and Eaton[174] from sequence alignments of the S-crystallins from mollusc lens. Although it is uncertain whether all these refractory proteins in the lens of the cephalopod eye are catalytically active, the sigma class SL11 crystallin does exhibit substantial activity toward CDNB.[204] Other class sigma GST have been identified in *Schistosoma mansoni*, *Onchocerca volvulus*, and *Ascaris suum*.[177] A class sigma GST from the digestive gland of the squid *Loligo vulgaris* has been purified, cloned,[148] and its X-ray crystal structure determined.[204]

Many of the GST in insects and in plants are only very distantly related to the mammalian class alpha, mu, pi, sigma, and theta enzymes. The GST in *Drosophila melanogaster* have been studied extensively by Tu and his co-workers.[159] These workers have defined a separate GST family in *Drosophila*, called D-class enzymes, which may be encoded by eight intronless genes. One of these GST from *Drosophila*, GST D1, may be related to plant GST because it shares 66% identity with maize GST III over a 40 amino acid region.[156] In plants it is apparent that at least two GST families exist. Cloning of cDNAs encoding maize GST I, II, III, and IV shows that they share 54% to 68% similarity but only 19% identity with carnation SR8[162] and 11% identity with tobacco NT103.[163]

A separate class of cytosolic GST may also be formed by the enzymes from *Escherichia coli* K-12[142] and *Proteus mirabilis*,[140] which share 54% identity.

B. Genes Encoding Membrane-Bound GST

In addition to the cytosolic GST, at least two membrane-bound GST exist in mammals. These are referred to as microsomal GST[205] and leukotriene C_4 synthase (LTC_4S).[60] The microsomal GST is involved in the detoxification of xenobiotics,whereas LTC_4S, as its name suggests, conjugates leukotriene A_4 with GSH; as far as is currently known, LTC_4S does not play a role in drug metabolism. Neither microsomal GST nor LTC_4S shares sequence identity[206–208] with the cytosolic enzymes and, as both of the membrane-bound enzymes lack obvious homology, it is assumed that they have each evolved separately. The gene for microsomal GST is located on human chromosome 12,[209] but the chromosomal location of the LTC_4S gene has not yet been reported. It appears unlikely that the microsomal GST is a member of a multigene family, as molecular cloning and protein purification have failed to reveal the existence of related proteins in any of the species examined. It is therefore concluded that the microsomal GST is encoded by a single, or very-low-copy, gene in all species. From examination of Southern blots, DeJong et al.[206,209] have suggested that the microsomal GST gene contains at least three exons and spans less than 12 kb. By contrast, molecular cloning of LTC_4S has revealed that this enzyme does possess homology (31% identity, 53% similarity) with 5-lipoxygenase-activating protein (FLAP), indicating that both proteins are members of the same multigene family.[207,208]

The microsomal GST is present in humans, rodents, and cows. Morgenstern

et al.[210] noted high levels of CDNB-GSH-conjugating activity in the microsomal fraction from livers of chickens, toads, and pike but they did not observe any immuno-cross-reactivity with antibody raised against the rat microsomal GST. Further work is required to determine how widely distributed the gene for microsomal GST is in nature. To date, the species distribution of LTC_4S has not been reported.

IV. GST ISOENZYMES

A. Purification of Cytosolic GST

Since the first reports in 1961 of GST activity in rat liver, the cytosolic GST have been the subject of extensive study.[211,212] The characterization of cytosolic GST has been greatly facilitated by the availability of affinity chromatography gels to which these enzymes bind. The multifunctional nature of GST has allowed a variety of affinity gels to be designed that can be used to isolate GST. These include agarose containing immobilized bromosulfophthalein, cholic acid, glutathione, *S*-hexylglutathione, *S*-octylglutathione, thyroxine, and triazine dye.[213] Among these affinity gels two matrices in particular, glutathione-agarose[214] and *S*-hexylglutathione-agarose,[215] have been widely used to purify class alpha, mu, pi, and sigma GST as they display both excellent specificity and yield of these enzymes. Class mu, pi, and sigma GST are adsorbed efficiently by both glutathione-agarose and *S*-hexylglutathione-agarose. By contrast, the class alpha GST do not display strong affinity for *S*-hexylglutathione-agarose but most isoenzymes of this class are efficiently adsorbed by glutathione-agarose;[216] at least one murine class alpha GST binds neither of these affinity gels, but can be isolated by affinity chromatography on bromosulfophthalein-agarose.[217] The class theta GST have proven to be more difficult to purify than the other classes of GST because they are labile and are the least abundant family. Many of the class theta GST are retained by neither glutathione-agarose nor *S*-hexylglutathione-agarose, but can be purified by affinity chromatography on the triazinyl dye gels, Orange A matrix and Blue Sepharose.[176,218,219] The reason for the failure of currently used glutathione-affinity chromatography matrices to purify theta class GST may be because this class possesses a much deeper active site than the alpha, mu and pi classes.[55] Therefore, GSH immobilized to an agarose support via a longer spacer arm may provide an effective affinity gel for class theta GST.[220] Following affinity purification of GST, the individual isoenzymes are normally resolved by exploiting differences in their charge using either ion-exchange chromatography, chromatofocusing, or isoelectric focusing.[56,58] Alternatively, adsorption chromatography on hydroxyapatite can be a highly effective purification step should ion-exchange chromatography not provide homogeneous protein.[221]

A potentially useful feature of the glutathione-agarose and the *S*-hexylglutathione-agarose gels that has not been exploited is that gradient elution allows resolution of different GST isoenzymes. The elution order of GST isoenzymes from *S*-hexylglutathione-agarose is dependent on the K_m value for GSH (i.e., the lower the K_m value of the enzyme the higher the concentration of *S*-hexylglutathione required to elute it from the gel).[222] Gradient elution of GST from glutathione-agarose also allows resolution of a large number of isoenzymes, but the concentration of GSH required to elute the various enzymes does not correlate with any single kinetic property.[213] The selective desorption of GST from affinity resins is

another approach that can yield highly purified protein and the elution of a single GST isoenzyme from glutathione-agarose using GSSG as a counter ligand has been described.[223]

An apparently homogeneous soluble 34-kDa protein that displays both carbonyl reductase and GST activity has been purified from rat ovary by glutathione-agarose chromatography.[224] This enzyme exhibits only low amounts of activity toward CDNB (0.16 μmol/min/mg) and its *N*-terminus shares sequence homology to an internal region of carbonyl reductase, suggesting that the hypothesis that this protein represents a new class of GST should be treated with caution. As this glutathione-binding protein is larger than most cytosolic GST, the possibility exists that a novel GST has arisen through a domain of carbonyl reductase becoming fused to class alpha, mu, pi, sigma, or theta transferase. Alternatively, glutathione-agarose chromatography may have resulted in the copurification of GST and carbonyl reductase; presumably, in this case, cleavage of carbonyl reductase would have had to occur prior to amino acid sequencing.

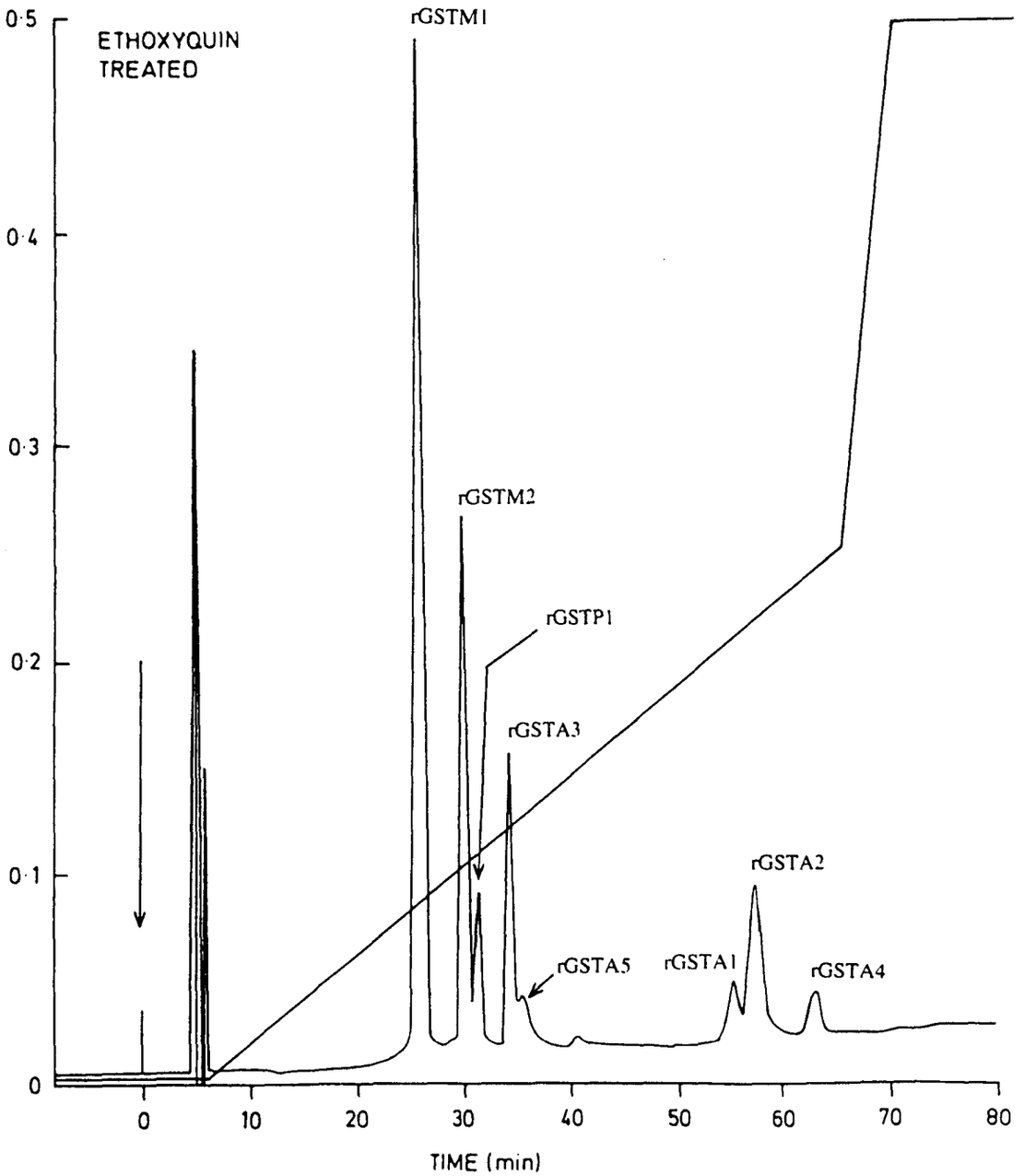
As a note of caution, several other proteins, including glyoxalase I,[222,225] Δ^3,Δ^2-enoyl-CoA isomerase,[226] MIF[119] are also eluted along with GST from the *S*-hexylglutathione-agarose column. These proteins can all be readily distinguished by sodium dodecyl sulfate/polyacrylamide-gel electrophoresis (SDS/PAGE).[227] Neither glyoxalase I, Δ^3,Δ^2-enoyl-CoA isomerase, nor MIF has been reported to bind to glutathione-agarose.

B. Identification of Cytosolic GST Subunits

The cytosolic GST in all vertebrate species comprise two subunits and exist as either homodimers or heterodimers. From an experimental point of view, the existence of multiple cytosolic GST has necessitated the use of high-specificity or high-resolution analytical techniques, or both, to allow identification of isoenzymes and the subunits they comprise. The analytical methods that have proved valuable in this context include SDS/PAGE,[227] isoelectric focusing,[42,228–230] reversed-phase high-pressure liquid chromatography (HPLC),[231] electrospray mass spectrometry,[232] Western blotting,[233] and immunoassay with either polyclonal antibody against purified GST, specific GST peptides, or monoclonal antibodies.[234-238] The GST subunits from rats have been most commonly identified by SDS/PAGE as they are readily resolved by this method. GST subunits from other rodent species and from humans can also be resolved by SDS/PAGE, but the relative mobility of certain subunits is dependent on the amount of cross-linker in the polyacrylamide gel.[227] More recently, reversed-phase HPLC has been increasingly employed to identify GST subunits (Figure 9): satisfactory resolution of GST subunits can be obtained using a μ-Bondapak C_{18} column developed with a 40 to 60% gradient of acetonitrile in 0.1% trifluoroacetic acid. However, for best results, particle size (less than 10 μm) and flow rate are critical.[239,240] This technique allows resolution of most of the major rat GST subunits. Using published extinction coefficients, reversed-phase HPLC can be used to quantitate GST. Furthermore, the method can be used preparatively to obtain purified GST subunits for amino acid sequencing or, following renaturation, for enzyme assay.[241]

C. Cytosolic GST Subunit Structure and Residues Responsible for Subunit Dimerization

A knowledge of the subunit composition of cytosolic GST is a prerequisite to



A

FIGURE 9. Resolution of rat GST subunits by reversed-phase HPLC. Hepatic GST were purified from rats that had been fed on a diet containing 0.5% ethoxyquin. (a) Absorbance profile at 220 nm obtained following elution of a μ-Bondapak C_{18} column with a linear 40 to 55% acetonitrile gradient. (b) SDS/PAGE analysis of the individual peaks obtained from the HPLC column. See Table 1 and Section IV.D.1 for a discussion of GST nomenclature.

understanding the catalytic properties of individual isoenzymes, because each subunit in the dimeric protein functions independently.[242,243] Cytosolic GST subunits contain a G-site, (or GSH-binding site), and an H-site, the second substrate-binding site.

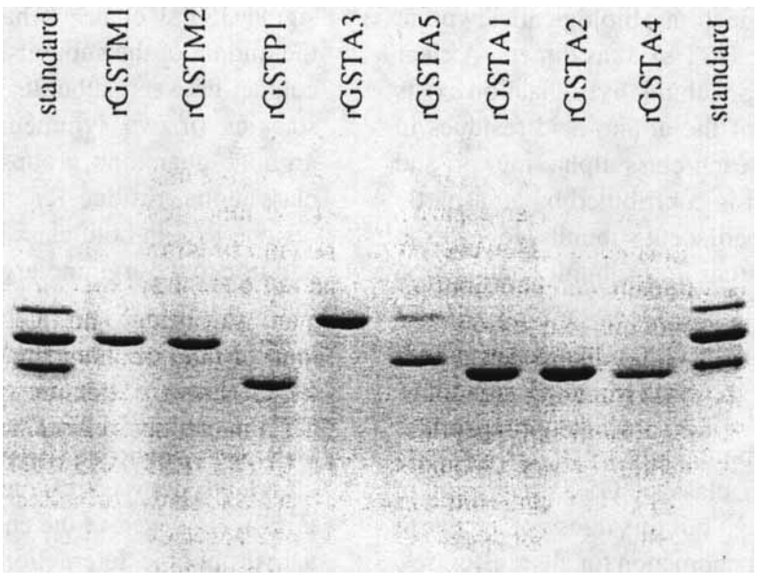


FIGURE 9B

Elucidation of the three-dimensional structure of the class alpha, mu, pi, sigma, and theta GST has demonstrated that each subunit comprises two domains: the smaller *N*-terminal α/β domain, domain I (comprising residues 1 to 78 of class alpha GST, residues 1 to 82 of class mu GST, residues 1 to 74 of class pi GST, residues 1 to 74 of class sigma GST, residues 1 to 78 of class theta GST), contains most of the amino acids that form the G-site; the larger α domain, domain II (residues 86 to 222 of class alpha GST, residues 90 to 217 of class mu GST, residues 81 to 207 of class pi GST, residues 81 to 202 of class sigma GST, residues 85 to 208 of class theta GST), contains most of the amino acids that form the H-site.[46,51–55,204]

Within the G-site the formation of the GS^- thiolate anion by class alpha, mu, pi, and sigma GST is facilitated primarily by a conserved tyrosine (Y9 of class alpha,[244,245] Y6 of class mu,[53] Y7 of class pi,[246] Y7 in class sigma[204]), whereas this role is performed by a serine residue (S9) in class theta GST.[55,247] A conserved G-site aspartate (D101 in class alpha, D105 in class mu, D98 in human class pi, D96 in class sigma) is also involved in catalysis by aiding proton release from certain transition-state conjugates, such as occurs during conjugation between CDNB and GSH.[246,248]

The substrate specificity of GST isoenzymes can be determined, at least in certain instances, by a relatively small number of residues in the H-site. Armstrong and coworkers[46] showed that different residues within the H-site of class mu GST are important for determining whether the enzyme is active primarily toward epoxides, halogenated benzenes, or α,β-unsaturated carbonyl-containing compounds. In class alpha GST, X-ray crystallography,[54] mutagenesis,[249–251] and photoaffinity labeling[252] have indicated that the *C*-terminal portion of the protein may be of particular importance in determining substrate specificity. Selection *in vivo* for loss of function of class alpha GST has shown that E32, E97, and G98 are essential for activity.[253]

It is unclear from a biological viewpoint why cytosolic GST exist as dimers. A clear requirement for subunit hybridization exists because one of the amino acid residues in the G-site of each class alpha, mu, pi, and sigma subunit is contributed by an aspartic acid from the adjacent subunit. However, it is not certain that intersubunit contribution to the G-site represents the sole reason for dimerization as it seems likely that a perfectly satisfactory GSH-binding site could have evolved from a single polypeptide. Structural communication between subunits of the human class pi GST can result in cooperativity,[254] but this does not appear to be a general phenomenon for all transferases. Other reasons why cytosolic GST exist as dimeric proteins might have nothing to do with catalysis, but may concern the putative sequestration activity of GST. The association between two subunits (homodimer or heterodimer) may generate an intrasubunit binding site for large bulky ligands that would not be able to bind to a monomeric GST, or the formation of heterodimers might be advantageous because it could enable a glutathione *S*-conjugate formed by one subunit to be sequestered by the adjacent subunit, thereby limiting product inhibition. Subunit dimerization might also help shield the H-site from large hydrophobic macromolecules, possibly preventing adsorption of the enzyme onto membrane lipid. The dimeric structure of cytosolic GST, with the increase in size of the complex, may be important in limiting diffusion of GST across membranes.

Analysis of the crystal structure of GST has allowed study of the dimer interface and identification of the residues involved in dimerization. Both hydrophilic and hydrophobic interactions are involved in the dimerization of the class alpha, mu, and pi GST subunits, whereas only hydrophobic interactions are involved in dimerization of class sigma GST. Ji et al.[52,204] have shown that, in the middle of the subunit-subunit interface, contact between subunits is created by the stacking of two symmetrically equivalent arginine guanidino groups (residue R69 in class alpha, residue R77 in class mu, and residue R68 in both class pi and sigma). In addition to the arginine-arginine interaction, many salt bridges and hydrogen bonds are found in the middle of the dimer interface for all known structures (for alpha class GST, interactions exist among residues R69, N73, Y82, R89, N93, E97, R155; for mu class GST, between residues Q71, R77, D97, E100). However, at the end of the interface a hydrophobic interaction occurs through the wedging of a phenylalanine from one monomer (F52 in alpha; F56 in mu; F47 in pi) into a hydrophobic pocket on the other side of the interface, formed by the side-chains of five residues belonging to helices $\alpha 4$ and $\alpha 5$ (alpha, M94, G98, A135, F136, V139; mu, I98, Q102, L136, Y137, F140; pi, M89, G93, P126, F127, L130). The interaction between phenylalanine in one subunit with the hydrophobic pocket in the other has been called a "lock-and-key" binding mechanism.[204]

In the class theta GST from insects, subunit interactions involve hydrogen bonding (Q49 with T103, W63 with Q95 and Y71 with K88), a salt bridge (E74 with R90), and stacking of the aromatic rings of Y98.[55]

D. Rat Cytosolic GST Isoenzymes

1. Nomenclatures

Many GST isoenzymes have been isolated from the cytosol fraction of various rat tissues and evidence suggests that the transferases in the rat are encoded by as many as

20 genes (Table 1). Class alpha, mu, pi, sigma, and theta GST are represented in the rat. Among these, the rat alpha class appears to be the most complex, containing possibly seven or eight genes, while it is estimated that the mu, pi, and theta families are composed of at least six, one, and three genes, respectively (the number of rat class sigma genes is unknown).

From a historical perspective, the use of SDS/PAGE has had a major impact on the characterization of GST isoenzymes in the rat. Originally, during SDS/PAGE of rat hepatic cytosol enriched for GST (referred to as a "Y" fraction or a "ligandin-containing" fraction), Bass et al.[256] resolved three electrophoretic bands that were designated Ya, Yb, and Yc according to their decreasing anodal mobility. It was subsequently found by others,[297-299] that the Ya and Yc bands represent class alpha GST, whereas the Yb band represents class mu GST.

Several nomenclatures have been proposed for rat GST subunits over the years; some of the most widely used are shown in Table 1. The Arabic numeral nomenclature for GST, devised by Jakoby et al.,[257] is of value because it is unambiguous and allows subunit combinations to be simply displayed, but suffers from the disadvantage that it is not immediately obvious to which gene family each subunit belongs. By contrast, the Y nomenclature is helpful because it groups GST by subfamily and allows immediate identification of subunits that will dimerize. Although the Y nomenclature has much in its favor, it is somewhat cumbersome and does not readily allow interspecies comparisons. A class-based subunit nomenclature has been proposed that groups subunits by gene family and numbers them according to their order of discovery; this system for defining GST was originally devised for the human transferases,[255] but it is generally applicable. In this nomenclature, single capital letter abbreviations are used to signify the alpha (A), the mu (M), the pi (P), the sigma (S), and the theta (T) classes, and Arabic numerals are employed for numbering each of the separate gene products; for example, class alpha subunits are called A1, A2, A3, etc. The dimeric GST isoenzymes are represented by the single letter suffix (signifying class) followed by hyphenated Arabic numerals (signifying each of the two subunits). Hence, the class alpha heterodimer formed between Ya_1 (A1) and Yc_1 (A3), GST Ya_1Yc_1, is designated GSTA1-3. GST are officially only admitted to the class-based subunit nomenclature once their primary structures have been determined, although it is common practice to give a preliminary class-number, designated by an asterisk, to a "new" subunit once there is sufficient evidence to indicate that the novel subunit is genetically distinct from previously described polypeptides (e.g., S1*). Also, as this system of nomenclature can be applied to describe the enzymes in other species, a single lower case prefix should be used when ambiguity might occur to indicate the origin of the enzyme; h, m, r signify human, mouse, and rat, respectively. Using this system the enzyme rGSTA1-3, originally called rat transferase B,[273] is the heterodimer formed between the rat A1 and A3 subunits.

The ability of different subunits to form heterodimers has not been studied systematically. Not all GST subunits that are included in the alpha class appear capable of hybridizing with other class alpha GST subunits, nor are all the class mu GST subunits able to hybridize with other class mu subunits. Specifically, among class alpha subunits rGSTA1, A2, A3 and A5 form heterodimers with each other,[260,270] whereas rGSTA4 is only found as a homodimer.[272] Among class mu subunits rGSTM1, M2, M3, M4 and M5* form heterodimers, but

TABLE 1
Rat GST Subunits

Class	Class-based[a] subunit nomenclature	"Y" SDS/PAGE [b] subunit terminology	Number[c] of subunit	Original[d] name of enzyme (homodimer)	cDNA clone	Chromosome localization	Ref.
Alpha	rGSTA1	Ya_1	1a	Ligandin	pGTR261	—	255–260
Alpha	rGSTA2	Ya_2	1b	Ligandin	pGTB38	8	260–262
Alpha	rGSTA3	Yc_1	2	GST AA	pGTB42	9	262–264
Alpha	rGSTA4	Yk (Y_α)	8	GST K	λGTRA8, X62660	—	216, 265, 266
Alpha	rGSTA5	Yc_2 (Y_{fetus}, Yx)	10	—	X78847	—	267–270
Alpha	n.i.	Yl* (Ya_3)	—	—	Not cloned	—	222
? Alpha	n.i.	Ys*	—	—	Not cloned	—	271
? Alpha	n.i.	—	—	GST A(6)*	Not cloned	—	272
Mu	rGSTM1	Yb_1	3	GST A	pGTA/C44, pGTR200	2	273–278
Mu	rGSTM2	Yb_2	4	GST D	pGTR187, J02592	2	278–281
Mu	rGSTM3	Yb_3 (Yn_1, Y_β)	6	—	J02744	—	282–284
Mu	rGSTM4*	Yb_4*	—	—	λGTR15-2 (genomic clone)	—	184
Mu	rGSTM5*	Yn_2*	9	—	Not cloned	—	285
Mu	rGSTM6*	Yo*	11	—	Not cloned	—	222, 286
Pi	rGSTP1	Yf (Yp, Y_δ)	7	GST P	pGP5	—	282, 283, 287–290
Sigma	rGSTS1*	—	—	PGDS*	Not cloned	—	62, 177
Theta	rGSTT1	—	5	GST E	X67654	—	176, 202, 291
Theta	rGSTT2	Yrs (also Yrs')	12	GST M	pYrs (originally Theta-1)	—	176, 218, 292–295
Theta	rGSTT3*	—	13	Mitochondrial GST	Unpublished	—	241, 296
membrane-bound GST		—	—	Microsomal GST	λrMGST1, J03746	—	205, 206

Note: Subunits whose cDNAs have not been cloned are designated by an asterisk (*). An entry of n.i., not included, indicates that a firm designation cannot be made because of either lack of certainty about the class or lack of proof that the subunit is genetically distinct. The form designated Yrs' represents a posttranslational modified variant.[295]

[a] The class-based subunit nomenclature for rat GST is based on the proposal of Mannervik et al.[255] which was originally devised for human GST.
[b] The "Y"-based nomenclature is based on the work of Bass et al.[256]
[c] The numbering of GST subunits is based on the proposed nomenclature of Jakoby et al.[257]
[d] Abbreviation: PGDS, prostaglandin D synthetase (GSH-dependent isoenzyme).

the rGSTM6* subunit is only found as a homodimer.[286] One class theta GST heterodimer has been reported in the rat, formed between two different T2-type subunits[294] that represent posttranslationally modified forms of a single gene product.[295] Through subunit hybridization, more than 15 class alpha, 15 class mu, and 5 class theta GST isoenzymes are formed in the rat (Table 2).

2. Model Substrates

The GST isoenzymes display marked differences in their abilities to conjugate GSH with various electrophiles. The model GST substrates that display selectivity for particular subunits are often used in a "diagnostic" sense to identify isoenzymes (Figure 10). Compounds that are used for this purpose are as follows: Δ^5 androstene-3,17-dione, selective for rGSTA1 and/or A2 subunits; 4-hydroxynonenal, selective for rGSTA4; 1,2-dichloro-4-nitrobenzene (DCNB), selective for rGSTM1; *trans*-4-phenyl-3-buten-2-one (tPBO), selective for rGSTM2; 1,2-epoxy-3-(*p*-nitrophenoxy) propane (EPNP); selective for rGSTT1; and 1-menaphthyl sulfate, selective for rGSTT2. It is apparent that, in the case of class alpha and mu GST polypeptides, which can form heterodimers, a subunit dose effect is observed in the specific activities of GST for particular highly selective substrates. For example, Table 3 shows that rGSTM1-1 has twice the activity of rGSTM1-2 toward DCNB and rGSTM2-2 has twice the activity of rGSTM1-2 and rGSTM2-3 for tPBO.

GST isoenzymes can demonstrate remarkable stereospecificity. It has, for example, been observed that rat GST containing the A5 subunit have high activity for

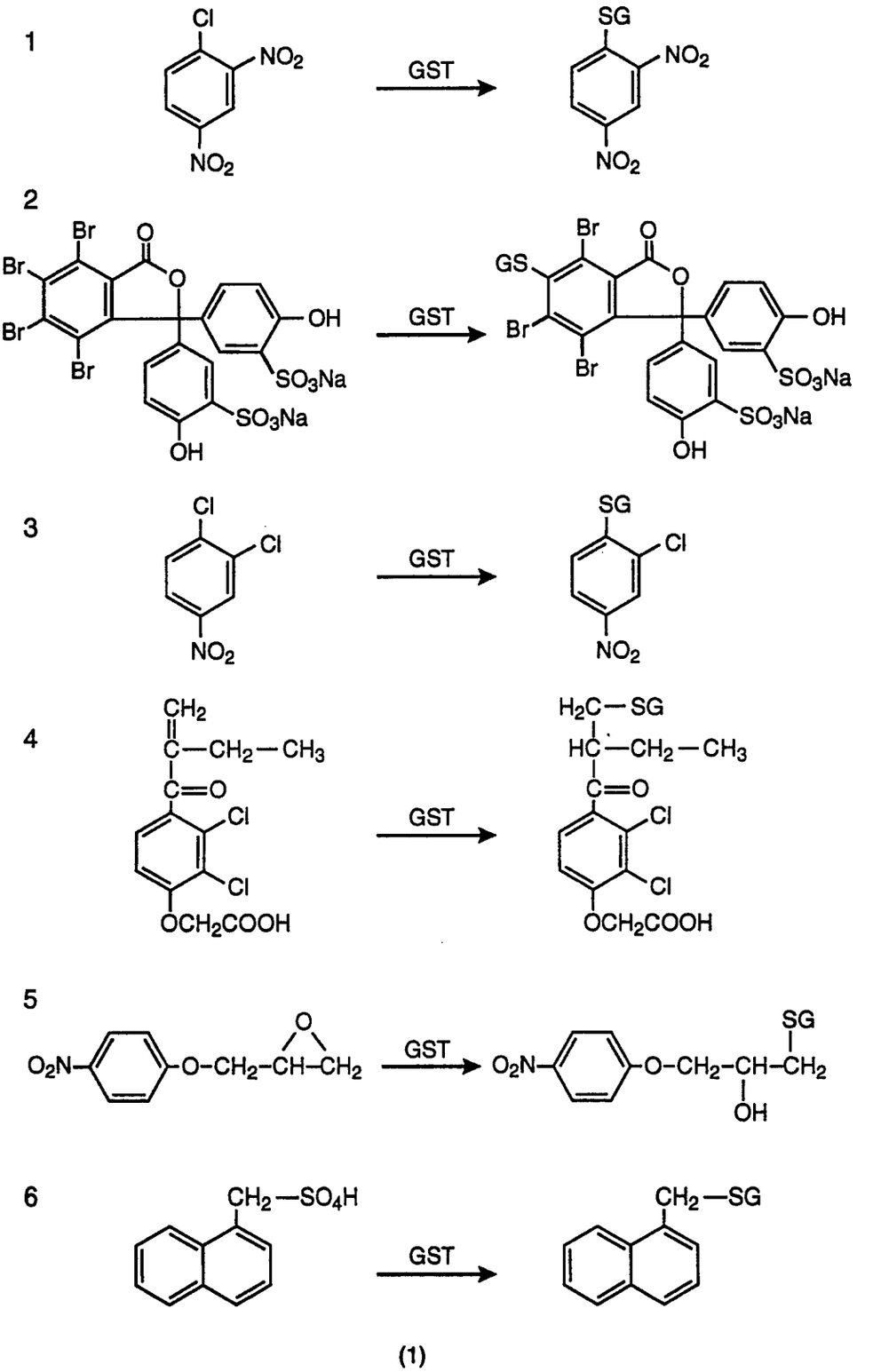
TABLE 2
Rat GST Isoenzymes and Their Tissues of Origin

Class	Organ[a]	Quaternary structure of GST isoenzymes[b]
Alpha	Liver, male control	rGSTA1-2 [Ya_1Ya_2], rGSTA1-3 [Ya_1Yc_1], rGSTA2-3 [Ya_2Yc_1], rGSTA3-3 [Yc_1Yc_1], rGSTA4-4 [YkYk], GST A(6)
Alpha	Liver, male EQ	rGSTA1-5 [Ya_1Yc_2], rGSTA2-5 [Ya_2Yc_2], rGSTA3-5 [Yc_1Yc_2]
Alpha	Liver, male PB	rGSTA2-2 [Ya_2Ya_2]
Alpha	Kidney	Ya_1Yl, Yc_1Yl
Alpha/sigma	Spleen	YsYs, rGSTS1*-1* [PGDS]
Mu	Liver, male control	rGSTM1-1 [Yb_1Yb_1], rGSTM1-2 [Yb_1Yb_2], rGSTM2-2 [Yb_2Yb_2]
Mu	Testis	rGSTM1-3 [Yb_1Yb_3], rGSTM2-3 [Yb_2Yb_3], rGSTM3-5* [Yb_3Yn_2], rGSTM6*-6* [YoYo], rGSTM4-4 [Yb_4Yb_4]
Mu	Brain	rGSTM3-3 [Yb_3Yb_3]
Pi	Kidney	rGSTP1-1 [YfYf]
Theta	Liver, male control	rGSTT1-1 [GST 5-5], rGSTT2-2 [YrsYrs], rGSTT2-2′ [YrsYrs′], rGSTT2′-2′ [Yrs′Yrs′], rGSTT3-3 [GST 13-13]
Microsomal	Liver	Trimeric enzyme

Note: The quaternary structure of rat GST isoenzymes is indicated where known; the subunit composition of GST A(6) has not been determined. Square brackets contain isoenzyme designations based on the "Y" subunit numbering nomenclature. As the cDNAs encoding GSTS1*, GSTA(6), Yl, Yo, Ys, and subunit 13 (T3) have not been isolated, their inclusion in the table is preliminary, but has been made for completeness.

[a] GST isolated from organs of male rats fed control diet, ethoxyquin-containing diet (EQ), or administered phenobarbital (PB).

[b] GSTM4-4, identified as a genomic clone by Lai et al.,[184] is expressed in rat testis. However, it is not yet clear how many mu class subunits it can hybridize with. The data are from References 62, 177, 205, 222, 241, 271, 272, 282, 294, 300–302.



(1)

FIGURE 10. Model substrates used for analyses of GST: (1) CDNB; (2) bromosulfophthalein; (3) DCNB; (4) ethacrynic acid; (5) EPNP; (6) 1-menaphthyl sulfate; (7) 4-NBC; (8) 4-nitrophenylacetate; (9) 4-nitrophenylbromide; (10) *trans*-4-phenyl-3-buten-2-one; (11) styrene-7,8-oxide; (12) cumene hydroperoxide.

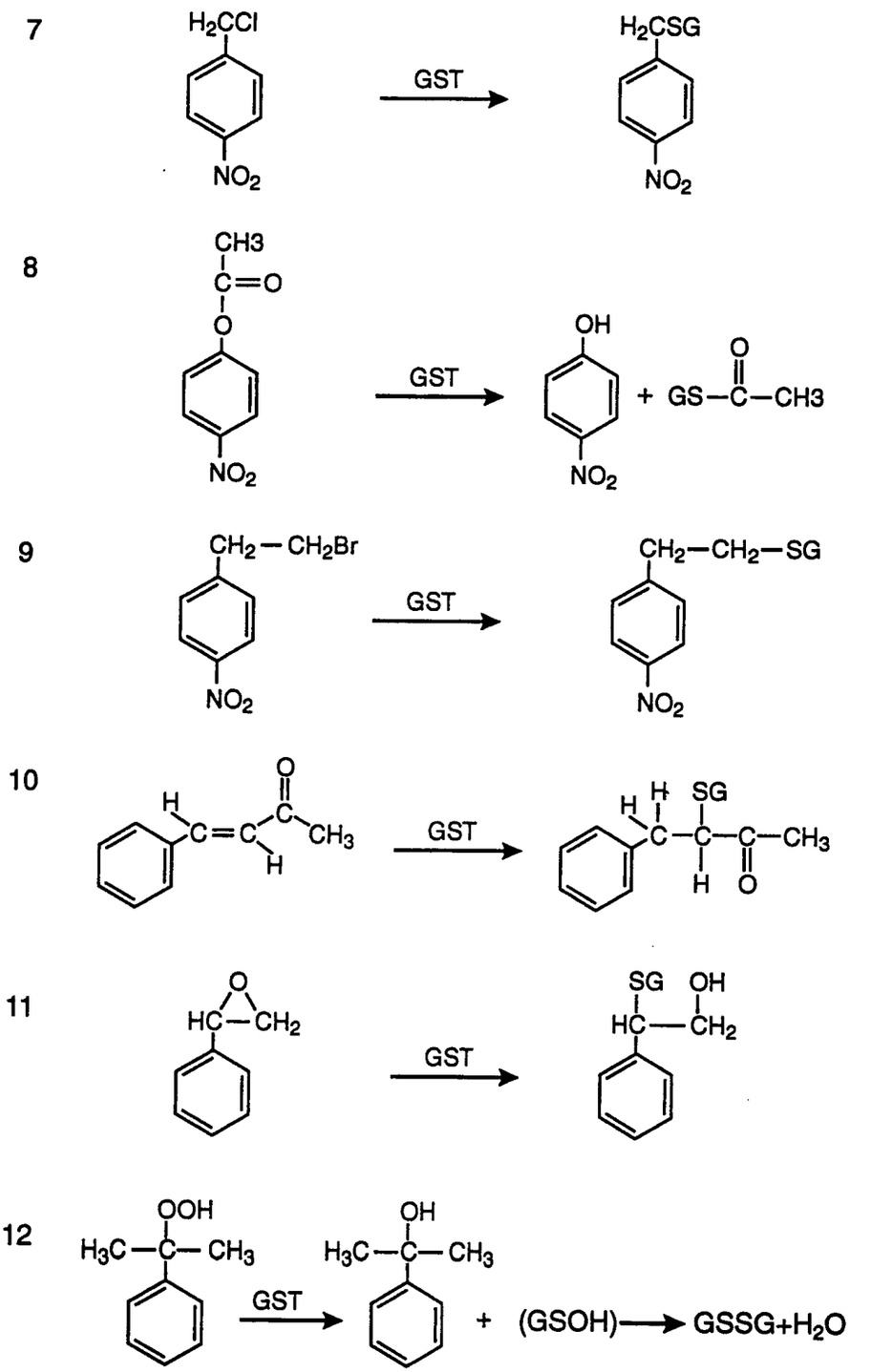


FIGURE 10(2)

TABLE 3
Catalytic Activities of Rat GST Isoenzymes

Isoenzyme	Specific activity (μmol/min per mg of protein)														
	CDNB	Δ^5AD	BSP	DCNB	EA	EPNP	4-HNE	MS	4-NBC	4-NPA	4-NPB	tPBO	SO	CuOOH	LiOOH
rGSTA1-2 [Ya_1Ya_2]	50.0	2.3	0	0	0.1	<0.1	2.6	—	1.1	0.8	—	0	3.3	3.4	3.0
rGSTA1-3 and A2-3 [$Ya_{1+2}Yc_1$]	25.0	0.9	0	0	0.5	<0.1	1.6	—	—	0.3	—	0	—	8.9	—
rGSTA3-3 [Yc_1Yc_1]	18.0	<0.01	0	0	1.2	<0.1	0.7	—	0.3	0.2	—	0	3.3	13.7	1.6
rGSTA3-5 [Yc_1Yc_2]	14.0	<0.01	—	0	0.8	—	0.4	—	—	—	—	0.005	—	15.5	—
rGSTA4-4 [YkYk]	10.0	0.06	0	0.1	7.0	—	170.0	—	1.1	0.1	—	0.1	—	1.1	0.2
YsYs	3.0	—	0	0	0.9	0.3	0.1	0	—	0	0	0	—	0.5	—
GST A(6)	10.0	—	—	0.3	2.5	—	320.0	—	—	—	—	1.0	—	0.8	—
rGSTM1-1 [Yb_1Yb_1]	58.0	0.02	0.8	5.3	0.1	0.5	2.7	—	14.0	1.0	—	0.1	133.0	0.4	0.2
rGSTM1-2 [Yb_1Yb_2]	45.0	0.01	0.3	3.2	0.3	0.9	2.9	—	—	0.6	—	0.6	—	0.5	—
rGSTM2-2 [Yb_2Yb_2]	17.0	0.002	0.04	0.2	0.6	1.4	6.9	—	14.0	0.3	—	1.2	104.0	0.7	0.2
rGSTM1-3 [Yb_1Yb_3]	64.0	—	0.7	4.9	0.5	<0.1	—	—	—	0.6	—	0.2	—	<0.2	—
rGSTM2-3 [Yb_2Yb_3]	45.0	—	—	2.3	0.6	—	0	—	—	—	—	0.6	—	0.45	—
rGSTM3-3 [Yb_3Yb_3]	190.0	—	—	2.9	0.06	—	—	—	—	0.2	—	0.02	—	0.19	—
rGSTM3-5* [Yb_3Yb_5]	170.0	—	0	2.4	0	0	—	—	1.7	0.05	—	0.2	—	0.04	0.06
rGSTM6*-6* [YoYo]	30.0	—	—	0.3	0	0.1	—	—	0.5	—	—	—	—	—	—
rGSTP1-1 [YfYf]	19.0	—	0.02	0.2	4.2	0.1	1.6	—	—	—	—	0.05	55.0	0.06	1.5
rGSTS1*-1* [PGDS]	70.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
rGSTT1-1 [5-5]	<0.5	—	—	—	—	180.0	—	—	86.0	—	65.0	—	—	41.0	—
rGSTT2-2 [YrsYrs]	<0.1	—	—	0	0.4	<0.1	—	0.4	0	0	—	0.02	—	2.0	9.7
rGSTT2-2′ [YrsYrs′]	<0.1	—	—	0	1.6	<0.1	—	0.4	0	0	—	—	—	2.0	—
rGSTT2′-2′ [Yrs′Yrs′]	<0.1	—	—	0	2.5	<0.1	—	0.4	0	0	—	—	—	2.0	0.9
rGSTT3-3 [13-13]	82.0	—	—	0	26.0	0	0	0	0	—	—	0	—	0	—
microsomal GST	30.0	0.07	<0.01	0.06	<0.01	<0.01	0.7	—	0.1	0.9	—	0.001	—	0.8	0.6

Note: Horizontal dashes are included to signify substrates that have not been tested with a particular isoenzyme. The GST-catalyzed reactions are all depicted in Figures 3, 8, and 10. Data for cytosolic GST are from References 62, 176, 216, 241, 271, 272, 294, 301, 303–305. The data for microsomal GST are from the *N*-ethylmaleimide-activated enzyme.[306,307] PGDS, prostaglandin D synthetase (GSH-dependent isoenzyme); CDNB, 1-chloro-2,4-dinitrobenzene; Δ^5AD, Δ^5 androstene-3,17-dione; BSP, bromosulfophthalein; DCNB, 1,2-dichloro-4-nitrobenzene; EA, ethacrynic acid; EPNP, 1,2-epoxy-3-(*p*-nitrophenoxy)propane; 4-HNE, 4-hydroxynonenal; MS, 1-menaphthyl sulfate; 4-NBC, 4-nitrobenzylchloride; 4-NPA, 4-nitrophenylacetate; 4-NPB, 4-nitrophenylbromide; tPBO, *trans*-4-phenyl-3-buten-2-one; SO, styrene-7,8-oxide; CuOOH, cumene hydroperoxide; LiOOH, linoleate hydroperoxide.

aflatoxin B_1 *exo*-8,9-epoxide, but essentially no activity toward aflatoxin B_1 *endo*-8,9-epoxide. Conversely, rGSTM2-2 exhibits about 10-fold greater activity toward aflatoxin B_1 *endo*-8,9-epoxide than the *exo*-8,9-epoxide.[308] Stereospecificity has been observed with other epoxides besides those of aflatoxin. All rat GST display a 97% selectivity for (+)-7β,8α-dihydroxy-9α,10α-oxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene rather than (–)-7β,8α-dihydroxy-9α,10α-oxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene.[309]

3. Structure and Function of Rat Isoenzymes

Examination of the primary structure of class alpha GST from the rat shows that they comprise three subfamilies (Figure 11). The suggestion that rGSTA1 and A2 represent a separate subfamily from rGSTA3 and A5 is supported by the fact that the genes for these two groups are located on different chromosomes.[262] Sequence alignment of the class alpha GST, which can form heterodimers with each other, shows that the nine residues involved in subunit dimerization are all conserved; a single conservative arginine to lysine substitution is found in rGSTA5. However, a comparison between rGSTA4 with the other class alpha GST subunits reveals that four of the nine residues involved in dimerization differ. Of these, the N73S and R155W changes may be critical in preventing rGSTA4 from dimerizing with other class alpha GST subunits. It is not known which residues in class alpha transferases are responsible for the marked differences in their activities. X-ray crystallography of hGSTA1-1[54] has indicated that the H-site is composed of residues 107 to 111 and residues 208 to 222. It is clear from Figure 11 that the H-site residues in the various rat class alpha GST are hypervariable, but it remains to be established what structural features in this enzyme class are responsible for determining the specificity of subunits for particular electrophiles. It has been suggested that Y108 and D208 in rGSTA5 might be responsible for the high activity of this subunit for aflatoxin B_1 *exo*-8,9-epoxide.[270] In this context, an H-site tyrosine has also been implicated in the activity of certain class mu GST toward phenanthrene-9,10-oxide, where it acts as an electrophile to stabilize the transition state for the addition of GSH to epoxides.[310] The role of M208 in the catalysis of hGSTA1-1 has been investigated.[251] Although it was shown that mutation of this residue influenced activity toward CDNB, aflatoxin B_1 was not studied. Replacement of methionine with the charged residue glutamate (M208E) decreased catalysis and increased K_m values for CDNB and 4-nitrobenzyl chloride (4-NBC), but a M208D mutant was not included in the study.

Many class mu isoenzymes exist in the rat and these can be divided into two subfamilies, namely, those containing rGSTM1, M2, M3, M4 and M5* subunits and rGSTM6*-6*. The primary structures of these subunits (Figure 12) indicate that rGSTM1 and rGSTM4 are more closely related to each other than to rGSTM2 and rGSTM3. Lai et al.[184] proposed that class mu GST have arisen by gene conversion because of the existence of a remarkable homology in both intron 3 and intron 4 of the *rGSTM2* and *rGSTM4* genes. About half of the primary structure of the rGSTM6* subunit has been determined and it has been found to be more closely related to M2, with which it shares 67% identity, than to M1, with which it shares 62% identity. The rGSTM1, M2, M3, and M5* subunits can all dimerize. Examination of the primary structures of class mu GST reveals that all of the 14 residues involved in dimerization,

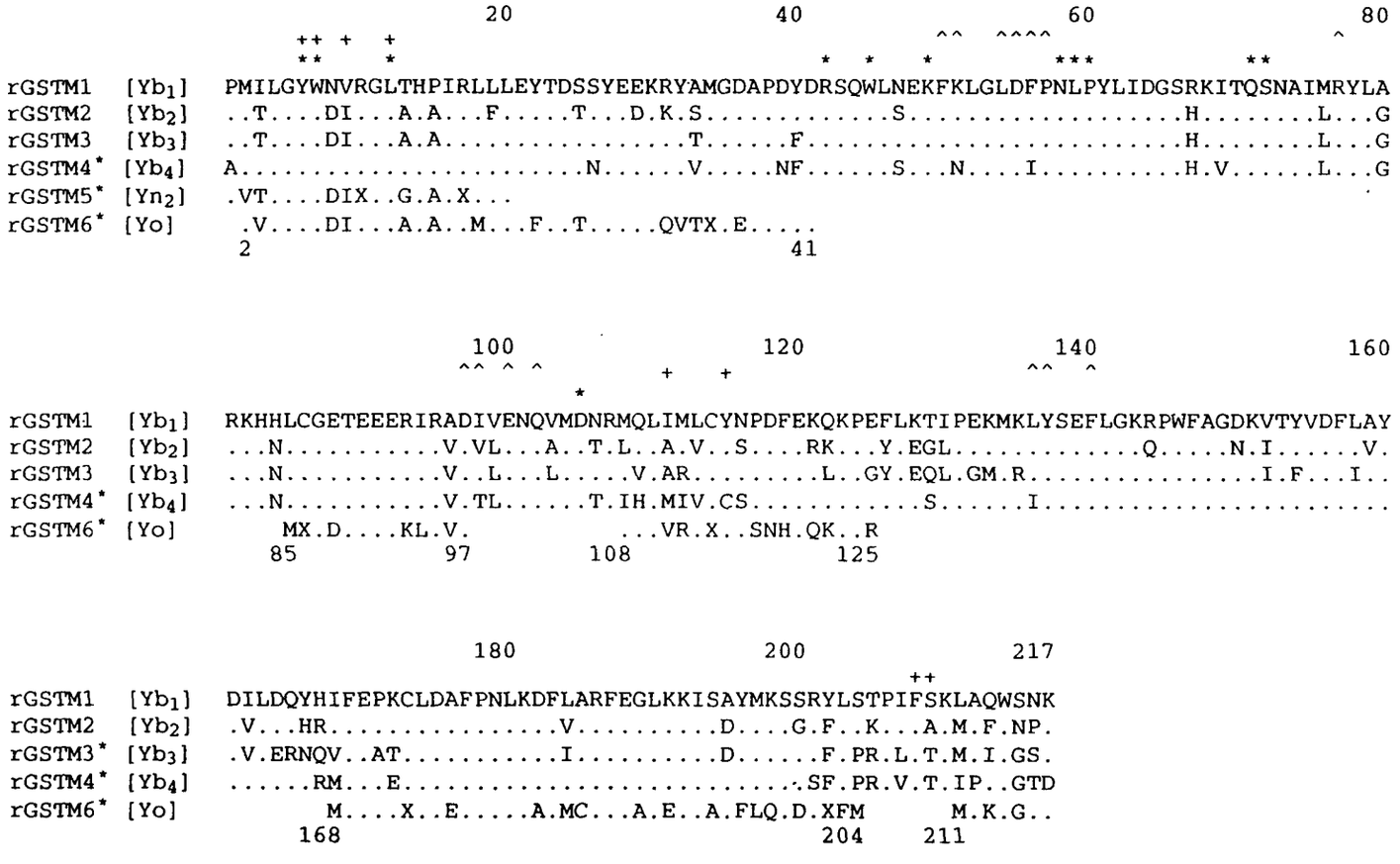
```
                                  20                  40                  60                  80
                           + +++                                  ^^                  ^   ^
                          *    *                          *        **          **
rGSTA1   [Ya1]   MSGKPVLHYFNARGRMECIRWLLAAAGVEFDEKFIQSPEDLEKLKKDGNLMFDQVPMVEIDGMKLAQTRAILNYIATKYD
rGSTA2   [Ya2]   ............................E..L.............................................
rGSTA3   [Yc1]   .P........DG.....P............E.Q.LKTRD..AR.RN..S...Q............V............N
rGSTA5   [Yc2]   .P........DG.....P............E.N.LKTRD..AR.RS..S...E............V..K.........N
rGSTA4   [Yk]    .EV..K.Y..QG.....S......T.....E.E.LETR.QY...Q...C.L.G...L......L.T......S.L.A..N

                                 100                 120                 140                 160
                  ^      ^    ^^  ^^         ++ ++                  ^^                  ^
                                 *  *                          •
rGSTA1   [Ya1]   LYGKDMKERALIDMYTEGILDLTEMIMQLVICPPDQKEAKTALAKDRTKNRYLPAFEKVLKSHGQDYLVGNRLTRVDIHL
rGSTA2   [Ya2]   ...............S..........I..........R.....................................
rGSTA3   [Yc1]   ...............A..VA..D.IVLHYPYI..GE...SL.KI..KAR...F.....................S.A.VY.
rGSTA5   [Yc2]   ...............A..VA..EL.VLYYPYM..GE...SL.KI..KAR...F..Y...............K.S.A.VS.
rGSTA4   [Yk]    .....L...VR....AD.TQ..MM..IGAPFKA.QE..ESL...VK.A....F.V...I..D..EAF....Q.SWA..Q.

                                 180                 200                 222
                                                                  +    +  +   + +
rGSTA1   [Ya1]   LELLLYVEEFDASLLTSFPLLKAFKSRISSLPNVKKFLQPGSQRKLPMDAKQIEEARKIFKF
rGSTA2   [Ya2]   ..............................................PA...........V...
rGSTA3   [Yc1]   VQV.YH...L.P.A.AN......LRT.V.N..T............PLE.E.CV.S.V...S
rGSTA5   [Yc2]   V...YH...M.PGIVDN......LRT.V.N..T............PFD.E.CV.S.K...S
rGSTA4   [Yk]    ..AI.M...VS.PV.SD....Q...T...NI.TI.....:.....P.P.GHYVDVV.TVL..
```

The primary structures of rGSTA1, A2, A3, A5 and A4 are from references 259, 261, 264, 270 and 266, respectively. The numbering of the amino acid residues includes the initiator methionine because acetylated methionine represents the *N*-terminus of rGST A4[272]. However, by contrast, proline represents the *N*-terminus of rGSTA3 and A5 The positions of G-site residues are indicated by an asterisk (*), the positions of H-site residues are indicated by a plus sign (+) and the residues associated with subunit dimerisation are indicated by a circumflex (^). Asp 101 from one subunit forms part of the G-site of the other monomer with which it hybridises; the G-site, H-site and subunit dimerisation residues were identified by Sinning *et al*[54].

FIGURE 11. Primary structure of rat class alpha GST.



Primary structures of rGSTM1, M2, M3, M4*, M5* and M6* are from references 276, 281, 284,184, 285 and 286 respectively. Sequences for the rGSTM5* and M6* subunits were obtained by automatated Edman degradation and residues that could not be assigned with confidence are designated X. Ding *et al*[275] have obtained a distinct allelic cDNA clone for rGSTM1 (pGTA/C44) that encodes N198 and C199 instead of K198 and S199 shown above. The numbering of the amino acid residues does not include the initiator methionine. Characteristically the class mu enzymes contain an additional heptapeptide, called the "mu loop" between residues 35 and 41, that is not found in other families. Positions of G-site residues are indicated by an asterisk (*), the positions of H-site residues are indicated by a plus sign (+) and the residues associated with subunit dimerisation are indicated by a circumflex (^). Asp 105 from one subunit forms part of the G-site of the other monomer with which it hybridises; the G-site, H-site and subunit dimerization residues were identified by Ji et al.[52]

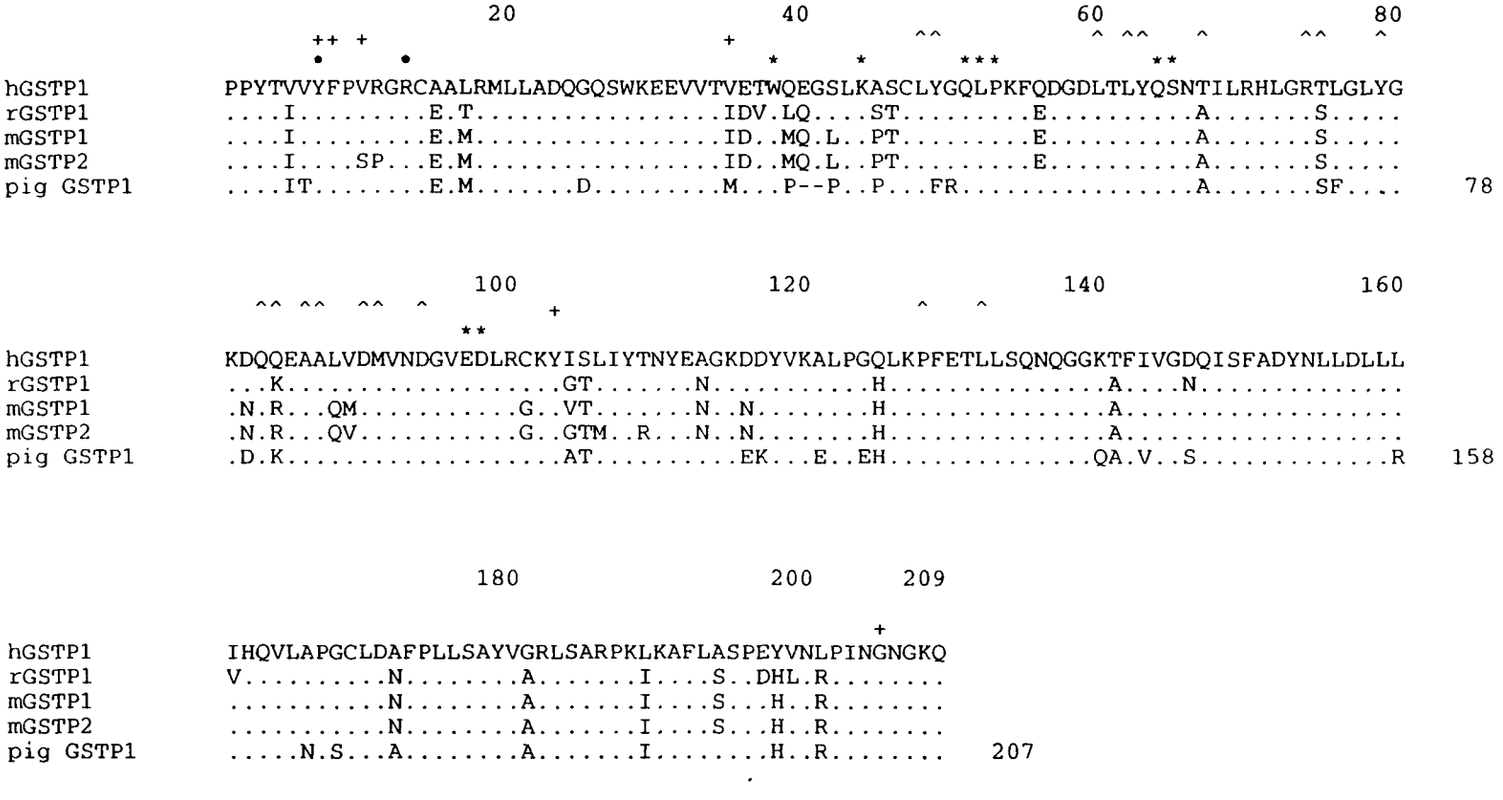
FIGURE 12. Primary structure of rat class mu GST.

namely F50, K51, L54, D55, F56, P57, R77, D97, I98, E100, Q102, L136, Y137, and F140 are conserved in rGSTM1 and rGSTM3, whereas rGSTM2 contains a single I98V substitution. Among the dimerization residues, four substitutions exist in rGSTM4 (N51K, I56F, T98I, and I136L), but it is not known whether rGSTM4 is able to hybridize with other class mu subunits. It appears unlikely that the M6* subunit hybridizes with other class mu subunits, but, to date, the regions of rGSTM6* involved in subunit dimerization have not been sequenced.

The molecular basis for the catalytic specificity of class mu transferases has been examined using X-ray crystallography and site-directed mutagenesis (SDM). The H-site in rGSTM1-1 is defined by residues Y6, W7, V9, and L12 from domain I, and I111, Y115, F208, and S209 from domain II.[311] Evidence has been presented showing that V9 and I111 are responsible for the stereoselectivity of class mu enzymes, whereas Y115 is responsible for activity toward epoxides.[312] Thus, Y115 is essential for the activity of rGSTM1-1 with phenanthrene-9,10-oxide, but is not required for activity with CDNB.[311] The Y115F GSTM1-1 mutant exhibited only about 1.5% of the activity toward phenanthrene-9,10-oxide as the wild-type rGSTM1-1, but 3.6-fold greater activity toward CDNB than the wild-type enzyme. Through only two mutations, it was possible to confer on rGSTM1-1 the catalytic activity that is typical of rGSTM2-2. The introduction of a V9I mutation into rGSTM1-1 resulted in selectivity toward enones and epoxides, and the introduction of a I111A mutation increased efficiency of the enzyme toward tPBO. Hence, the double V9I/I111A rGSTM1-1 mutant exhibited stereoselectivity and k_{cat}/K_m^{tPBO} similar to wild-type rGSTM2-2.

A single class pi GST homodimer exists in the rat. This subunit does not hybridize with subunits from other classes. The rat class pi GST has a broader substrate specificity than most of the other rat isoenzymes but has particularly high activity toward (+)-7β,8α-dihydroxy-9α,10α-oxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene[309] and glycidoxycoumarin.[313] It is also generally insensitive to inhibition by a wide range of organic anions such as bilirubin, hematin, bromosulfophthalein, indocyanine green, sulfasalazine, deoxychlolate and cholate.[304] The enzyme is readily inactivated by thiol-reactive agents through modification of cysteine 47, a reaction which results in steric hindrance of substrate binding to the H-site rather than modification of the active site. Class pi GST appear to be highly conserved in all mammalian species (Figure 13).

The *N*-terminal amino acid sequence of glutathione-dependent prostaglandin D synthase (PGDS) shares about 40% identity with class alpha GST and has therefore previously been included within the alpha class. However, as PGDS is now known to possess up to 50% identity with class sigma GST from *Schistosoma japonicum, Schistosoma mansoni, Onchocerca volvulus,* and *Ascaris suum* (Figure 14),[177] it should be designated rGSTS1*-1*. Although it is clear that rGSTS1*-1* is active toward prostaglandins and CDNB,[62,177] an exhaustive study of its substrate specificity has not yet been described. It is not known how many class sigma GST exist in the rat and it will be interesting to establish the structural and functional relationships between this class and other cytosolic GST classes. The relationship between the Ys subunit from rat spleen[271] and rGSTS1* requires clarification. The Ys subunit has been designated a class alpha enzyme,[271] but it may be identical to rGSTS1*; Ys has been included in Table 1 separately from rGSTS1* because Ys apparently possesses a blocked *N*-terminus, whereas rGSTS1*



The numbering of the amino acid residues does not include the initiator methionine. The primary structures are from references 191, 193, 290, and 314. The positions of G-site residues are indicated by an asterisk (*), the positions of H-site residues are indicated by a plus sign (+) and the residues associated with subunit dimerization are indicated by a circumflex (^). Asp 98 from one subunit forms part of the G-site of the other monomer with which it hybridizes. Porcine GSTP1 lacks amino acids equivalent to residues 39 and 40 in human, rat and murine pi-class GST; spaces (shown by - -) have been introduced into the primary structure of pig GSTP1 to maximize sequence homology. The designation X represents an amino acid whose identity could not be determined.The allelic variants of hGSTP1 described by Ali-Osman and Akande[201] contain a valine at residue 104 or a valine at residue 113.

FIGURE 13. Primary structure of class pi GST.

```
                                             10        20        30        40      48
                                            *
rGSTS1•                                PNYKLLYFNMRGRAEIIXYIFAYLDIKYEDHRIEQADWPKIKPTLPFG
Schistosoma japonicum 28 kDa GST        V..I...G.....P.RM.LVAAGVEF..E...FQ........I.G.
Ascaris suum GST                       .Q...T..DI..LG.GARL..HQAGV.F..N.LKRE...AL..KT...
Schistosoma mansoni 28 kDa GST       AGEHI.VI..DG.....S.RMTLVAAGVD...E..SFQ........I.G.
Squid lens crystallin SL11 GST         .S.T.Y...G......CRML..VASVQ.Q.K...L.E.TQF.TKM.CH
Onchocerca volvulus GST                EK.T.T...G.....V.RLL..LANVS...N..TRDE.KYL..RT...
```

The amino acid sequence data for class sigma GST are from references 148, 150, 152, 153 and 177. The position of the active site tyrosine is indicated by an asterisk (*). The designation X represents an amino acid whose identity could not be determined.

FIGURE 14. Primary structure of class sigma GST.

is amenable to automated amino acid sequencing.

In the rat, several class theta GST have been described. Two of these were among the first GST for which purification schemes were reported. These enzymes were called transferase E[291], because of its activity with epoxides, and transferase M,[292] because of its activity with 1-menaphthyl sulfate. Transferases E and M have also been called 5-5 and YrsYrs, but are now called rGSTT1-1 and rGSTT2-2, respectively. In more recent years, cDNA clones encoding these enzymes have been isolated and, as they share only about 50% identity, it is clear that they represent two separate subfamilies within class theta (Figure 15). In the T2 theta subfamily, heterogeneity has been observed by several research groups. Two distinct forms of the T2 subunit have been described that dimerize to give rise to T2-2, T2-2′, and T2′-2′; the T2 and T2′ subunits represent posttranslationally modified forms of a single polypeptide.[295] Most significantly, T2 and T2′ have distinct catalytic activities toward ethacrynic acid and linoleic acid hydroperoxide (Table 3). Meyer et al.[176] have isolated an enzyme, called GST 12-12, from rat liver that has a similar, although not identical, *N*-terminal amino acid sequence to T2; it is possible that either strain variation or sequencing errors account for the differences in primary structures between T2 and subunit 12.

A further transferase, GSTT3*-3* (13-13), has been isolated from rat liver mitochondria and found to have activity toward CDNB and ethacrynic acid.[241] Automated amino acid sequencing and molecular cloning[296] have shown that, over the first 33 *N*-terminal residues, rGSTT3* shares about 30% identity with the rGSTT2 subunit (Figure 15). Although rGSTT3*-3* has been included as a class theta GST, it is clearly separate from the other two class theta subfamilies. The rGSTT3* subunit possesses an additional *N*-terminal peptide sequence that might serve as a mitochondrial import signal.

4. Posttranslational Modification of Rat Cytosolic GST

Various reports exist in the literature suggesting that GST are subject to phosphorylation,[315,316] methylation,[239] glycosylation,[317] and autoxidation.[299] Much of the data suggesting that these enzymes may be posttranslationally modified have been obtained from *in vitro* experiments, and therefore the *in vivo* biological significance of such postsynthetic events is unclear.

A preparation containing the rGSTA1 and A2 subunits provides a good *in vitro* substrate for protein kinase C, and phosphorylation, which occurred in stoichiometric amounts, decreased the affinity of these subunits for bilirubin;[315] presumably phosphorylation will reduce the affinity of GSTA1 and A2 for other nonsubstrate ligands such as bile acids and fatty acids. Human LTC_4S also contains a protein kinase C phosphorylation site, and Nicholson[316] has presented data suggesting that this enzyme is phosphoregulated *in vivo*; phosphorylation may inhibit the production of LTC_4 in HL-60 cells.

The GSTM6* subunit can be methylated *in vitro*. Although the maximal level of methylation achieved was only 22%, it resulted in a significant reduction in activity toward CDNB, suggesting a functional significance for this modification.[239]

The human class pi GST is glycosylated[317] and human LTC_4S contains a potential *N*-linked glycosylation site,[60] but to date no evidence has been provided that rat GST subunits are glycosylated.

ROS have also been shown to modulate GST activity *in vitro*. Class alpha and pi

```
                                  20                  40                  60                  80
                                  *                   *                 * *                 ***
hGSTT1          GLELYLDLLSQPCRAVYIFAKKNDIPFELRIVDLIKGQHLSDAFAQVNPLKKVPALKDGDFTLTESVAILLYLTRKYKVP
rGSTT1          V.............I.......N...QMHT.E.R..E..........M.....M...G...C.........AH.....
hGSTT2          ....F...V...S.........G..L...T...V....K.KE.L.I.S.G.L.T......I....S...I..SC..QT.
rGSTT2          ............S.........G...Q..T...L......EQ.S...C.....V....S.V....T...I..SS..Q.A
rGSTT3*   CPAPRV...FY.V..PYSWLGFEVL--------------CRY...WNI-----K..LR...LT.IMKDSGNQPPAMA
                1                         25              26      34    35                    58

                                100                 120                 140                 160
                                                    *
hGSTT1          DYWYPQDLQARARVDEYLAWQHTTLRRSCLRALWHKVMFPVFLGGPVSPQTLAATLAELDVTLQLLEDKFLQNKAFLTGP
rGSTT1          .H..............................T............EQIR.EM......D...NV.V...Q...D.D..V..
hGSTT2          .H...S........H...G.HADCI.GTFGIP..VQ.LG.L-I.VQ.PEEKVERNRTAM.QA..W......GDRP..A.Q   159
rGSTT2          .H...A......Q.H...G.HADNI.GTFGVL..T..LG.L-I.VQ.PEEKVERNRNSMVLA..R......RDR..IA.Q   159

                                180                 200                 220                 239
hGSTT1          HISLADLVAITELMHPVGAGCQVFEGRPKLATWRQRVEAAVGEDLFQEAHEVILKAKDFPPADPTIKQKLMPWVLAMIR
rGSTT1          ......V...........G..P......R..A.YR.......K...L.........VR.C.....V.......R..T..Q
hGSTT2          QVT....M.LE...Q..AL.YEL.....R..A..G....FL.AE.C....SI..SILEQAAKKTLPTPSPEAYQAMLL.   238
rGSTT2          QVT....MSLE..IQ..AL..NL.....Q.TA..E....FL.AE.C....NP.MSVLGQAAKKTLPVPPPEAHASM.L.   238

hGSTT2          IARIP     244
rGSTT2          .....     244
```

A comparison is shown between the T1-type and the T2-type class theta transferases. The numbering of the amino acid residues does not include the initiator methionine. A single amino acid gap has been inserted in human GSTT2 and rat GSTT2 between residues 121 and 122 to maximise the similarity between the sequences. Hence, residues 123-239 of hGSTT1 are aligned with residues 122-238, respectively, of hGSTT2. The sequence of the rat mitochondrial GSTT3* is shown to demonstrate that it is separate from the other class theta GST; a14 amino acid gap has been introduced in rGSTT3* between residues 25 and 26 as well as a 5 amino acid gap between residues 34 and 35 to increase homology within this GST and other class theta enzymes. The sequence data for hGSTT1, rGSTT1, hGSTT2, rGSTT2 and rGSTT3* are from references 44, 202, 200, 293 and 296, respectively. The positions of the G-site residues in the mammalian class theta GST that are equivalent to those in the *Lucilia cuprina*, Australian sheep blowfly, GST whose crystal structure has been solved[55] are indicated by an asterisk (*). Two of these residues, S10 and H39, are conserved in the *Lucilia* GST and the mammalian cytosolic and mitochondrial class theta GST. Two further residues, E65 and S66, are conserved between *Lucilia* GST and the mammalian cytosolic theta GST.

FIGURE 15. Primary structure of rat and human class theta GST.

GST are inhibited by ROS, whereas the activity of certain class mu GST is stimulated by treatment with ROS.[318] The rGSTM1 and rGSTM2 subunits appear to be sensitive to superoxide, or other ROS, as incubation with xanthine and xanthine oxidase can increase the specific activity of GSTM1-1, M1-2 and M2-2 approximately five fold toward CDNB.[318] All class pi GST contain a reactive cysteine residue (C47) that, when modified by ROS or thiol agents, results in inactivation of the protein. The activity of the microsomal GST can be stimulated dramatically by treatment with ROS,[319] and in this case activation is achieved by modification of C49, possibly by inter-subunit disulfide interchange.

In addition to activation by ROS, the microsomal GST can be activated by treatment with thiol agents,[320] again mediated by C49, or by proteolysis at K41.[321] The activation of microsomal GST by thiol agents results in increased catalytic efficiency *in vitro* against essentially all substrates at low GSH levels,[306] but increased activity is less obvious when high GSH levels are employed. It appears likely that the increased activity of microsomal GST produced by thiol agents represents a rapid adaptive response to chemical stress *in vivo*, but the biological significance of activation by proteolysis is not clear. The physical properties of the rat GST subunits and putative post-translational modifications are summarized in Table 4.

E. Mouse Cytosolic GST Isoenzymes

By comparison with the rat enzymes, relatively little is known about murine GST. As might be expected for two species of rodent, obvious similarities exist between the rat and mouse GST. The class alpha, mu, pi, and theta GST are all represented in the mouse (Table 5). Also, among the class alpha murine GST, each of the three subfamilies observed in the rat has been identified. The cDNA encoding several class mu subunits have been cloned and these are closely similar to those found in the rat. One of the surprising features about the mouse enzymes is that two distinct class pi GST subunits exist,[191] whereas the rat possesses only one class pi GST.[190] Like the rat, the mouse possesses both the T1 and T2 subfamilies of class theta GST.

Mouse GST have been the subject of many investigations and various nomenclatures have been used to describe the enzymes. Talalay and his co-workers[337] first employed isoelectric focusing to identify inducible GST from the livers of female mice, and the forms they obtained were designated according to their pI values (e.g., GT-8.7, GT-9.3).[329] Subsequently, Lee et al.[338] purified three enzyme-containing peaks (F1, F2, and F3) from the livers of mice that had not been treated with inducing agents. Table 6 shows the quaternary structure of murine GST along with the nomenclatures that were used previously to describe the isoenzymes. It is now widely recognized that livers from normal male mice express substantial amounts of class alpha, mu, and pi GST, whereas livers from normal female mice express predominantly only class alpha and mu GST. The treatment of mice with xenobiotics can result in the induction of GST subunits that are not expressed constitutively and hence the isoenzyme profile can change dramatically in mice that have been administered drugs.

Comparison between the catalytic activities of the mouse GST and the activities of the rat enzymes reveals considerable conservation of structure and function among orthologous subunits from the two species (Table 7). Among class alpha enzymes, the mouse and rat GSTA3 have characteristically high peroxidase activity with cumene

TABLE 4
Physical Properties of Rat GST Subunits

Subunit class-based nomenclature	Alternative term	Apparent mol wt. (SDS/PAGE)[a]	Predicted mol wt. (cDNA)	Mol wt. from electrospray mass spectrometry[b]	Posttranslational modification[c]
rGSTA1	Ya_1	25,500	25,522	25,520	*N*-acetylation, ? phosphorylation
rGSTA2	Ya_2	25,500	25,469	25,473	*N*-acetylation, ? phosphorylation
rGSTA3	Yc_1	27,500	25,188	25,188	Not modified
rGSTA4	Yk	25,000	25,500	25,553	*N*-acetylation
rGSTA5	Yc_2	25,800	25,216	25,211	Not modified
—	Ys*	26,000	Not cloned	—	Blocked *N*-terminus
—	GST A(6)*	26,500	Not cloned	—	Blocked *N*-terminus
rGSTM1	Yb_1	26,300	25,782	25,782	Not modified
rGSTM2	Yb_2	26,300	25,571	25,571	Not modified
rGSTM3	Yb_3 (Yn_1)	26,000	25,551	25,551	Not modified
rGSTM4*	Yb_4*	Not reported	Genomic clone only	—	—
rGSTM5*	Yn_2*	26,000	Not cloned	—	—
rGSTM6*	Yo*	26,500	Not cloned	—	Blocked *N*-terminus, ? methylated
rGSTP1	Yf (Yp)	24,800	23,308	23,308	Intrasubunit disulfide bond
rGSTS1*	PGDS	26,500	Not cloned	—	—
rGSTT1	5	26,200	27,340	—	—
rGSTT2	Yrs	26,500	27,311	—	—
rGSTT3*	13*	26,500	Not cloned	—	—
Microsomal	—	17,000	17,430	—	—

[a] The apparent molecular weights of GST subunits determined by SDS/PAGE are based on the data taken from Hayes and Mantle.[227]
[b] The electrospray analyses were described by Yeh et al.[232]
[c] The methylation and phosphorylation studies were described by Johnson et al.[239] and by Taniguchi and Pyerin,[315] respectively.

TABLE 5
Mouse GST Subunits

Class		"Y" SDS/PAGE subunit terminology[a]	Subunit mol wt. by SDS/PAGE[b]	cDNA clone	Chromosomal localization[c]	Ref.
Alpha	(mGSTA1)	Ya_1	25,600	λmYa1	(9)	180, 217, 322, 323
Alpha	(mGSTA2)	Ya_2	25,600	pGT41	(9)	322, 323, 324
Alpha	(mGSTA3)	Yc	25,800	pmusGSTYc, X65021	(9)	322, 323, 325, 326
Alpha	(mGSTA4)	Yk	25,000	—		327, 328
Mu	(mGSTM1)	Yb_1	26,400	pGT875, pmGT10, J04632	—	221, 329, 330
Mu	(mGSTM2)	Yb_2	26,200	pmGT2, J04696	—	330, 331
Mu	(mGSTM3)	Yb_3	—	pGT55a	—	324
Mu	(mGSTM4*)	Yb_5^*	26,500	—	—	331
Mu	(mGSTM5*)	Yb_4*(Ft)	—	—	—	332
Pi	(mGSTP1)	Yf_1	24,800	X53451, X76143	1	322, 333
Pi	(mGSTP2)	Yf_2	24,800	X76144	1	191, 192, 322
Theta	(mGSTT1)	—	—	—	—	219, 334
Theta	(mGSTT2)	Yrs	25,400	—	—	219, 334, 335
Microsomal GST		—	17,000	—	—	336

[a] The "Y"-based nomenclature that was devised by Bass et al.[256] has been applied to the mouse.[217,221]

[b] The apparent molecular weights of GST subunits determined by SDS/PAGE are based on data taken from Hayes and Mantle.[227]

[c] As the localization of alpha class GST genes to murine chromosome 9 was made using a heterologous probe[322] (i.e., cDNA encoding hGSTA1), it is not certain which of the mouse GST genes reside on this chromosome and therefore the most likely candidate genes are entered in parentheses.

TABLE 6
Quaternary Structure of Mouse GST Isoenzymes and Various Nomenclatures

Class	Alternative name of isoenzymes: Class-based nomenclature	"Y"-based nomenclature	Preparations obtained through protein purification by[a]: A	B/P/T	L	M	Ref.
Alpha	mGSTA1-2	Ya_1Ya_2	—	—	—	—	217, 323
Alpha	mGSTA1-3	Ya_1Yc	—	GT-10.3	—	—	217, 339
Alpha	mGSTA2-3	Ya_2Yc	—	GT-10.3	—	—	217, 339
Alpha	*m*GSTA3-3	YcYc	Peak I	GT-10.6	F4	MI	323, 339–342
Alpha	*m*GSTA4-4	YkYk	GST 5.7	—	—	—	328, 343, 344
Mu	mGSTM1-1	Yb_1Yb_1	Peak III	GT-8.7	F3	MIII	221, 329, 332, 341, 342
Mu	mGSTM1-2	Yb_1Yb_2	—	—	—	—	331
Mu	mGSTM1-4	Yb_1Yb_5	—	—	—	—	331
Mu	*m*GSTM3-3	Yb_3Yb_3	—	GT-9.3	—	—	329
Mu	mGSTM5*-5*	$Yb_4^*Yb_4^*$	—	—	Ft	—	332
Pi	mGSTP1-1	Yf_1Yf_1	Peak II (male)	GT-9.0	F1	MII	339-342
Pi	mGSTP1-2	Yf_1Yf_2	Peak II (female)	—	—	—	342
Theta	mGSTT1-1	—	—	—	—	—	219, 334
Theta	mGSTT2-2	YrsYrs	—	—	—	—	219, 334, 335
Theta	*m*GSTT2-2′	YrsYrs′	—	—	—	—	335
Microsomal GST		—	—	—	—	—	217, 336

[a] Abbreviations for principal laboratories that have published nomenclatures for mouse GST are as follows: A, Awasthi; B, Benson; L, Lee; M, Mannervik; P, Pearson; T, Talalay.

hydroperoxide, and both mouse and rat GSTA4 have high activity for 4-hydroxynonenal. The mouse and rat GSTM1 subunits both metabolize 1,2-dichloro-4-nitrobenzene, and the GSTM2 subunit from both species is active with *trans*-4-phenyl-3-butene-2-one. The mouse and rat GSTP1 subunits have high activity toward ethacrynic acid. Three class theta T2-containing GST can be resolved from mouse liver[335] and, like the rat enzymes, these display activity toward 1-menaphthyl sulfate. However, several substantial differences exist in the catalytic properties of certain orthologous mouse and rat GST. Analysis of the basis for these differences can give a valuable insight into the residues involved in substrate selectivity. The specific activity of the mGSTA1 and mGSTA2 subunits for Δ^5-androstene-3,17-dione is only 2.5% of that exhibited by the rGSTA1 and rGSTA2 subunits. An even more remarkable difference in activity is observed between the two different murine class pi GST subunits which, although possessing 97% identity (Figure 13), exhibit approximately 1000-fold difference in activity toward CDNB. By using SDM, it has been shown that the amino acid differences at positions 10 (V10S), 11 (R11P), and 104 (V104G) are responsible. for the reduced activity of mGSTP2-2.[345]

It appears likely that the marked variation in the activity of the P1 and P2 subunits is responsible for the differences in the specific activity of class pi GST (peak II) from male and female mouse liver that was reported by Singhal et al.[342] These data suggest that the enzyme in the livers from the male mouse comprises primarily mGSTP1-1, whereas the class pi enzyme from the liver of the female mouse comprises both the active P1 subunit and the inactive P2 subunit. Because the class pi GST is found in approximately tenfold greater amounts in the male mouse liver than in the female mouse liver,[340] and activity toward CDNB is greater in hepatic cytosol from male than in hepatic cytosol from

TABLE 7
Catalytic Activities of Mouse GST Isoenzymes

	Specific activity (μmol/min/mg of protein)												
Isoenzyme	**CDNB**	**Δ^5AD**	**BSP**	**DCNB**	**EA**	**EPNP**	**4-HNE**	**MS**	**4-NBC**	**4-NPA**	**tPBO**	**CuOOH**	**LiOOH**
mGSTA1-2 [Ya_1Ya_2]	3.1	0.05	0	0	0.86	0	—	—	—	0.70	0	1.06	—
mGSTA1/2-3 [$Ya_{1,2}Yc$]	6.0	—	0	0	0.23	—	—	—	0.92	—	0.01	1.21	—
mGSTA3-3 [YcYc]	15.1	0.04	0	0.06	0.10	0.23	1.1	—	—	0.01	0.01	11.6	—
mGSTA4-4 [YkYk]	12.0	—	0.08	0.40	1.90	2.76	55.4	—	1.16	—	0.01	0.70	1.14
mGSTM1-1 [Yb_1Yb_1]	148.0	0.04	0.58	4.40	0.12	0.48	6.0	—	—	0.59	0.04	0.10	—
mGSTM1-2 [Yb_1Yb_2]	81.0	—	—	2.80	—	—	—	—	—	—	0.39	—	—
mGSTM1-4 [Yb_1Yb_5]	74.0	—	—	3.20	—	—	—	—	—	—	0.08	—	—
mGSTM3-3 [GT-9.3]	22.2	—	0.01	0.08	0.01	—	—	—	0.50	—	0.08	—	—
mGSTM4-4 [Ft]	60.0	—	—	0.12	—	0.12	—	—	0.04	—	—	—	—
mGSTP1-1 [Yf_1Yf_1]	119.0	0.14	0.01	0.14	4.30	0.77	2.6	—	1.24	0.21	0.01	0.14	—
mGSTP2-2, recombinant	0.12	0	0	0	0.04	0	—	—	0.05	0	0	0.01	—
mGSTT2-2 [YrsYrs]	0	—	—	—	—	—	—	1.6	—	—	—	0	—
mGSTT2-2′ [YrsYrs′]	0	—	—	—	—	—	—	1.7	—	—	—	0	—
Microsomal GST	42.0	—	<0.01	0.10	<0.01	—	0	—	—	—	—	1.90	—

Note: Horizontal dashes are included to signify substrates that have not been tested with a particular isoenzyme. Data for recombinant mGSTP2-2 are from Bammler et al.[345] All the reactions are shown in Figures 3, 8, and 10. All other data for cytosolic GST are from References 323, 331, 332, 335, 339, 341, 346. The data for microsomal GST are from the *N*-ethylmaleimide-activated enzyme: Andersson et al.[336] Abbreviations used: CDNB, 1-chloro-2,4-dinitrobenzene; Δ^5AD, Δ^5 androstene-3,17-dione; BSP, bromosulfophthalein; DCNB, 1,2-dichloro-4-nitrobenzene; EA, ethacrynic acid; EPNP, 1,2-epoxy-3-(*p*-nitrophenoxy)propane; 4-HNE, 4-hydroxynonenal; MS, 1-menaphthyl sulfate; 4-NBC, 4-nitrobenzylchloride; 4-NPA, 4-nitrophenylacetate; tPBO, *trans*-4-phenyl-3-buten-2-one; CuOOH, cumene hydroperoxide; LiOOH, linoleate hydroperoxide.

female mice, it would appear that mGSTP1-1 rather than mGSTP2-2 is the male-specific class pi transferase in the mouse.

F. Human Cytosolic GST Isoenzymes

The GST in humans have been studied by many research groups and it is thought that at least 20 isoenzymes exist. Over the years several different nomenclatures have been used to define the various enzymes (Table 8). The currently used class alpha, mu, pi, and theta designations, now used in all mammalian species, are based on the trivial names GST α, GST μ, GST π, and GST θ that were originally used to describe the best known human isoenzyme in each of the four families of cytosolic enzyme.

The GST isoenzymes in the human were first purified by Kamisaka et al.[347] who described the isolation of a number of class alpha enzymes that were designated transferases α to ε. These were all believed to represent deamidation products. Subsequently, it was demonstrated that the multiple class alpha forms found in the liver are homodimers or heterodimers formed between at least two distinct subunits;[348] these subunits are now referred to as A1 and A2 and dimerization gives rise to hGSTA1-1, hGSTA1-2, and hGSTA2-2.[255] Besides the class alpha GST, an additional class mu enzyme is present in certain, but not all, human liver specimens. This enzyme, which was originally called GST μ, is highly polymorphic in the population. Besides the "null" phenotype due to a gene deletion,[362] two allelic charge variants exist that differ only in the amino acid present at residue 173. The more basic variant subunit (hGSTM1a) contains K173, whereas the more acidic subunit (hGSTM1b) contains N173. These two subunits can dimerize to form hGSTM1a-1a, hGSTM1a-1b, and hGSTM1b-1b isoenzymes, which possess essentially identical catalytic activities[365] but differ in having isoelectric points (pI) of 6.1, 5.8, and 5.5, respectively.[363] On the basis of mobility in starch gels, the existence of another class mu GST, called GSTM1 3, has been reported but this appears to be relatively uncommon and has not been characterized.[385,386] It is possible that GSTM1 3 is identical to GST ϕ, a transferase with a pI of 4.6 and subunit size 26 700 Da, which was found in only 1 of a series of 20 livers.[384]

Since the characterization of these major hepatic cytosolic transferases, additional class alpha GST have been characterized in several extrahepatic tissues. For example, Del Boccio et al.[355] reported the presence of a highly basic class alpha enzyme of pI 9.9 (skin GST 9.9) in human skin that appears to comprise polypeptides orthologous to the rat A3 and A5 subunits. Also, Awasthi and his co-workers[353] identified an acidic alpha class GST of pI 5.8 (hGSTA4*-4*) that is expressed in many tissues and appears to comprise polypeptides orthologous to rGSTA4. The catalytic properties of these enzymes, along with those of hGSTA1-1, A1-2, and A2-2, are shown in Table 9. Like certain class alpha GST in rats, those in humans show relatively high activity for Δ^5 androstene-3,17-dione and cumene hydroperoxide. The hGSTA4*-4* isoenzyme is particularly noteworthy because of its high activity toward 4-hydroxynonenal and ethacrynic acid, substrates toward which rGSTA4-4 displays greatest activity. Amino acid sequencing of hGSTA4-4* has shown that it possesses substantial homology with rat and mouse GSTA4-4.[354] Genomic cloning of human class alpha GST has resulted in the characterization of a human gene encoding a polypeptide that had not previously been identified;[183] this subunit is re-

TABLE 8
Human GST Enzymes

Class (cytosolic GST)	Isoenzyme[a]	Alternative name of isoenzyme[b]: Zymogen staining B	Preparations obtained through protein purification by the following laboratories: A	H	J	K	M	S	Previous subunit designation	cDNA clone (corresponding to homodimer)	Chromosome localization	Ref.
Alpha	hGSTA1-1	GST2 type 1	—	B_1B_1	ε	$\alpha_x\alpha_x$		Y_1Y_1	Ha_1, α_x	M15872, M21758	6	196, 231, 347–351
Alpha	hGSTA1-2	GST2 type 1-2	—	B_1B_2	δ			Y_1Y_4	—	—	—	348
Alpha	hGSTA2-2	GST2 type 2	—	B_2B_2	α-γ	$\alpha_y\alpha_y$		Y_4Y_4	Ha_2, α_y	pGTH2, M16594	6	351, 352
Alpha	hGSTA3-3*	—	—	—	—	—	—	—	—	L13275	6	183
Alpha	hGSTA4-4*	—	GST 5.8	—	—	—	—	—	—	Not cloned	nd	353, 354
Alpha	GST 9.9*	—	—	—	—	—	Skin 9.9	—	—	Not cloned	nd	355
Alpha	GST ω*	—	ω	—	—	—	—	—	—	Not cloned	nd	356, 357
Mu	hGSTM1a-1a	GST1 type 2	—	$N_1{}^aN_1{}^a$		μ	μ	M_3M_3	Hb	J03817	1	197, 358–362
Mu	hGSTM1a-1b	GST1 type 1-2	—	$N_1{}^aN_1{}^b$		—	μ	—	—	—	—	363-366
Mu	hGSTM1b-1b	GST1 type 1	φ	$N_1{}^bN_1{}^b$		—	μ	—	Hb	X08020	1	362, 366–368
Mu	hGSTM1b-2	—	—	$N_1{}^aN_2$	—	—	—	M_3N_2	—	—	—	361, 369
Mu	hGSTM2-2	GST4	ζ	N_2N_2	—	—	—	N_2N_2	N_2	M63509	1	361, 369–272
Mu	hGSTM2-3	—	—	N_2N_3	—	—	—	—	—	—	—	369
Mu	hGSTM3-3	GST5	—	N_3N_3	—	—	—	—	N_3	J05459	1	373
Mu	hGSTM4-4	—	—	—	—	—	—	—	—	M99422, M96234	1	188, 374
Mu	hGSTM5-5	GST6	—	—	—	—	—	—	—	L02321	1	187, 375
Pi	hGSTP1-1	GST3	—	λ	ρ	π	π	Y_xY_x	Yf	X08094-X08096	11	193, 194, 198, 216, 376–379
Theta	hGSTT1-1	—	—	—	—	θ	—	—	—		22	44, 176, 380
Theta	hGSTT2-2	—	—	T2-2	—	—	—	—	—	L38503	22	200, 381
—	Microsomal	—	—	Microsomal		—	—	—	—	J03752	12	205, 382, 383
—	LTC_4S		—	—		—	—	—	—	—	nd	60, 207, 208

[a] The isoenzyme designation is based, where possible, on the quaternary structure of the protein using the nomenclature proposed by Mannervik et al.[255] Enzymes that are marked by an asterisk (*) represent forms for which corresponding cDNAs have not been isolated. The existence of GST A3-3* has been predicted from genomic cloning.[183] GST 9.9* was isolated from human skin by Del Boccio et al.[355] and its *N*-terminal amino acid sequence has shown it to be related to rat Yc-type subunits (rGSTA3 and rGSTA5). GST ω* described by Awasthi and his co-workers[356,357] is a heterodimer comprising subunits that are closely related, but not identical, to A1 and A2.

[b] Abbreviations for principal laboratories that have published nomenclatures for human GST are as follows: A, Awasthi; B, Board; H, Hayes; J, Jakoby; K, Ketterer; M, Mannervik; S, Sato. It should be noted that Tsuchida et al.[361] have isolated two mu class GST heterodimers, M_1M_2 and M_2N_1, that are distinct from those listed in the table. During automated amino acid sequencing of these GST they were found to contain either glutamic acid (M_2N_1) or glutamic acid and glutamine (M_1M_2) at residue 8 rather than aspartic acid or asparagine, the residues present in M1 to M5 subunits. The M_1M_2 and M_2N_1 enzymes require further structural characterization to confirm their identity and they have not been included in the table, as the designations given by Tsuchida et al.[361] would cause confusion with the nomenclature proposed by Mannervik et al.[255] The human hepatic class mu enzyme with pI 4.6 that was designated GST φ[384] has not been included in the table, as it is a rare polymorphic variant at the *GSTM1* locus that probably represents hGSTM1c-1c and has also been called GSTM1 3.[385,386]

TABLE 9
Catalytic Activities of Human GST Isoenzymes

	Specific activity (μmol/min/mg of protein)														
Isoenzyme	**CDNB**	**Δ^5AD**	**BSP**	**DCNB**	**EA**	**EPNP**	**4-HNE**	**MS**	**4-NBC**	**4-NPA**	**4-NPB**	**tPBO**	**SO**	**CuOOH**	**LiOOH**
hGSTA1-1	82.0	4.0	—	0.25	0.1	0	—	—	—	0.7	—	0	0.02	3.1	—
hGSTA1-2	—	—	—	0.8	—	—	—	—	—	—	—	0	—	9.2	—
hGSTA2-2	80.0	—	—	0.9	0.1	0	—	—	—	0.2	—	0	—	10.4	—
hGSTA4-4*	12.5	—	0.07	0.91	2.8	2.4	168.0	—	0.6	—	—	0.03	—	0.6	0.3
skin GST 9.9	—	—	—	—	0.3	—	—	—	—	—	—	—	—	4.3	—
hGSTM1a-1a	190.0	0.12	0	0	0.1	0.1	3.3	—	2.7	0	—	0.21	—	0.3	—
hGSTM1a-1b	161.0	—	0	0	—	—	2.3	—	2.2	—	—	0.13	—	0.3	—
hGSTM1b-1b	172.0	—	0	0	—	—	2.5	—	2.2	—	—	0.16	—	0.3	—
hGSTM1b-2	203.0	—	0	1.7	—	—	3.0	—	2.6	—	—	0.13	—	0.04	—
hGSTM2-2	276.0	—	0	2.0	0.2	0	3.6	—	0	1.7	—	0	—	0.1	—
hGSTM2-3	172.0	—	0	2.1	—	—	3.3	—	0	—	—	0	—	0.1	—
hGSTM3-3	15.2	—	0	0	0.2	0	1.8	—	0	0.2	—	0	—	0.05	—
hGSTM4-4	1.4	0	—	0	0.1	0	—	—	—	0.03	—	—	—	—	—
GST M_1M_2*	32.6	—	<0.02	0.94	0.7	<0.02	—	—	—	<0.02	—	0.64	—	0.9	—
GST M_2N_1*	46.5	—	<0.02	0.92	0.4	<0.02	—	—	—	<0.02	—	0.53	—	<0.02	—
hGSTP1-1	103.0	—	<0.02	0.14	1.22	0.5	1.6	—	—	—	—	0.02	0.14	0.03	—
hGSTT1-1	0	—	—	—	—	>1.9	—	—	—	—	>0.5	—	—	—	—
hGSTT2-2	0	—	—	—	—	0	—	0.5	0	—	—	—	—	6.9	—
Microsomal	4.5	0.03	—	0.6	—	—	—	—	0.6	—	—	—	—	0.92	—

Note: Horizontal dashes are included to signify substrates that have not been tested with a particular isoenzyme. The GST-catalyzed reactions in this table are all depicted in Figures 3, 8, and 10. Data for cytosolic GST are from References 176, 355, 361, 366, 369, 374, 381, 387. CDNB, 1-chloro-2,4-dinitrobenzene; Δ^5AD, Δ^5 androstene-3,17-dione; BSP, bromosulfophthalein; DCNB, 1,2-dichloro-4-nitrobenzene; EA, ethacrynic acid; EPNP, 1,2-epoxy-3-(*p*-nitrophenoxy)propane; 4-HNE, 4-hydroxynonenal; MS, 1-menaphthyl sulfate; 4-NBC, 4-nitrobenzylchloride; 4-NPA, 4-nitrophenylacetate; 4-NPB, 4-nitrophenylbromide; tPBO, *trans*-4-phenyl-3-buten-2-one; SO, styrene-7,8-oxide; CuOOH, cumene hydroperoxide; LiOOH, linoleate hydroperoxide.

ferred to as A3 but its substrate specificity is unknown. Thus, there is evidence for as many as five different human class alpha genes, namely, those encoding A1, A2, A3, A4, and skin GST 9.9.

Certain class mu enzymes that are not expressed in the liver are found in human muscle, testis, and brain. In addition to the hGSTM1a and M1b subunits, hGSTM2, M3, M4, and M5 subunits have been obtained from extrahepatic tissues and cell lines. In general, these GST share greater than 80% identity at the amino acid level, although hGSTM3 (with only about 70% identity) is more distantly related to the other class mu subunits. The hGSTM1a, M2, M4, and M5 are closely related to the rat class mu subunits, but differ significantly from their rat counterparts in their substrate specificities. The hGSTM2 subunit has highest activity with CDNB, whereas hGSTM4 is almost inactive toward this compound.[374] The hGSTM1a and M1b subunits are active with 4-NBC and *trans*-4-phenyl-3-buten-2-one, whereas hGSTM2 and M3 are unable to conjugate GSH with these compounds.[366] By contrast, hGSTM2 has high activity with DCNB, but hGSTM1a, M1b, M3, and M4 are not active with this compound.[366,374] To date, the activity of hGSTM5 has not been described. It appears possible that further human class mu GST exist. In particular, Tsuchida et al.[361] have purified several heterodimeric transferases, GST M_1M_2 and GST M_2N_1, from human aorta that are distinct from other class mu GST in having basic pI values. Furthermore, during automated amino acid sequencing GST M_1M_2 and GST M_2N_1 yielded E and Q at residue 8, rather than D or N, which are found in other class mu GST.

The class pi transferase, hGSTP1-1, has been purified from many extrahepatic organs and the older literature contains many different designations for the same enzyme: preparations from erythrocytes were called GST ρ,[376] preparations from placenta were called GST π,[377] preparations from lung were called GST λ,[216] and preparations from kidney were called YπYπ.[378] It has also been simply called acidic or anionic GST.[388] This enzyme has activity toward ethacrynic acid as a substrate, but its characteristic feature is high activity toward acrolein, crotonaldehyde, and base propenals.[26] Crystallography and SDM studies of hGSTP1-1 have shown that, although R13, K44, Q51, and Q64 all contribute to binding of GSH, R13 also has an important structural role as R13A demonstrates low stability.[246] Mutagenesis has also suggested a catalytic role for D98 as the k_{cat}/K_m-vs.-pH profile for the D98N mutant is shifted by 0.5 pH unit, suggesting that this aspartate residue participates in proton release during catalysis.

Two class theta transferases, GSTT1-1 and GSTT2-2, have been isolated from human liver. Meyer et al.[176] reported that hGSTT1-1 is inactive with CDNB, but can utilize EPNP and 4-nitrophenyl bromide as substrates. Hepatic hGSTT2-2 is also inactive with CDNB but has activity with 1-menaphthyl sulfate and cumene hydroperoxide.[381] The hGSTT1 and hGSTT2 proteins possess only about 48% sequence identity.[44,200] However, the hGSTT1 and hGSTT2 subunits are more closely related to the rGSTT1 and rGSTT2 subunits than to each other; hGSTT1 and rGSTT1 share 79% identity, whereas hGSTT2 and rGSTT2 share 78% identity.

The class theta hGSTT1 is polymorphic.[44] Red cells from individuals with the GSTT1$^+$ phenotype can conjugate GSH with methyl bromide, dichloromethane, and ethylene oxide, whereas individuals with the GSTT1$^-$ phenotype cannot catalyze these reactions,[104] suggesting that, besides EPNP and 4-nitrophenyl bromide, hGSTT1-1 is also active with methyl bromide, dichloromethane, and ethylene oxide.

G. Membrane-Bound GST Enzymes

The separate origins of the two membrane-bound GST and the cytosolic class alpha, mu, pi, sigma, and theta enzymes is emphasized by the marked differences in their subunit molecular weights. The subunit, which the microsomal GST comprises, contains 154 amino acids,[206] whereas that of LTC_4S is composed of 150 amino acids.[207,208] By contrast, the rat cytosolic GST comprise subunits containing between 209 and 244 amino acids. The fact that microsomal GST and LTC_4S subunits are not only smaller than those of the soluble enzymes but that microsomal GST and LTC_4S are also integral membrane proteins suggests that the two membrane-bound enzymes have a markedly different three-dimensional structure from the cytosolic GST. The possession of a hydrophobic *N*-terminal region in both microsomal GST and LTC_4S, which spans the membrane, makes it difficult to envisage that these enzymes contain structurally separate G- and H-sites that are similar to domains I and II of the cytosolic GST; should each subunit of either microsomal GST or LTC_4S contain both a G- and an H-site, then they must be substantially more compact than those of the cytosolic GST. Furthermore, the fact that proteolysis of microsomal GST at K41 yields a functional protein[321] indicates that the active center is contained within just 113 residues, a polypeptide almost half the size of the cytosolic enzymes. A significant functional difference exists between the G-site of the microsomal GST and that of the cytosolic enzymes, as microsomal GST can utilize *N*-acetylcysteine as a substrate.[389] As far as is known, this thiol-containing compound cannot be used by cytosolic enzymes. Chemical modification of microsomal GST suggests that histidine and arginine residues are possibly involved in the active center of this isoenzyme,[390] but further experiments using SDM are required to confirm the involvement of these residues in catalysis. It would be interesting to know whether tyrosine or serine might also be involved in the catalytic mechanism of microsomal GST, as is the case for cytosolic GST.

The microsomal GST is the major binding site for LTC_4 in cellular membranes.[391] The stoichiometry of binding indicates that one molecule of LTC_4 is bound per trimer. Although it is not known whether each subunit of microsomal GST has its own H-site, or whether the H-site is generated between the subunit interfaces, the stoichiometry of the binding of LTC_4 to microsomal GST suggests the latter possibility. The biological role of LTC_4 binding by microsomal GST is not obvious. It might sequester newly formed LTC_4 until appropriate events occur to signal release. Alternatively, LTC_4 might attenuate microsomal GST activity and govern its *in vivo* functions.

A comparison between the membrane topology of microsomal GST and LTC_4S is unfortunately not currently possible. However, Andersson et al.[392] have shown that the *N*-terminus of microsomal GST is located on the luminal side of the endoplasmic reticulum, whereas the active site faces the cytoplasm.

V. ROLE OF GST ISOENZYMES IN PROTECTION AGAINST NOXIOUS CHEMICALS AND CHEMOTHERAPEUTIC AGENTS

GST contribute to the detoxification of a number of potentially harmful chemicals that we encounter daily, either in the air we breathe, the food we eat, or the medication we receive. Table 10 lists some of the compounds detoxified by GST and indicates the subunit, if known, that is responsible for the activity.

TABLE 10
GST Isoenzyme Activities Associated with the Detoxification and Activation of Xenobiotics

Toxic chemicals	Reaction	GST subunits with activity				Ref.
		Rat	Mouse	Human	Other species	
Carcinogens/Mutagens detoxified						
N-Acetoxy-PhIP	Conjugation	A1, A2, A3, T2	—	A1, T2	—	393
Aflatoxin B_1-8,9-*exo*-epoxide	Conjugation	A5	A3	—	*Aspergillus flavus*	145, 301, 325, 394
Aflatoxin B_1-8,9-*endo*-epoxide	Conjugation	M2	—	M1	Hamster GST Yb	308, 395
Benzo[*a*]pyrene-4,5-oxide	Conjugation	M1, M2	M1	M1, P1	—	341, 359, 396
+*Anti* Benzo[*a*]pyrene-7,8-diol-9,10-oxide	Conjugation	M2, P1	P1	M1, P1	—	309, 397
+*Anti* Benz[*a*]anthracene-3,4-diol-1,2-oxide	Conjugation	M2	—	—	—	398
Benz[*a*]anthracene-5,6-oxide	Conjugation	M2	—	—	—	399
Benz[*a*]anthracene-8,9-diol-10,11-oxide	Conjugation	M2	—	—	—	399
Butadiene monoepoxide	Conjugation	Yes	Yes	Yes	—	400
+*Anti* Chrysene-1,2-diol-3,4-oxide	Conjugation	M2	—	—	—	398
5-Hydroxymethylchrysene sulfate	Conjugation	T2	—	—	—	218
DHBA-sulfate	Conjugation	T2	—	—	—	218
7-HMBA sulfate	Conjugation	T2	—	—	—	218
1-Methyl-2-nitro-1-nitrosoguanidine	Denitrosation	—	—	—	—	401
1-Nitropyrene-4,5-oxide	Conjugation	M1, M2	—	—	—	402
1-Nitropyrene-9,10-oxide	Conjugation	M1, M2	—	—	—	402
4-Nitroquinoline 1-oxide	Conjugation	M3	M1, P1	P1	—	403, 404
Carcinogens bound covalently						
Benzo[*a*]pyrene	Covalent binding	A1, A2, A3	M1, P1	—	—	405, 406
Dimethylaminoazobenzene	Covalent binding	A1, A2	—	—	—	123, 407
7,12-Dimethylbenz[*a*]anthracene	Covalent binding	—	"h-Protein"	—	—	408
5,9-Dimethyl-7H-dibenzo[*c,g*]carbazole	Covalent binding	—	—	—	—	409
3-Methylcholanthrene	Covalent binding	—	"h-Protein"	—	—	410
Environmental pollutants and pesticides detoxified						
Acetochlor	Conjugation	—	—	—	Maize	411
Acifluorfen	Conjugation	—	—	—	Soybean	412
Alachlor	Conjugation	—	—	—	Maize GST II, III, IV	166, 167, 413
Aldrin	Conjugation	—	—	—	Tobacco budworms	414
Atrazine	Conjugation	—	P1	—	Maize GST I	415–417
Azinphosmethyl	Conjugation	—	—	—	Predaceous mite, housefly, apple moth	418–420
1,4-Benzoquinone	Conjugation	—	—	—	Daphnia magna	421
Chlorimuron ethyl	Conjugation	—	—	—	Soybean	422
CuOOH	Reduction	A3, P1, T1	A3	A1, A2, T2	—	176, 221, 300, 381, 387

TABLE 10 (continued)
GST Isoenzyme Activities Associated with the Detoxification and Activation of Xenobiotics

Toxic chemicals	Reaction	GST subunits with activity				Ref.
		Rat	Mouse	Human	Other species	
DDT	Dehydrochlorinase	—	—	—	Housefly, *Drosophila* D1, mosquito	423–425
N,N-Diallyl-2-chloroacetamide	Conjugation	Yes	—	—	—	426
Diazinon	Conjugation	—	—	—	Housefly	427, 428
Dichlobenil	Conjugation	Yes	—	—	—	429
Dichlofluanid	Thiolysis	—	—	—	Strawberry	430
2,4-Dichlorophenoxyacetic acid	Binding	—	—	—	Daphnia magna	421
EPTC sulfoxide	Thiolysis	Yes	Yes	—	Maize GST	431, 432
Ethylene oxide	Conjugation	—	—	T1	—	104
Ethylparathion	—	—	—		Housefly	433
Fenoxaprop-ethyl	Conjugation	—	—	—	Barley, oat, wheat	434
Fluorodifen	Thiolysis	Yes	—	—	Pea GST	426, 435
Lindane	Conjugation	—	—	—	Housefly	427, 436, 437
Malathion	—	—	—		Housefly	433, 438
Methyl bromide	Conjugation	—	—	T1	—	104
Methyl chloride	Conjugation	—	—	T1	—	104
Methyl parathion	—	—	—		Housefly, diamondback moth	427, 439, 440
Metolachlor	Conjugation	—	—	—	Sorghum GST	441
trans, trans-Muconaldehyde	Conjugation	—	A4, M1	—	—	442
Naphthaline 1,2-oxide	Conjugation	Yes	—	—	—	59
Parathion	—	—	—		Mediterranean fruitfly, diamondback moth	439, 443
Propachlor	—	Yes	—	—	—	426
Propetamphos	—	—	—	—	—	430
Styrene oxide	Conjugation	M1, M2	—	M1	Mediterranean fruitfly	443–445
Tetrachlorvinphos	Conjugation	—	—	—	Housefly	446
trans-Stilbene oxide	Conjugation	—	—	M1	—	447
Tridiphane	Conjugation	—	—	—	Maize, housefly	428, 448
Vinyl chloride	Conjugation	—	—	—	—	59
Antibiotics detoxified/bound						
Ampicillin	Binding	—	—	—	Bacterial (*Providencia stuartii* CH114)	449
Fosfomycin	Conjugation	—	—	—	Bacterial (*Serratia marcescens*)	136, 450, 451
Penicillin	Binding	A1	—	—	—	128

Anticancer drugs detoxified[a]						
BCNU	Denitrosation	M2, Mic GST	—	M3	—	452, 453, 454
Chlorambucil	Conjugation/binding	—	A3	A1, A2	—	135, 455, 456
Cyclophosphamide	Conjugation	—	—	A1	—	457
Ethacrynic acid	Conjugation	A3, A4, P1	P1	P1	—	16, 59
Mechlorethamine	Conjugation	A3	—	—	—	458
Melphalan	Conjugation	—	A3	—	Monkey GST	459–462
mitozantrone	Conjugation	Yes	—	—	—	463
Nitrogen mustard	Conjugation	Yes	—	A1	—	457, 464
Thiotepa	Conjugation	—	—	A1, M1	—	465
Oxidation products that are detoxified[a]						
Acrolein	Conjugation	—	—	P1	—	26
Adenine propenal	Conjugation	—	—	P1	—	26
Cholesterol α-oxide	Conjugation	A1, A2	—	—	—	466
Cytosine propenal	Conjugation	—	—	P1	—	26
Dilinoleoylphosphatidylcholine OOH	Reduction	Mic GST	A4	A1, A2, A4	—	344, 353, 467, 468
DLPEH	Reduction	Mic GST	A4	A1, A2, A4	—	307, 344, 353, 468
Dilinoleoylphosphatidylglycerol OOH	Reduction	—	A4	A1, A2, A4	—	344, 468
Epoxyeicosatrienoic acid	Conjugation	M1, T1	—	—	—	469
4-Hydroxynonenal	Conjugation	A4	A4	A4	—	344, 353, 470
Linoleic acid hydroperoxide	Reduction	Mic GST	A4	A4	—	307, 344, 353
Methyl linoleate ozonide	Reduction	Mic GST	—	—	—	306, 307
Thymine propenal	Conjugation	—	—	P1	—	26
Uracil propenal	Conjugation	—	—	P1	—	26
Compounds activated by GST						
Chlorotrifluoroethane	Conjugation	Mic GST	—	—	—	471
1,4-Dibromo-2,3-epoxybutane	Conjugation	T1	—	—	—	106
Dibromomethane	Conjugation	T1	—	—	—	105
1,3-Dichloroacetone	Conjugation	T1	—	—	—	106
Dichloroacetylene	Conjugation	Mic GST	—	—	—	107
Dichloromethane	Conjugation	T1	T1	—	—	99, 101, 102, 105, 219
1,2,3,4-Diepoxybutane	Conjugation	T1	—	—	—	106
1,2-Epoxy-4-bromobutane	Conjugation	T1	—	—	—	106
Ethylenedibromide	Conjugation	T1	—	—	—	105
Hexachlorobutadiene	Conjugation	Mic GST	—	Mic GST	—	382, 472

Note: BCNU, 1,3-*bis*(2-chloroethyl)-1-nitrosourea; DHBA, 7,12-dihydroxymethylbenzo(*a*)anthracene; DLPEH, dilinoleoylphosphatidylethanolamine hydroperoxide; EPTC, *S*-ethyl *N,N*-dipropylthiocarbamate; 7-HMBA, 7-hydroxymethyl-12-methylbenz(*a*)anthracene; PhIP, 2-amino-1-methyl-6-phenylimidazo(4,5-*b*)pyridine; DDT, dichlorodiphenyltrichloroethane; CuOOH, cumene hydroperoxide; Mic GST, microsomal GST.

[a] As metabolism of several anticancer drugs (including cyclophosphamide, adriamycin, and bleomycin) can result in oxidative stress, GST are therefore also probably involved indirectly in protection against such chemotherapeutic compounds.

The biochemical basis for protection by GST includes not only conjugation reactions, but also drug sequestration. Different GST may exhibit different activities for either a specific compound or metabolites formed from the particular compound. For example, whereas rGSTM2-2 will conjugate benzo[*a*]pyrene and benz[*a*]anthracene epoxides with GSH, rGSTA1-2 is able to bind covalently metabolites of these polycyclic aromatic hydrocarbons (PAH). Furthermore, if sulfated, the sulfate esters formed from methylated PAH can serve as substrates for rGSTT2-2.[218] Thus, through the concerted actions of several isoenzymes, the GST supergene family provides several tiers of defense against toxic chemicals.

Many carcinogens are substrates for the transferases. Aflatoxin B_1, produced by the mold *Aspergillus flavus*, is arguably the most potent naturally occurring hepatocarcinogen known.[473] It is activated by the human CYP1A2 and CYP3A4 isoenzymes to the ultimate carcinogen, aflatoxin B_1 *exo*-8,9-epoxide; it may also be oxidized by CYP to a less harmful metabolite, the *endo*-8,9-epoxide.[474,475] The *exo*-8,9-epoxide can be detoxified by conjugation with GSH, a reaction catalyzed by rGSTA5 or mGSTA3. The importance of GSH-mediated detoxification can be concluded from the fact that the mouse, which is intrinsically resistant to aflatoxin B_1, expresses high levels of mGSTA3 in the liver,[325,326] whereas the rat, which is sensitive to mycotoxin, expresses much lower amounts of rGSTA5.[270]

Heterocyclic amines, such as 2-amino-1-methyl-6-phenylimidazo(4,5-*b*)pyridine (PhIP), are formed during the cooking of protein-rich foods and are regularly consumed in the Western diet. The ultimate carcinogen produced from PhIP is the *N*-acetoxy derivative, formed in humans by the combined actions of CYP1A2 and an *O*-acetylase. The *N*-acetoxy-PhIP is a substrate for class alpha and theta transferases; rGSTA1-2, rGSTA3-3, and rGSTT2-2 have activity for this carcinogen, as does hGSTA1-1 and hGSTT2-2. In an *in vitro* system, these class alpha and theta GST inhibit the binding of *N*-acetoxy-PhIP to DNA.[393]

PAH represent a major group of chemical carcinogens, first identified in coal tar and associated with several occupational cancers.[476] These compounds are commonly encountered in combustion products such as car exhaust fumes, cigarette smoke, and coal soot. PAH include the compounds benzo[*a*]pyrene, benz[*a*]anthracene, 7-methylbenz[*a*]anthracene, 7,12-dimethylbenz[*a*]anthracene, and 3-methylcholanthrene (3-MC). Most PAH require activation by CYP isoenzymes before they are able to exert their harmful effects. The ultimate carcinogens of PAH are epoxide-containing metabolites, many of which are substrates for class mu and pi GST (Table 10). The ultimate carcinogens of methylbenz[*a*]anthracene and methylchrysene are sulfate esters, formed by the combined actions of CYP and sulfotransferase isoenzymes. These reactive sulfate esters are metabolized by class theta T2 subunits.[218]

It is evident from Table 10 that, whereas much has been published about herbicide and insecticide metabolism by GST from plants and insects, little is known about the metabolism of these compounds by mammalian GST. It might be expected that the mammalian class theta enzymes, which are more similar to the GST in invertebrates than the mammalian class alpha, mu, or pi enzymes, would be the most active class of mammalian enzyme toward many of the insecticides and herbicides that are metabolized by plant and insect GST enzymes.

Several antibiotics are bound by GST but, to date, only one enzyme, a GST from *Serratia marcescens*, has been reported to conjugate GSH with the epoxide-containing antibiotic fosfomycin.[136,450,451] Mammalian

class theta T1 subunits have a preference for small epoxide-containing compounds, such as 1,2-epoxybutane, 1,2-epoxypropane, 3,3,3-trichloro-1,2-epoxypropane, and epichlorohydrin, rather than the relatively large PAH-epoxides.[291] It is therefore possible that rodent and human GSTT1-1 can metabolize fosfomycin.

The ability of GST to detoxify anticancer drugs has attracted considerable interest. Class mu and microsomal GST catalyze the denitrosation of BCNU,[452–454] whereas class alpha GST catalyze the conjugation of GSH with the nitrogen mustards chlorambucil,[455,456] mechlorethamine,[458] and melphalan.[459–462] Human GST are active toward the aldophosphamide and aziridinium metabolites of cyclophosphamide.[457] Several other alkylating agents are used in cancer chemotherapy, such as triethylenemelamine and busulphan, that may also serve as GST substrates. Anticancer drugs, such as adriamycin and bleomycin, can give rise to ROS through redox-cycling, or, in the case of cyclophosphamide, may be metabolized to reactive carbonyl-containing compounds. GST, by their ability to reduce organic hydroperoxides or conjugate α,β-unsaturated aldehydes, may be involved in protecting against the cytotoxicity of adriamycin, bleomycin, and cyclophosphamide. Class pi GST have the highest activity toward the α,β-unsaturated aldehydes acrolein and base propenals,[26] whereas rGSTA4-4 and rat GSTA(6), mGSTA4-4 and hGSTA4*-4* have the highest activity with 4-hydroxynonenal.[346,353,468,470] By contrast, the microsomal and class alpha GST exhibit the highest peroxidase activities.[175,307,468]

The transferases that are primarily involved in activation reactions include microsomal and class theta GST (Table 10). In view of the possibility that class alpha, mu, and pi GST sequester GSH conjugates,[134] it would be interesting to know whether the presence of these enzymes can modulate the toxicity of compounds that are activated by conjugation. For example, methylene chloride is a hepatocarcinogen in mice but not rats.[100,101] It is not known whether this selective toxicity is due to the high activity of murine class theta GST for methylene chloride or failure of mouse liver to express constitutively the mGSTA1 and A2 subunits.

VI. INDUCTION OF GST AS PART OF AN ADAPTIVE RESPONSE TO CHEMICAL STRESS

A. Examples of GST Induction

GST activity is increased in many organisms following exposure to foreign compounds. In the fresh water mussel, GST is inducible by increased levels of pollutants in the environment.[477] Sorghum and maize GST isoenzymes are inducible by herbicide safeners, such as *N,N*-diallyl-2,2-dichloroacetamide and 1,8-naphthalic anhydride.[166,167,478–480] GST enzymes in plant-eating insects are inducible by the phytochemicals indole-3-carbinol, indole-3-acetonitrile and flavone.[481] GST activity in the flour beetle is inducible by phenobarbital (PB), 3-MC, *trans*-stilbene oxide, and hexachlorocyclohexane.[482] GST in the house fly are inducible by PB[483] and those in *Drosophila* are inducible by pentamethylbenzene.[484] Induction of GST has been most thoroughly studied in rodents and at least 100 different chemicals have served as inducing agents of rat and mouse GST.

The diversity of the organisms in which induction has been observed, and the spectrum of xenobiotics that can serve as induc-

ing agents, suggest that GST induction is part of an adaptive response mechanism to chemical stress that is widely distributed in nature. From studies of rodents, the adaptive response to chemical stress is clearly pleiotropic in character and involves the induction of many drug-metabolizing enzymes.[17,32-40] Collectively, these detoxification enzymes provide protection against a diverse spectrum of harmful compounds. Several distinct induction mechanisms exist and different xenobiotics can cause the induction of a different subset of detoxification enzymes. Evidence suggests that, besides providing protection against chemicals of foreign origin, GST are involved in protection against oxidative stress. In plants, the hypersensitive response caused by attempted infection by nonpathogenic microorganisms results in the rapid induction of GST due to the transient accumulation of H_2O_2.[485,486] A similar oxidative burst occurs in mammals during phagocytic activation, but it is not known whether this results in GST induction. However, in selenium- and copper-deficient rats chronically exposed to increased intracellular levels of H_2O_2 because of a lack of selenium-dependent glutathione peroxidase and superoxide dismutase, marked overexpression of hepatic GST isoenzymes is observed.[487]

B. The Chemical Nature of Xenobiotics Involved in GST Induction

Rats and mice have been used extensively as models to study GST induction. The chemicals that induce GST in these animals are extremely diverse and include PAH, azo dyes, phenolic antioxidants, flavonoids, thiocarbamates, dithiolethiones, indoles, and cinnamates; Figure 16 shows the structures of a number of chemicals that induce rodent GST. From a structural point of view, these inducing agents cannot be grouped by any single chemical feature that might account for induction. Talalay and his co-workers[32-35] have pointed out that many GST inducers are Michael reaction acceptors (alkenes conjugated to electron-withdrawing functions), or are metabolized to Michael acceptors. The reactivity of these soft electrophiles suggests that they are likely to cause chemical stress within the cell and, therefore, the induction of xenobiotic-metabolizing enzymes would represent an appropriate adaptive response. Indeed, the fact that Michael reaction acceptors are potential GST substrates supports the hypothesis that GST induction by this group of chemicals is an adaptive response.

Studies of the structural features required for chemicals to serve as inducers of GST have been hampered by the fact that many transformed cell lines do not exhibit an increase in GST activity following exposure to inducing agents. However, Talalay et al.[33] found that NAD(P)H:quinone oxidoreductase (NQO), an enzyme that is frequently coordinately regulated with GST *in vivo*, is highly inducible in Hepa 1c1c7 murine hepatoma cells. Using the Hepa 1c1c7 cells as a model system, these workers obtained data suggesting that among acrylates, crotonates, and cinnamates, the possession of an electrophilic center is essential for NQO induction. Furthermore, it was found that the level of induction parallels the potency of the electron-withdrawing group. Similarly, the level of NQO induction by coumarin analogs is related to the electrophilic nature of the α,β-unsaturated carbonyl function. Induction by diphenols, phenylenediamines, and quinones requires oxidation and is ascribed to their conversion to electrophilic quinones and electrophilic quinoneimines.

As many NQO inducers also act as GST inducers, the requirement for an electro-

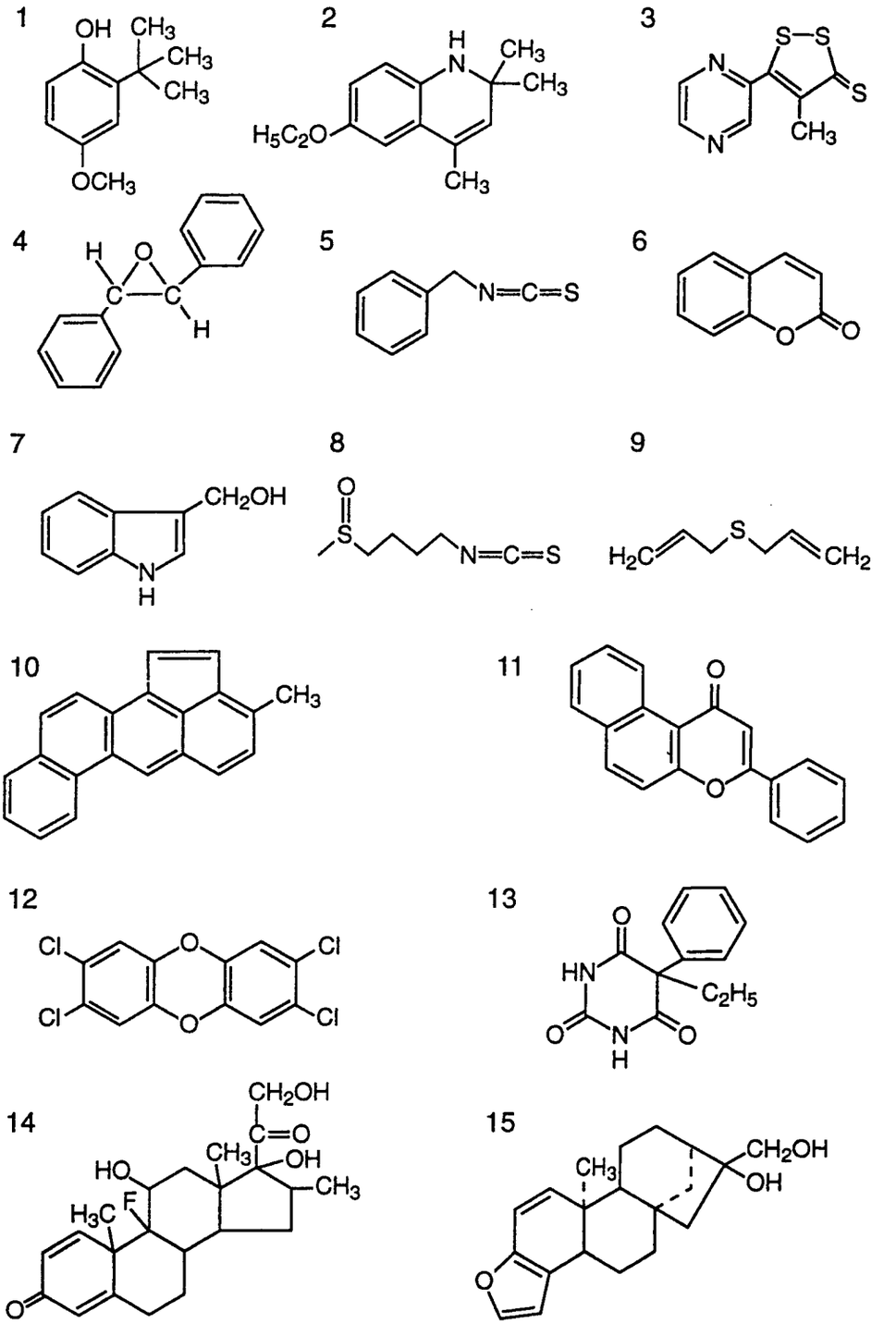


FIGURE 16. Structure of chemicals that induce GST: (1) BHA; (2) ethoxyquin; (3) oltipraz; (4) *trans*-stilbene oxide; (5) benzyl isothiocyanate; (6) coumarin; (7) indole-3-carbinol; (8) sulforaphane; (9) diallyl sulfide; (10) 3-MC; (11) β-NF; (12) tetrachlorodibenzo-*p*-dioxin; (13) PB; (14) dexamethasone; (15) kahweol.

philic center in NQO inducers would also appear to hold true for GST induction. Table 11 shows that BCNU, benzo[*a*]pyrene, DDT, hexachlorocyclohexane, *trans*-stilbene oxide, and organic isothiocyanates, which are all GST substrates, also increase GST activity in rodent organs. Thiocarbamates such as disulfiram, diethyldithiocarbamate, and bisethylxanthogen, which are GST inducers, can also form GSH conjugates. The inducer cyclophosphamide is metabolized by CYP to 4-hydroxycyclophosphamide and acrolein, both of which are substrates for class alpha and pi GST, respectively.[26,457] Similarly, *O*-deethylation and *O*-demethylation of the inducers ethoxyquin and butylated hydroxytoluene yield metabolites that can be conjugated with GSH.[530,531] Because model GST substrates serve as inducers of NQO in Hepa 1c1c7 cells[35] and dihydrodiol dehydrogenase in HT29 cells,[532-534] the existence of a broad-based adaptive response mechanism to electrophilic compounds in mammalian cells involves several enzyme systems.

When studying compounds that are subject to extensive biotransformation, it is difficult to be certain whether the parental compound or its metabolites are responsible for induction. For example, in the rat the major route of coumarin metabolism is the 3-hydroxylation pathway.[535,536] It is unclear whether induction of GST by coumarin is due entirely to the α,β-unsaturated carbonyl function of coumarin or whether generation of the electrophilic coumarin-3,4-epoxide as an intermediate during the formation of 3-hydroxycoumarin also contributes to induction. It should be possible to address this question using "gene knock-out" mice lacking CYP isoenzymes responsible for the metabolism of the inducing agent of interest.

The ability of inducing agents to generate pro-oxidant species may be equally as important as their proposed ability to form Michael reaction acceptors during the process of induction. For example, induction of GST by ethanol and by CCl_4 may occur through CYP metabolism producing ROS. Certain CYP isoenzymes, such as 2E1, are poorly coupled and are thereby likely to give rise to ROS; indeed, animals treated with inducers of CYP2E1 exhibit higher rates of H_2O_2 production.[537,538] It is therefore possible that the process of metabolizing xenobiotics, or the generation of a metabolic cascade, is an important factor in GST induction.

It is apparent that production of Michael reaction acceptors is not the only mechanism responsible for GST induction. For example, organic isothiocyanates, which can be potent inducers of GST, are not classified as Michael reaction acceptors (i.e., electrophilic alkenes). Also, GST inducers such as dexamethasone, PB and tetrachlorodibenzo-*p*-dioxin are not metabolized extensively to electrophiles nor do they generate ROS.

C. Biological Differences in GST Induction

It is apparent that the species, strain, age, sex, and organ all influence the responsiveness of rodent GST to inducing agents (Table 11). It appears likely that such biological differences in GST induction reflect variable adaptive response mechanisms that will possibly result in intrinsic differences in susceptibility to chemical insult.

Significant differences exist between the rat and mouse in the levels of GST induction achieved by certain drugs. Mouse hepatic GST activity toward CDNB is increased to a greater extent by butylated hydroxyanisole (BHA), dimethyl fumarate, ethoxyquin, oltipraz, and 1,4-*bis*[2-(3,5-dichloropyridyloxy)]benzene) (TCBOP) than rat hepatic GST. By contrast, *trans*-

TABLE 11
Induction of GST Activity in Rodents by Xenobiotics

Drug	Dose, route, and duration	Time elapsed (after last dose)	Increase in GST (enzyme activity or protein)	Species (sex in parentheses)	Organ	Ref.
Allobarbital	500 ppm in diet, oral, *ad libitum*, 14 d	Continuous	Approximate 2-fold increase in A1/A2	Fischer 344/NCr rat (m)	Liver	488
1-β-D-Arabinofuranosyl cytosine	200 mg/kg, i.p., single dose	48–96 h	1.9-fold increase with CDNB	CBA mouse (m)	Bone marrow	489
Barbital	1500 ppm in diet, oral, *ad libitum*, 14 d	Continuous	Approximate 2-fold increase in A1/A2	Fischer 344/NCr rat (m)	Liver	488
BCNU	25 mg/kg, i.p., single dose	13 d	1.3-fold increase with CDNB	CD-1 mouse (f)	Liver	490
Benzo[*a*]pyrene	50 mg/kg, orally, each day for 10 d	24 h	1.9-fold increase with CDNB	Sprague-Dawley rat (m)	Liver	491
3,4-Benzo[*a*]pyrene	6 mg/kg, 2 i.p. doses/day for 10 d	12 h	1.3-fold increase with NBC	Sprague-Dawley rat (m)	Liver	492
	3 mg/kg, 2 i.p. doses/day for 10 d	12 h	1.5-fold increase with EA	Sprague-Dawley rat (m)	Distal small intestine	493
Bisethylxanthogen	0.5% in diet, oral, *ad libitum*, 14 d	Continuous	3.6-fold increase with CDNB	CD-1 mouse (f)	Liver	494
			2.5-fold increase with CDNB	CD-1 mouse (f)	Forestomach	494
			4.0-fold increase with CDNB	CD-1 mouse (f)	Small intestine	494
Butylated hydroxyanisole	0.75% in diet, oral, *ad libitum*, 12 d	Continuous	11.1-fold increase with CDNB	CD-1 mouse (f)	Liver	337
	0.75% in diet, oral, *ad libitum*, 8 d	Continuous	2.0-fold increase with CDNB	Sprague-Dawley rat (m)	Liver	337
Butylated hydroxytoluene	0.5% in diet, oral, *ad libitum*, 14 d	Continuous	2.5-fold increase with CDNB	Fischer 344 rat (m)	Liver	495
	0.4% in diet, oral, *ad libitum*, 7 d	Continuous	3.1-fold increase with CDNB	Sprague-Dawley rat (m)	Liver	496
3,5-Di-*tert*-Butylcatechol	35 µmol, oral, gavage, daily for 5 d	24 h	4.7-fold increase with CDNB	DBA/2J mouse (f)	Liver	32
Tert-Butylhydroquinone	100 µmol, oral, gavage, daily for 5 d	24 h	2.8-fold increase with CDNB	CD-1 mouse (f)	Liver	497
			3.5-fold increase with DCNB	CD-1 mouse (f)	Glandular stomach	497
	75 µmol, oral, gavage, daily for 5 d	24 h	2.2-fold increase with CDNB	C57BL/6J mouse (f)	Liver	32
		24 h	2.7-fold increase with CDNB	DBA/2J mouse (f)	Liver	32
2-*n*-Butylthiophene	90 µmol, gavage, 3 doses on alternate days	24 h	1.8-fold increase with CDNB	A/J mouse (f)	Liver	498
			1.2-fold increase with CDNB	A/J mouse (f)	Forestomach	498
			2.2-fold increase with CDNB	A/J mouse (f)	Small intestine	498
Carbon (colloidal)	500 µl/kg single i.c. dose	6 h	5-fold increase in rGSTP1	Wistar rat (m and f)	Liver	499
Cisplatin	7.2 mg/kg, i.v., single dose	7 d	Approximate 3-fold increase in rGSTA3	Fischer 344 rat (m)	Liver	500
Clonazepam	1200 ppm in diet, *ad libitum*, 14 d	Continuous	Approximate 3-fold increase in rGSTA1/A2	Fischer 344/NCr (m)	Liver	488
Cyclophosphamide	75 mg/kg, i.p., single dose	5–8 d	2.5-fold increase with CDNB	CBA mouse (m)	Bone marrow	489
DDT	200 mg/kg, single i.p. dose	7 d	4.9-fold increase with CDNB	Wistar rat (m)	Liver	501
	500 ppm in diet, *ad libitum*, 14 d	Continuous	Approximate 2-fold increase in rGSTA1/A2	Fischer 344/NCr rat (m)	Liver	488
Dexamethasone	100 mg/kg, i.p., daily for 4 d	24 h	1.7-fold increase with DCNB	C57BL/6 mouse (f)	Liver	45
			1.8-fold increase with DCNB	DBA/2 mouse (f)	Liver	45
			2.3-fold increase with DCNB	C57BL/6 mouse (m)	Liver	45
			1.9-fold increase with DCNB	DBA/2 mouse (m)	Liver	45
Diethyldithiocarbamate	100 mg/kg, i.p., daily for 4 d	24 h	Approximate 5-fold increase in rGSTA2	Fischer 344 rat (m)	Liver	500
	0.5% in diet, oral, *ad libitum*, 14 d	Continuous	1.2-fold increase with DCNB	CD-1 mouse (f)	Liver	494
			1.6-fold increase with DCNB	CD-1 mouse (f)	Forestomach	494
			4.8-fold increase with DCNB	CD-1 mouse (f)	Small intestine	494
Diethyl maleate	500 mg/kg, single i.p. dose	24 h	1.9-fold increase with CDNB	Wistar rat (m)	Intestinal mucosa	502
Diethylnitrosamine	150 mg/kg, single i.p. dose	14 d	2.7-fold increase with CDNB	CD-1 mouse (f)	Liver	490

TABLE 11 (continued)
Induction of GST Activity in Rodents by Xenobiotics

Drug	Dose, route, and duration	Time elapsed (after last dose)	Increase in GST (enzyme activity or protein)	Species (sex in parentheses)	Organ	Ref.
5,6-Dihydro-2*H*-pyran-2-one	50 µmol, oral, gavage, daily for 5 d	24 h	2.5-fold increase with CDNB	CD-1 mouse (f)	Liver	33
			2.6-fold increase with CDNB	CD-1 mouse (f)	Forestomach	33
			4.4-fold increase with DCNB	CD-1 mouse (f)	Glandular stomach	33
Dimethyl fumarate	0.5% in diet, oral, *ad libitum*, 14 d	Continuous	4.5-fold increase with CDNB	CD-1 mouse (f)	Liver	503
			7.3-fold increase with CDNB	CD-1 mouse (f)	Forestomach	503
			6.2-fold increase with DCNB	CD-1 mouse (f)	Small intestine	503
	0.5% in diet, oral, *ad libitum*, 14 d	Continuous	1.5-fold increase with CDNB	Sprague-Dawley rat (f)	Liver	503
			6.0-fold increase with CDNB	Sprague-Dawley rat (f)	Forestomach	503
			3.4-fold increase with CDNB	Sprague-Dawley rat (f)	Small intestine	503
Dimethyl maleate	75 µmol, oral, gavage, daily for 5 d	24 h	3.6-fold increase with CDNB	CD-1 mouse (f)	Liver	33
			4.4-fold increase with CDNB	CD-1 mouse (f)	Forestomach	33
			6.2-fold increase with CDNB	CD-1 mouse (f)	Glandular stomach	33
Dimethyl itaconate	75 µmol, oral, gavage, daily for 5 d	24 h	2.8-fold increase with CDNB	CD-1 mouse (f)	Liver	33
			4.9-fold increase with DCNB	CD-1 mouse (f)	Forestomach	33
			7.8-fold increase with DCNB	CD-1 mouse (f)	Glandular stomach	33
Disulfiram	0.6% in diet, oral, *ad libitum*, 14 d	Continuous	1.7-fold increase with CDNB	ICR/Ha mouse (f)	Liver	504
			2.9-fold increase with CDNB	ICR/Ha mouse (f)	Small intestine	504
	0.5% in diet, oral, *ad libitum*, 14 d	Continuous	1.6-fold increase with DCNB	CD-1 mouse (f)	Liver	494
			2.1-fold increase with DCNB	CD-1 mouse (f)	Forestomach	494
			3.9-fold increase with DCNB	CD-1 mouse (f)	Small intestine	494
1,2-Dithiole-3-thione	0.075% in diet, oral, *ad libitum*, 7 d	Continuous	3.3-fold increase with CDNB	Fischer 344 rat (m)	Liver	505
	0.075% in diet, oral, *ad libitum*, 5 d	Continuous	2- fold increase in rGSTA2	Sprague-Dawley rat (f)	Liver	506
			3-fold increase in rGSTA3	Sprague-Dawley rat (f)	Stomach	506
			13-fold increase in rGSTA5 and M1	Sprague-Dawley rat (f)	Intestine	506
			2-fold increase in rGSTA3	Sprague-Dawley rat (f)	Lung	506
			5-fold increase in rGSTM1	Sprague-Dawley rat (f)	Kidney	506
			6-fold increase in rGSTA2	Sprague-Dawley rat (m)	Liver	506
			2-fold increase in rGSTA3	Sprague-Dawley rat (m)	Stomach	506
			10-fold increase in rGSTA5 and M1	Sprague-Dawley rat (m)	Intestine	506
			2-fold increase in rGSTA3	Sprague-Dawley rat (m)	Lung	506
			10-fold increase in rGSTM1	Sprague-Dawley rat (m)	Kidney	506
Erucin	15 µmol, oral, gavage, daily for 5 d	24 h	1.9-fold increase with CDNB	CD-1 mouse (f)	Liver	507
			2.5-fold increase with CDNB	CD-1 mouse (f)	Forestomach	507
			3.0-fold increase with CDNB	CD-1 mouse (f)	Small intestine	507
Erysolin	5 µmol, oral, gavage, daily for 5 d	24 h	1.1-fold increase with CDNB	CD-1 mouse (f)	Liver	507
			1.5-fold increase with CDNB	CD-1 mouse (f)	Forestomach	507
Ethanol	36% of calories, oral, dietary for 7 d	Continuous	1.8-fold increase with DCNB	Swiss-Webster mouse (m)	Liver	508

Ethoxyquin	0.5% in diet, oral, *ad libitum*, 12 d	Continuous	6.6-fold increase with DCNB	CD-1 mouse (f)	Liver	337
	0.5% in diet, oral, *ad libitum*, 7 d	Continuous	2.8-fold increase with CDNB	C57BL/6NCR mouse (m)	Liver	509
			2.9-fold increase with CDNB	DBA/2NCR mouse (m)	Liver	509
			5.3-fold increase with CDNB	SJL/JCR mouse (m)	Liver	509
	0.4% in diet, oral, *ad libitum*, 14 d	Continuous	4.1-fold increase with CDNB	Sprague-Dawley rat (f)	Liver	503
			1.2-fold increase with CDNB	Sprague-Dawley rat (f)	Forestomach	503
			3.0-fold increase with CDNB	Sprague-Dawley rat (f)	Small intestine	503
			1.1-fold increase with CDNB	Sprague-Dawley rat (f)	Lung	503
			3.2-fold increase with CDNB	Sprague-Dawley rat (f)	Kidney	50?
			4.8-fold increase with CDNB	Fischer 344 rat (m)	Liver	51
	0.5% in diet, oral, *ad libitum*, 5 d	Continuous	4.0-fold increase with CDNB	Fischer 344 rat (m)	Liver	3(
			1.5-fold increase with CDNB	Fischer 344 rat (m)	Lung	3(
			2.4-fold increase with CDNB	Fischer 344 rat (m)	Kidney	36
	200 mg/kg, i.p., daily for 3 d	24 h	1.9-fold increase with CDNB	Sprague-Dawley rat (m)	Liver	511
			2.5-fold increase with EA	Sprague-Dawley rat (m)	Kidney	511
5-Ethyl-5-phenylhydantoin	500 ppm in diet, oral, *ad libitum*, 14 d	Continuous	Approximate 2-fold increase in rGSTA1/A2	Fischer 344/NCr rat (m)	Liver	488
2-*n*-Heptylfuran	50 µmol, gavage, 3 doses on alternate days	24 h	2.0-fold increase with CDNB	A/J mouse (f)	Liver	498
			1.5-fold increase with CDNB	A/J mouse (f)	Forestomach	498
			2.7-fold increase with CDNB	A/J mouse (f)	Small intestine	498
	80 µmol, gavage, 3 doses on alternate days	24 h	1.4-fold increase with CDNB	A/J mouse (f)	Liver	498
			1.6-fold increase with CDNB	A/J mouse (f)	Forestomach	498
			6.1-fold increase with CDNB	A/J mouse (f)	Small intestine	498
α-Hexachlorocyclohexane	320 ppm in diet, oral *ad libitum*, 14 d	Continuous	Approximate 5-fold increase in rGSTA1/A2	Fischer 344/NCr rat (m)	Liver	488
γ-Hexachlorocyclohexane	360 ppm in diet, for 3 months	Continuous	5.8-fold increase with CDNB	CF1 mouse (f)	Liver	512
			1.9-fold increase with CDNB	CF1 mouse (m)	Liver	512
Hexachlorobenzene	0.1% in diet, oral, *ad libitum*, 14 d	Continuous	3.5-fold increase with CDNB	Wistar rat (m)	Liver	513
3,4,5,3′,4′,5′-Hexachlorobiphenyl	50 mg/kg, single oral dose	72 h	1.4-fold increase with SO	Charles River rat (m)	Liver	514
			2.0-fold increase with BPO	Charles River rat (m)	Liver	514
2,4,5,2′,4′,5′-Hexachlorobiphenyl	50 mg/kg, single oral dose	72 h	1.5-fold increase with SO	Charles River rat (m)	Liver	514
			1.4-fold increase with BPO	Charles River rat (m)	Liver	514
	30 mg/kg, 2 i.p. doses on days 1 and 4	3 d	Approximate 2-fold increase in rGSTA1/A2	Fischer 344/NCr rat (m)	Liver	488
2,3,5,2′,3′,5′-Hexachlorobiphenyl	50 mg/kg, single oral dose	72 h	1.5-fold increase with SO	Charles River rat (m)	Liver	514
			1.5-fold increase with BPO	Charles River rat (m)	Liver	514
Interferon-α/β	2×10^5 mouse EA cell interferon, s.c.		2.2-fold increase with EA	nu/nu BALB/c mouse (f)	Liver	343
Iproplatin	30 mg/kg, single i.v. dose	7 d	Approximate 3-fold increase in rGSTA3	Fischer 344 rat (m)	Liver	500
Isosafrole	150 mg/kg, i.p., 3 d	24 h	4-fold increase in rGSTA1 and A2	Fischer 344 rat (m)	Liver	500
Lead acetate	100 µmol/kg, single i.c. injection	2–8 d	10-fold increase in rGSTP1	Wistar rat (m and f)	Liver	499
Lead acetate (low Ca^{++})	500 ppm in drinking water, 3 weeks	Continuous	30-fold increase in rGSTM1	Sprague-Dawley rat	kidney	515
			12-fold increase in rGSTP1	Sprague-Dawley rat	Kidney	515
p-Methoxyphenol	0.36% in diet, oral, *ad libitum*, 14 d	Continuous	2.3-fold increase with CDNB	ICR/Ha mouse (f)	Osophagus	516

TABLE 11 (continued)
Induction of GST Activity in Rodents by Xenobiotics

Drug	Dose, route, and duration	Time elapsed (after last dose)	Increase in GST (enzyme activity or protein)	Species (sex in parentheses)	Organ	Ref.
Methyl acrylate	25 µmol, oral, gavage, daily for 5 d	24 h	1.6-fold increase with CDNB	CD-1 mouse (f)	Liver	33
			3.0-fold increase with DCNB	CD-1 mouse (f)	Forestomach	33
			4.7-fold increase with DCNB	CD-1 mouse (f)	Glandular stomach	33
3-Methylcholanthrene	20 mg/kg, i.p., daily for 4 d	24 h	2.1-fold increase with t-PBO	NMRI mouse (m)	Liver	517
	20 mg/kg, i.p., daily for 5 d	24 h	2.2-fold increase with CDNB	Sprague Dawley rat (m)	Liver	518
	30 mg/kg, i.p., daily for 3 d	24 h	1.7-fold increase with CDNB	Wistar rat (m)	Liver	513
	40 mg/kg, oral, daily for 14 d	Continuous	1.6-fold increase with CDNB	Sprague Dawley rat (m)	Liver	519
			1.3-fold increase with CDNB	Sprague Dawley rat (m)	Small intestine	519
	20 mg/kg, 3 i.p. doses on each alternate day for 7 d	24 h	2.3-fold increase with CDNB	Sprague Dawley rat (f)	Liver	520
			1.9-fold increase with CDNB	Sprague Dawley rat (m)	Liver	520
2-Methylene-4-butyrolactone	50 µmol, oral, gavage, daily for 5 d	24 h	2.3-fold increase with CDNB	CD-1 mouse (f)	Liver	33
			2.3-fold increase with CDNB	CD-1 mouse (f)	Forestomach	33
			3.3-fold increase with DCNB	CD-1 mouse (f)	Glandular stomach	33
Methyl selenocyanate	2 mg/kg, injection		Approximate 2-fold increase in GST	Rat	Liver	521
Musk xylene	200 mg/kg, i.p. daily for 5 d	24 h	1.9-fold increase with CDNB	Wistar rat (m)	Liver	522
β-NF	5 µmol, i.p., daily for 5 d	24 h	2.2-fold increase with CDNB	C57BL/6J mouse (f)	Liver	32
			1.3-fold increase with CDNB	DBA/2J mouse (f)	Liver	32
	0.2% in diet, oral for 2 weeks	Continuous	2.0-fold increase with CDNB	ICR/Ha mouse (f)	Liver	504
			3.9-fold increase with CDNB	ICR/Ha mouse (f)	Small intestine	504
Oltipraz	4 mmol/kg, single i.g. dose	48 h	7.5-fold increase with CDNB	CD-1 mouse (f)	Liver	523
			2.5-fold increase with CDNB	CD-1 mouse (f)	Small intestine	523
			1.6-fold increase with CDNB	CD-1 mouse (f)	Lung	523
			1.8-fold increase with CDNB	CD-1 mouse (f)	kidney	523
	0.1% in diet, oral, *ad libitum*, 14 d	Continuous	3.3-fold increase with CDNB	Fischer 344 rat (m)	Liver	523
	0.075% in diet, oral, *ad libitum*, 7 d	Continuous	2.8-fold increase with CDNB	Fischer 344 rat (m)	Liver	505
PB	80 mg/kg, i.p., daily for 4 d	24 h	1.3-fold increase with CDNB	NMRI mice (m)	Liver	517
	0.2% in diet, oral, *ad libitum*, 4 d	Continuous	2.6-fold increase with CDNB	NMRI mice (m)	Liver	517
	300 ppm in diet, oral, *ad libitum*, 14 d	Continuous	Approximate 4-fold increase in rGSTA1/A2	Fischer 344/NCr rat (f)	Liver	524
			Approximate 1.5-fold increase in rGSTA1/A2	Zucker Lean rat (f)	Liver	524
	60 mg/kg, i.p., daily for 7 d	24 h	2.0-fold increase with CDNB	Sprague-Dawley rat (f)	Liver	520
			2.6-fold increase with CDNB	Sprague-Dawley rat (m)	Liver	520
	0.1% in drinking water, *ad libitum*, 7 d	Continuous	2.2-fold increase with CDNB	Wistar rat (m)	Liver	513
Phenylbutyl isothiocyanate	1.0 mmol/kg, single i.g. dose	24 h	1.4-fold increase with CDNB	Fischer 344 rat (m)	Liver	525
Phenylhexyl isothiocyanate	1.0 mmol/kg, single i.g. dose	24 h	1.3-fold increase with CDNB	Fischer 344 rat (m)	Liver	525
Phorone	250 mg/kg, single s.c. dose	24 h	1.1-fold increase with CDNB	Wistar rat (m)	Liver	502
			2.7-fold increase with CDNB	Wistar rat (m)	Small intestine	502
Polychlorinated biphenyl(s)	500 mg/kg, single i.p. dose	138 h	3.4-fold increase with CDNB	Long Evans rat (m)	Liver	526

Propylthiouracil	1.5 mmol/kg, i.p., 14 d	24 h	1.7-fold increase with CDNB	Sprague Dawley rat	Liver	527
Rifampicin	20 mg/kg, oral, daily for 5 d	24 h	2.2-fold increase with CDNB	Wistar rat (m)	Liver	501
Trans-Stilbene oxide	400 mg/kg, i.p., daily for 4 d	24 h	1.3-fold increase with CDNB	NMRI mouse (m)	Liver	517
	400 mg/kg, i.p., daily for 5 d	24 h	3.7-fold increase with CDNB	Sprague Dawley rat (m)	Liver	518
Streptozotocin	200 mg/kg, i.p., single dose	15 d	2.4-fold increase with CDNB	CD-1 mouse (f)	Liver	490
Sudan I	56.8 µmol/kg, single i.g. dose	42 h	1.5-fold increase with CDNB	Long Evans rat (m)	Liver	526
Sudan III	5 µmol, i.p., daily for 5 d	24 h	1.5-fold increase with CDNB	C57BL/6J mouse (f)	Liver	32
			0.9-fold increase with CDNB	DBA/2J mouse (f)	Liver	32
	56.8 µmol/kg, single i.g. dose	42 h	1.6-fold increase with CDNB	Long Evans rat (m)	Liver	526
Sudan IV	56.8 µmol/kg, single i.g. dose	42 h	1.4-fold increase with CDNB	Long Evans rat (m)	Liver	526
TCBOP	3 mg/kg, single i.p. injection	2-4 weeks	3.8-fold increase with DCNB	C57BL/6 mouse (f)	Liver	45
			2.6-fold increase with DCNB	DBA/2 mouse (f)	Liver	45
			4.0-fold increase with DCNB	C57BL/6 mouse (m)	Liver	45
			3.5-fold increase with DCNB	DBA/2 mouse (m)	Liver	45
Tetrachlorodibenzo-*p*-dioxin	10 µg/kg, two i.p. injections on days 1 and 7	6 D	1.5-fold increase with CDNB	SPF-Wistar rat (m)	Liver	528
2,3,5,6-Tetrafluorophenol	0.3% in diet, oral for 2 weeks	Continuous	1.1-fold increase with CDNB	ICR/Ha mouse (f)	Liver	504
	0.4% in diet, oral for 2 weeks	Continuous	1.2-fold increase with CDNB	ICR/Ha mouse (f)	Forestomach	516
1-(2-Thiazolylazo)-2-naphthol	5 µmol, i.p., daily for 5 d	24 h	2.6-fold increase with CDNB	C57BL/6J mouse (f)	Liver	32
			1.9-fold increase with CDNB	DBA/2J (f)	Liver	32
Vinylidene chloride	50 ppm in air for 6 h each day for 8 d	24 h	1.3-fold increase with CDNB	Swiss Webster mouse (f)	Liver	529
			1.4-fold increase with CDNB	Swiss Webster mouse (m)	Liver	529
	200 ppm in air for 6 h each day for 8 d	24 h	1.7-fold increase with CDNB	Sprague Dawley rat (m)	Liver	529

Note: The change in GST is expressed as a ratio of specific activities of treated to control. Duration of treatment is given in days (d) or hours (h). Route of drug administration: i.c., intracardiac; i.g., intragastric; i.p., intraperitoneal; i.v., intravenous; s.c., subcutaneous. BCNU, 1,3-*bis*(2-chloroethyl)-1-nitrosourea; BPO, benzo[*a*]pyrene-4,5-oxide; CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene; DDT, dichlorodiphenyltrichloroethane; EA, ethacrynic acid; β-NF, β-naphthoflavone; NBC, 4-nitrobenzylchloride; tPBO, *trans*-4-phenyl-3-buten-2-one; TCBOP, 1,4-*bis*[2-(3,5-dichloropyridyloxy)]benzene; SO, styrene-7,8-oxide; PB, phenobarbital.

stilbene oxide is a better inducer of GST in the rat than in the mouse.

Strain differences in GST induction in the mouse have been studied primarily with a view to establishing whether the arylhydrocarbon (Ah)-receptor is involved in the regulation of GST. To this end, the C57BL/6 mouse, which possesses a functional Ah-receptor, and the DBA/2 mouse, which lacks a functional Ah-receptor, have been investigated, and it has been found that the responsiveness of hepatic GST in these two inbred mouse strains varies with the inducing agent. The greatest contrast between the hepatic levels of GST induction in these two strains of mice is observed following treatment with β-naphthoflavone (β-NF) and the azo dye sudan III,[32] both of which show substantially greater levels of induction in C57BL/6 than in DBA/2 mice (Table 11). However, the C57BL/6 mouse also shows a modestly greater increased level of induction of GST by 1-(2-thiazolylazo)-2-naphthol and TCBOP than is observed in the DBA/2 mouse. Little difference is observed in the levels of GST induction in C57BL/6 and DBA/2 mice following treatment with *tert*-butylhydroquinone (tBHQ), dexamethasone, or ethoxyquin. On the basis of these data, it is thought that induction of GST by tBHQ and ethoxyquin is independent of the Ah-receptor, but that induction of GST by β-NF and sudan III requires a functional Ah-receptor; as discussed below, it should be noted that a metabolite of β-NF appears to be capable of induction of GST by a mechanism independent of the Ah-receptor.

Other strain differences in induction have been observed that are independent of the Ah-receptor. A larger increase in the level of GST enzyme activity following ethoxyquin treatment has been reported in the SJL/JCR mouse[509] when compared with either C57BL/6 DBA/2 mice. Although the molecular basis for this strain difference in induction is not known, it appears to be due to lower basal levels of GST in SJL/JCR mice rather than to the production of abnormally elevated GST levels by ethoxyquin. In the rat, a lesser response to induction by PB has been observed in the livers of Zucker rats than in Fischer 344 rats,[524] but the basis for this difference is not certain.

The responsiveness of GST in rat liver toward PB is greatest in animals of about 4 weeks of age.[539] Our laboratory has studied the induction of GST by 3-MC on several occasions and variable results have been obtained. Although a marked induction of rGSTA5 is apparent in rats of 2 to 3 months of age, it has been difficult to demonstrate induction of GST in rats of about 8 months of age.[495]

The level of GST induction can be influenced by the sex of the animal. Both PB and 1,2-dithiole-3-thione are better GST inducers in male than in female Sprague-Dawley rats,[506,520] whereas 3-MC is a slightly better inducer of hepatic GST in female than male Sprague-Dawley rats.[520] In the mouse, GST activity is increased to a greater extent by BHA in female than in male livers. In this context, it is important to remember that basal expression of GST differs in male and female mice,[340] and the sexual dimorphism in the expression of class pi GST largely disappears in BHA-treated mice.[540]

The route of drug administration is an important factor in determining the levels of GST induction observed in different organs. For example, hepatic GST activity in the mouse is increased to a much greater extent by PB administered orally than by the barbiturate administered intravenously. When given orally, inducing agents frequently produce a greater increase in GST levels in the stomach or small intestine than in the liver; bisethylxanthogen, 2-*n*-butylthiophene, diethyldithiocarbamate, dimethyl

fumarate, disulfiram, erucin, methyl acrylate, and methylene-4-butyrolactone increase GST activity to a greater extent in the stomach and small intestine than the liver.[33,494,498,503,507]

D. Natural Chemicals Responsible for GST Induction

Components in the diet can influence susceptibility to carcinogenesis and a large number of nonnutrient phytochemicals have induced GST in rodents.[541] The most active naturally occurring GST inducers are α-angelicalactone, allyl isothiocyanate, allyl methyl disulfide, benzyl isothiocyanate, *n*-butyl phthalide, cafestol, β-caryophyllene, coumarin, flavone, indole-3-acetonitrile, kahweol, D-limonene, nomilin, sedanolide, and valencene (Table 12). The majority of these compounds cause maximal increase of GST activity in the small intestine with lower levels of GST induction in the liver, lung, and stomach. However, *n*-butyl phthalide, coumarin, flavone, indole-3-acetonitrile, and D-limonene induce GST activity more in the liver than in the intestine.

Dietary lipid can influence GST activity significantly. Hietanen et al.[559] showed that feeding rats a diet containing 2% cholesterol results in a 1.8-fold increase in hepatic GST activity toward styrene oxide. Furthermore, these workers reported that 2% dietary cholesterol enhances induction of GST by PB and CCl_4. The effect of dietary cholesterol on GST may be indirect as it increases significantly the levels of CYP, epoxide hydrolase, and UDP-glucuronosyl transferase in rat liver.

For ethical reasons, few studies have been reported demonstrating induction of GST in humans. In healthy volunteers who consumed 300 g of Brussels sprouts per day for 3 weeks, a 1.4-fold increase in the plasma levels of class alpha GST has been reported;[560] it was suggested that the increase in plasma GST reflected increased *de novo* synthesis of GST in liver rather than hepatotoxicity. In a follow-up study, rectal biopsies taken from volunteers placed for 1 week on a diet containing 300 g of Brussels sprouts per day were found to contain a 1.3-fold greater increase in class alpha GST than biopsies from volunteers placed on a glucosinolate-free diet for a similar length of time.[561] Although the inducing agent was not identified in these studies, Brussels sprouts contain significant amounts of allyl isothiocyanate and goitrin, substances that induce rodent GST.

E. Induction of GST Subunits

Not all GST subunits are induced to the same extent by drugs. Following treatment of rats with xenobiotics, it is generally found that the hepatic concentration of the rGSTA2 and M1 subunits are increased to the greatest amount, whereas the rGSTA1 and A3 subunits show a less dramatic increase. By contrast, xenobiotics usually elicit only modest increases in the levels of rGSTA4 and M2 in liver. The rGSTA5 and P1 subunits, which are expressed at relatively low levels in the liver of adult male rats, can be induced dramatically by certain chemicals; the synthetic antioxidant ethoxyquin and the naturally occurring lactone coumarin are good inducers of hepatic rGSTA5 and P1, respectively. Meyer et al.[506] examined GST subunit induction by 1,2-dithiole-3-thione in intestine, kidney, liver, lung, and stomach and showed that the pattern of induction varies significantly in different organs, but that induction could be demonstrated in all organs studied. Microsomal GST does not appear to be inducible by xenobiotics.

Relatively little is known about GST subunit induction in the mouse. Substantial induction of mGSTM1 is observed in the liver following treatment with BHA, PB,

TABLE 12
Induction of GST Activity in Rodents by Naturally Occurring Dietary Constituents

Inducer	Source	Dose and duration	Time elapsed	Increase in GST (enzyme activity)	Species (and sex)	Organ	Ref.
Trans-Anethole	Fennel, anise, star anise	250 mg/kg, i.g. daily for 10 d	24 h	1.4-fold with CDNB	Rat	Liver	542
α-Angelicalactone	*Archangelica officinalis*	0.3% in diet, oral for 2 weeks	Continuous	4.2-fold with CDNB	ICR/Ha mouse (f)	Liver	504
				2.0-fold with CDNB	ICR/Ha mouse (f)	Forestomach	516
				2.5-fold with CDNB	ICR/Ha mouse (f)	Small intestine	504
		0.5% in diet, oral for 2 weeks	Continuous	2.0-fold with CDNB	Wistar rat (m)	Liver	543
				1.5-fold with CDNB	Wistar rat (m)	Esophagus	544
				2.3-fold with CDNB	Wistar rat (m)	Stomach	544
				2.9-fold with CDNB	Wistar rat (m)	Small intestine	543
				1.2-fold with CDNB	Wistar rat (m)	Pancreas	544
				1.4-fold with CDNB	Wistar rat (m)	Large intestine	543
Allyl methyl disulfide	Garlic oil	40 µmol, 2 i.g. doses 2 d apart	48 h	1.8-fold with CDNB	A/J mouse (f)	Liver	545
				2.1-fold with CDNB	A/J mouse (f)	Forestomach	545
				3.6-fold with CDNB	A/J mouse (f)	Small intestine	545
				1.6-fold with CDNB	A/J mouse (f)	Lung	545
Allyl methyl trisulfide	Garlic oil	15 µmol, 2 i.g. doses 2 d apart	48 h	1.9-fold with CDNB	A/J mouse (f)	Liver	546
				1.6-fold with CDNB	A/J mouse (f)	Forestomach	546
				2.1-fold with CDNB	A/J mouse (f)	Small intestine	546
				1.6-fold with CDNB	A/J mouse (f)	Lung	546
Allyl isothiocyanate	Brussels sprouts	0.1% in diet, oral for 4 weeks	Continuous	2.2-fold with CDNB	Fischer 344 rat (m)	Liver	547
				3.9-fold with CDNB	Fischer 344 rat (m)	Small intestine	547
Benzyl isothiocyanate	Garden cress[a]	0.45% in diet, oral for 2 weeks	Continuous	3.2-fold with CDNB	ICR/Ha mouse (f)	Liver	504
				4.4-fold with CDNB	ICR/Ha mouse (f)	Small intestine	504
		0.45% in diet, oral for 2 weeks	Continuous	2.5-fold with CDNB	ICR/Ha mouse (f)	Forestomach	516
		0.5% in diet, oral for 2 weeks	Continuous	2.0-fold with CDNB	Wistar rat (m)	Liver	513
n-Butyl phthalide	Celery seed oil	20 mg/ 2 d, 3 i.g. doses over 6 d	24 h	5.9-fold with CDNB	A/J mouse (f)	Liver	548
				2.0-fold with CDNB	A/J mouse (f)	Forestomach	548
				4.3-fold with CDNB	A/J mouse (f)	Small intestine	548
Cafestol	Green coffee beans	10 µmol, i.g. daily for 3 d	24 h	2.0-fold with CDNB	ICR/Ha mouse (f)	Liver	549
				1.2-fold with CDNB	ICR/Ha mouse (f)	Forestomach	549
				3.8-fold with CDNB	ICR/Ha mouse (f)	Small intestine	549
				1.0-fold with CDNB	ICR/Ha mouse (f)	Lung	549
Cafestol acetate	Green coffee beans	2.5 mg/animal, single i.g. dose	28 h	1.6-fold with CDNB	ICR/Ha mouse (f)	Small intestine	550
β-Caryophyllene	Orange oil	10% in diet, oral for 10 d	24 h	5-fold with CDNB	ICR/Ha mouse (f)	Liver	551
				6-fold with CDNB	ICR/Ha mouse (f)	Small intestine	551
Coumarin	*Leguminosae* spp.	0.45% in diet, oral for 2 weeks	Continuous	5.3-fold with CDNB	ICR/Ha mouse (f)	Liver	504
				1.7-fold with CDNB	ICR/Ha mouse (f)	Small intestine	504
		0.45% in diet, oral for 2 weeks	Continuous	1.8-fold with CDNB	ICR/Ha mouse (f)	Forestomach	516
		0.25% in diet, oral for 2 weeks	Continuous	2.4-fold with CDNB	Wistar rat (m)	Liver	543
				2.4-fold with CDNB	Wistar rat (m)	Esophagus	544
				1.4-fold with CDNB	Wistar rat (m)	Stomach	544
				1.8-fold with CDNB	Wistar rat (m)	Small intestine	543
				1.1-fold with CDNB	Wistar rat (m)	Pancreas	544
				1.2-fold with CDNB	Wistar rat (m)	Large intestine	543

Curcumin	Turmeric	1% in diet, oral for 2 weeks	Continuous	1.1-fold with CDNB	Wistar rat (m)	Liver	543
				1.5-fold with CDNB	Wistar rat (m)	Small intestine	543
Cyanohydroxybutene	Cruciferous vegetables	100 mg/kg, i.g. daily for 7 d	24 h	1.7-fold with CDNB	Wistar rat (m)	Liver	552
				2.7-fold with CDNB	Wistar rat (m)	Pancreas	552
Diallyl sulfide	Garlic oil	20 mmol, 2 i.g. doses 2 d apart	48 h	1.2-fold with CDNB	A/J mouse (f)	Liver	545
				1.3-fold with CDNB	A/J mouse (f)	Forestomach	545
				1.5-fold with CDNB	A/J mouse (f)	Small intestine	545
				1.1-fold with CDNB	A/J mouse (f)	Lung	545
Diallyl trisulfide	Garlic oil	20 µmol, 2 i.g. doses 2 d apart	48 h	1.4-fold with CDNB	A/J mouse (f)	Liver	545
				1.9-fold with CDNB	A/J mouse (f)	Forestomach	545
				2.1-fold with CDNB	A/J mouse (f)	Small intestine	545
				1.3-fold with CDNB	A/J mouse (f)	Lung	545
Ellagic acid	Grapes, strawberries	50 mg/kg, i.p. dose daily for 5 d	24 h	1.4-fold with CDNB	C57BL/6N mouse (f)	Liver	553
				1.3-fold with CDNB	DBA/2N mouse (f)	Liver	553
				1.4-fold with CDNB	BALB/c mouse (f)	Liver	553
		1% in diet, oral for 2 weeks	Continuous	1.4-fold with CDNB	Wistar rat (m)	Liver	543
				1.1-fold with CDNB	Wistar rat (m)	Esophagus	544
				1.1-fold with CDNB	Wistar rat (m)	Stomach	544
				1.9-fold with CDNB	Wistar rat (m)	Small intestine	543
				1.1-fold with CDNB	Wistar rat (m)	Pancreas	544
				1.2-fold with CDNB	Wistar rat (m)	Large intestine	543
Eugenol	Cloves, cinnamon, basil	1 g/kg, i.g. daily for 10 d	24 h	1.5-fold with CDNB	Rat	Liver	542
Ferulic acid	Plums, apple, cabbage	1% in diet, oral for 2 weeks	Continuous	1.4-fold with CDNB	Wistar rat (m)	Liver	543
				1.4-fold with CDNB	Wistar rat (m)	Small intestine	543
				1.2-fold with CDNB	Wistar rat (m)	Large intestine	543
Flavanone	Citrus fruit	0.2 mmol/kg, i.p. daily for 7 d	24 h	2.4-fold with DCNB	Fischer 344 rat	Liver	554
Flavone	Citrus fruit	0.25% in diet, oral for 2 weeks	18 h	2.8-fold with CDNB	SPF Wistar rat (m)	Liver	555
		0.5% in diet, oral for 2 weeks	Continuous	3.1-fold with CDNB	Wistar rat (m)	Liver	543
				1.3-fold with CDNB	Wister rat (m)	Esophagus	544
				0.9-fold wih CDNB	Wistar rat (m)	Stomach	544
				1.9-fold with CDNB	Wistar rat (m)	Small intestine	543
				1.0-fold with DCNB	Wistar rat (m)	Pancreas	544
				1.2-fold with CDNB	Wistar rat (m)	Large intestine	543
Goitrin	Brussels sprouts	0.02% in diet, oral for 4 weeks	Continuous	2.3-fold with CDNB	Fischer rat (m)	Liver	547
Indole-3-acetonitrile	Cruciferous vegetables	0.6% in diet, oral for 2 weeks	Continuous	3.6-fold with CDNB	ICR/Ha mouse (f)	Liver	504
				1.8-fold with CDNB	ICR/Ha mouse (f)	Small intestine	504
Indole-3-carbinol	Cruciferous vegetables	0.6% in diet, oral for 2 weeks	Continuous	2.8-fold with CDNB	ICR/Ha mouse (f)	Liver	504
				3.5-fold with CDNB	ICR/Ha mouse (f)	Small intestine	504
		0.5% in diet, oral for 2 weeks	Continuous	1.4-fold with CDNB	Wistar rat (m)	Liver	556
				1.6-fold with CDNB	Wistar rat (m)	Small intestine	556
Kahweol	Green coffee beans	10 µmol, i.g. daily for 3 d	24 h	3.0-fold with CDNB	ICR/Ha mouse (f)	Liver	549
				2.1-fold with CDNB	ICR/Ha mouse (f)	Forestomach	549
				7.3-fold with CDNB	ICR/Ha mouse (f)	Small intestine	549
				1.0-fold with CDNB	ICR/Ha mouse (f)	Lung	549
Kahweol acetate	Green coffee beans	2.5 mg/animal, single i.g. dose	28 h	5.0-fold with CDNB	ICR/Ha mouse (f)	Small intestine	550
Kahweol palmitate	Green coffee beans	2.5 mg/animal, single i.g. dose	28 h	5.5-fold with CDNB	ICR/Ha mouse (f)	Small intestine	550
D-Limonene	Citrus fruit oils	20 mg/2 d, 3 i.g. doses over 6 d	24 h	2.8-fold with CDNB	A/J mouse (f)	Liver	548
				1.1-fold with CDNB	A/J mouse (f)	Forestomach	548
				1.6-fold with CDNB	A/J mouse (f)	Small intestine	548

TABLE 12 (continued)
Induction of GST Activity in Rodents by Naturally Occurring Dietary Constituents

Inducer	Source	Dose and duration	Time elapsed	Increase in GST (enzyme activity)	Species (and sex)	Organ	Ref.
Limonin	Grapefruit seeds	10 mg, 3 i.g. doses on alternate days	24 h	1.4-fold with CDNB	ICR/Ha mouse (f)	Liver	557
				0.9-fold with CDNB	ICR/Ha mouse (f)	Forestomach	557
				1.4-fold with CDNB	ICR/Ha mouse (f)	Small intestine	557
p-Mentha-2,8-dien-1-ol	Celery seed oil	20 mg/2 d, 3 i.g. doses over 6 d	24 h	3.7-fold with CDNB	A/J mouse (f)	Liver	548
				1.6-fold with CDNB	A/J mouse (f)	Forestomach	548
				3.7-fold with CDNB	A/J mouse (f)	Small intestine	548
p-Mentha-8(9)-en-1,2-diol	Celery seed oil	20 mg/2 d, 3 i.g. doses over 6 d	24 h	1.7-fold with CDNB	A/J mouse (f)	Liver	548
				0.9-fold with CDNB	A/J mouse (f)	Forestomach	548
				2.7-fold with CDNB	A/J mouse (f)	Small intestine	548
Nomilin	Grapefruit seeds	10 mg, 3 i.g. doses on alternate days	24 h	3.4-fold with CDNB	ICR/Ha mouse (f)	Liver	557
				1.2-fold with CDNB	ICR/Ha mouse (f)	Forestomach	557
				4.2-fold with CDNB	ICR/Ha mouse (f)	Small intestine	557
Phenethyl isothiocyanate	Turnips, water cress*	1 mmol/kg, single i.g. dose	24 h	1.4-fold with CDNB	Fischer rat (m)	Liver	525
Quercetin	Citrus fruit	1% in diet, oral for 2 weeks	18 h	1.2-fold with CDNB	SPF Wistar rat	Liver	555
		1% in diet, oral for 2 weeks	Continuous	1.0-fold with CDNB	Wistar rat (m)	Liver	543
				1.2-fold with CDNB	Wistar rat (m)	Small intestine	543
				1.8-fold with CDNB	Wistar rat (m)	Large intestine	543
Sedanolide	Celery seed oil	20 mg/2 d, 3 i.g. doses over 6 d	24 h	4.7-fold with CDNB	A/J mouse (f)	Liver	548
				1.3-fold with CDNB	A/J mouse (f)	Forestomach	548
				4.5-fold with CDNB	A/J mouse (f)	Small intestine	548
Sulforaphane	Broccoli*	15 µmol, daily i.g. dose for 5 d	24 h	1.9-fold with CDNB	CD-1 mouse (f)	Liver	507
				2.0-fold with CDNB	CD-1 mouse (f)	Forestomach	507
				3.0-fold with CDNB	CD-1 mouse (f)	Glandular stomach	507
				2.1-fold with CDNB	CD-1 mouse (f)	Small intestine	507
				1.2-fold with CDNB	CD-1 mouse (f)	Lung	507
Tannic acid	Tea	1% in diet, oral for 4 weeks	Continuous	1.6-fold with CDNB	Sencar mouse (f)	Stomach	558
		1% in diet, oral for 2 weeks	Continuous	1.0-fold with CDNB	Wistar rat (m)	Liver	543
				1.4-fold with CDNB	Wistar rat (m)	Small intestine	543
				1.2-fold with CDNB	Wistar rat (m)	Large intestine	543
Valencene	Orange oil	10% in diet, oral for 10 d	24 h	5-fold with CDNB	ICR/Ha mouse (f)	Liver	551
				6-fold with CDNB	ICR/Ha mouse (f)	Small intestine	551

Note: i.g., intragastric; i.p., intraperitoneal; CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene.

The change in GST is expressed as a ratio of specific activities of treated to control.

trans-stilbene oxide, cafestol palmitate, and bisethylxanthogen.[339,517] The levels of the mGSTA1 and A2 subunits, which are not expressed constitutively in mouse liver,[217] are also markedly increased by BHA and are possibly induced to a greater extent than mGSTM1 (Figure 17). The hepatic mGSTA1, A2, and M1 subunits are induced significantly by β-NF in the C57BL/6 mouse.[323] The mGSTA3 subunit appears to be largely unresponsive to drugs,[217] but mGSTA4, M2, M3, and M4 are inducible by BHA.[328,331] Studies of the regulation of the mGSTP1 and P2 subunits have been complicated both by their sex-specific regulation and by the fact that they are difficult to resolve because of their close sequence identity.[191] Available evidence suggests that

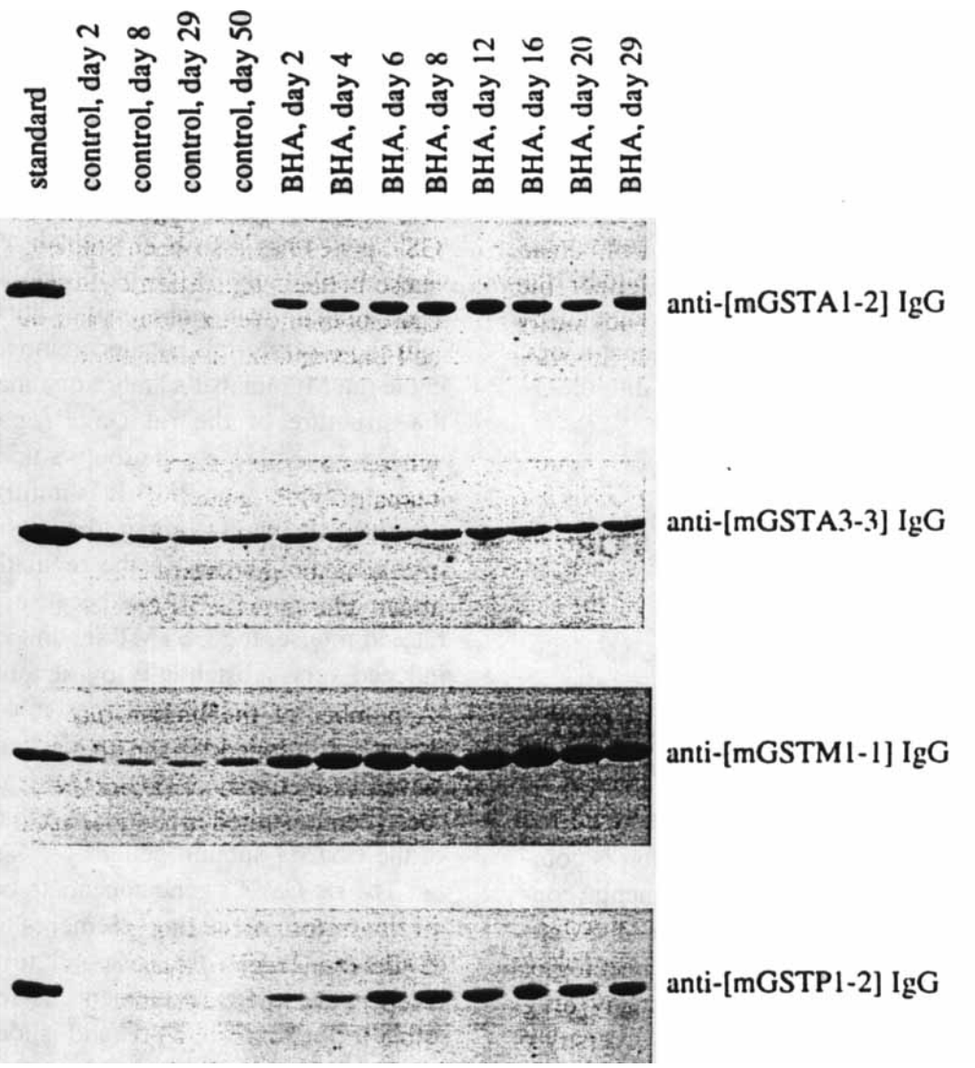


FIGURE 17. Induction of mouse hepatic GST by BHA. Mature female BALB/c mice were fed on a diet containing 0.75% BHA for either 2, 4, 6, 8, 12, 16, 20, or 29 d. Hepatic cytosols were prepared and probed with antibodies raised against murine class alpha, mu, and pi GST. The blots show that hepatic cytosol from control female mice (lanes 2 to 5 from the left-hand side) contain essentially no mGSTA1-2 or mGSTP1-2. Furthermore, the induction of mGSTP1-2 appears to follow a different time course than mGSTA1-2 and mGSTM1-1. (Adapted from Reference 217.)

both mGSTP1 and mGSTP2 are inducible; certainly, Western blotting shows a dramatic induction of class pi GST following BHA treatment (Figure 17).

Analysis of the human biopsy specimens described above suggests that GST subunits are inducible in humans. Using human primary hepatocytes, Morel et al.[562] reported that the steady-state class alpha GST (either A1 and/or A2) mRNA are increased significantly by the two dithiolethiones 1,2-dithiole-3-thione and oltipraz. PB and 3-MC also induced class alpha GST in hepatocytes from certain individuals, but a marked variation was observed in different culture specimens. Furthermore, PB and 3-MC were not as potent inducers of human GST as the dithiolethiones. Neither the hGSTM1 nor the hGSTP1 were obviously inducible in primary human hepatocytes cultured in the presence of 1,2-dithiole-3-thione, oltipraz, PB, or 3-MC.

VII. MOLECULAR BASIS FOR REGULATION OF GST EXPRESSION

A. Enhancers Identified in GST Genes

Regulation of GST expression is complex; they are subject to developmental control, their expression is tissue and sex specific, they are responsive to physiological stress, and are inducible by many drugs and chemicals. The structural diversity of the compounds that increase the expression of GST suggests that several distinct mechanisms might be responsible for induction. In particular, induction of GST by PAH, Michael acceptors, ROS, dexamethasone, and PB is likely to involve distinct mechanisms. From what is now known about enhancers within GST genes, these inducers can be grouped into four broad categories that regulate GST by distinct mechanisms: (1) PAH, (2) phenolic antioxidants, Michael reaction acceptors, ROS, organic isothiocyanates, and trivalent arsenicals (3) barbiturates, and (4) synthetic glucocorticoids.

The GSTA1 and A2 subunits in rodent liver are markedly inducible by drugs and their regulation has been studied by several research groups. Pickett's laboratory studied regulation of the rat *GSTA2* gene[178,179] and Daniel's laboratory studied the mouse *GstA1* gene.[180] The regulation of class pi GST genes has also been studied, not because of their regulation by drugs but because of their overexpression in many tumor cell lines and during hepatocarcinogenesis in the rat. Muramatsu's laboratory analyzed the structure of the rat *GSTP1* gene,[190] whereas several research groups studied the human *GSTP1* gene.[193,194] It is unfortunate that little is known about the molecular mechanisms involved in the regulation of rodent class mu GST genes because, in the rat and mouse, the GSTM1 subunit can be induced very substantially by xenobiotics. A number of the human class mu genes have been cloned,[187] and the existence of several functional *cis*-acting elements has been demonstrated in the 5′-flanking region of the *GSTM4* subunit gene.[563]

The rat *GSTA2* gene appears to contain at least four *cis*-acting elements in the 5′-flanking region that respond to xenobiotics. One of these elements is responsible for induction by PAH and is identical to the xenobiotic-responsive element (XRE) found in the rat *CYP1A1* gene.[564] A second element in the rat *GSTA2* gene has been designated the antioxidant-responsive element (ARE), because it mediates responsiveness to phenolic antioxidants;[565] the

consensus sequence of the ARE is similar, although not identical, to that of the 12-*O*-tetradecanoyl phorbol 13-acetate (TPA)-responsive element (the TPA responsive element is designated the TRE and is also called the AP-1-binding site). The third element identified in this gene is identical to the glucocorticoid-responsive element (GRE) and may render expression of *GSTA2* responsive to dexamethasone.[566] A fourth element exists in rat *GSTA2,* which is responsible for the induction of this gene by barbiturates. Responsiveness to PB might be mediated by the Barbie box element,[567] and several potential elements can be identified in the 5′-flanking region of the rat *GSTA2* gene.

In the murine *GstA1* gene, an electrophile-responsive element (EpRE), which comprises two adjacent, nonidentical, 9 base pair (bp) motifs, has been identified.[568,569] One of these motifs is identical to the ARE in the rat *GSTA2* gene and the other motif, although not identical, also contains the ARE consensus sequence; hence, the EpRE comprises essentially two tandemly arranged ARE that are separated by 6 bp. A putative Barbie box element has been identified in the 5′-flanking region of mouse *GstA1*.[567]

The rat *GSTP1* gene contains an enhancer, called GPE1 (i.e., glutathione transferase P enhancer 1), which consists of two elements that are related to the TRE.[190,570] The overexpression of *GSTP1* that accompanies hepatocarcinogenesis in the rat occurs through GPE1. The rat class pi gene also contains several negative regulatory elements, approximately 400 bp upstream from the CAP site, that appear to control basal expression of this GST. The 5′-flanking region of the human *GSTP1* gene contains a regulatory element, C1, that contains both an ARE and a TRE (see below).

B. Regulation of GST Expression by PAH

1. The XRE and its Modulation Through the Aryl Hydrocarbon (Ah) Receptor

Early studies indicated that the hepatic level of the rGSTA2 subunit is increased in animals that had been treated with 3-MC, and nuclear run-on experiments indicated that the increase in rGSTA2 protein was accompanied by an elevation in the level of its mRNA.[571] To allow analysis of the mechanism responsible for transcriptional activation, 1.6 kb of the 5′-flanking region of *rGSTA2* was ligated to a chloramphenicol acetyl transferase (CAT) reporter gene and the construct transfected into rat, mouse, and human hepatoma cell lines.[572] The 1.6-kb fragment was capable of supporting expression of the CAT gene and, furthermore, this activity could be upregulated by treating the transfected cells with β-NF, a compound that can be bound by the Ah-receptor. These data suggest that the 1.6 kb region in the 5′-flanking region of the rat *GSTA2* gene contains *cis*-acting regulatory elements responsible for both basal and inducible activity from the promoter.

A putative DNA binding site for a protein factor that responds to PAH exposure was located in the flanking region of the rat *GSTA2* gene between –905 and –885 bp.[573] To confirm this observation, an oligonucleotide encompassing the region –910 to –875 bp was cloned in front of the minimal promoter fused to the CAT reporter gene and the resulting construct was transfected into HepG2 cells. Constructs containing this region maintained responsiveness to 3-MC, whereas those lacking the element did not. The region between –905 and –885 bp showed significant homology

to the XRE described in a number of inducible *CYP* genes.[574–576] Observation that the rat GSTA2-CAT constructs are not responsive to β-NF when transfected into cells lacking the Ah-receptor[572] is consistent with the hypothesis that this receptor is responsible for induction by PAH.

2. Involvement of the Ah-Receptor in the Induction of XRE-Containing Genes

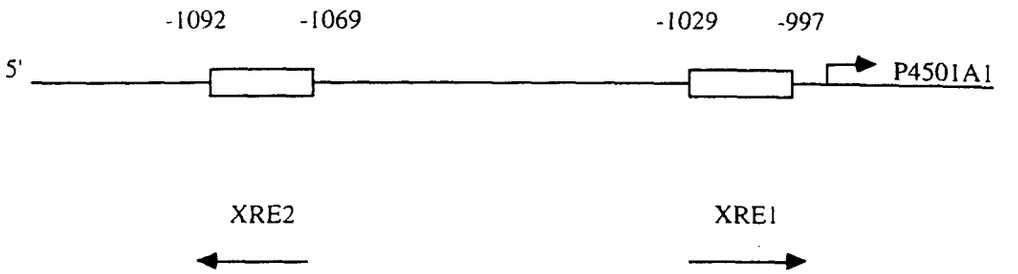
Inducible expression of *CYP1A1* by PAH is mediated through the *cis*-acting XRE. In the flanking region of the *CYP1A1* gene, multiple XRE exist and induction by PAH occurs through the Ah-receptor interacting with these elements. The expression of the rGSTA2 subunit is also increased in liver in response to PAH and identification of a regulatory sequence with homology to the XRE in the *CYP1A1* gene suggested that a similar regulatory mechanism may operate in both *GST* and *CYP* genes.[573]

In the absence of PAH, the Ah-receptor is normally maintained in the cytosol of the cell as an inactive form in association with Hsp90. Binding of PAH to the Ah-receptor:Hsp90 complex allows dissociation of the receptor from Hsp90, resulting in the formation of a ligand-Ah-receptor complex that has increased affinity for DNA.[577] Initial observations suggested that, once the PAH ligand-Ah-receptor complex dissociates from Hsp90, the receptor plus ligand is able to bind an 84 kDa nuclear translocation factor known as Ah-receptor nuclear translocator (ARNT). This hypothesis was supported by the observation that cells harboring a mutation in "nuclear targeting" of the Ah-receptor complex were defective in the expression of CYP1A1.[578] Isolation of a cDNA clone that was able to rescue the mutant cell phenotype and conferred responsiveness to PAH, appeared to confirm a translocation role for ARNT. However, the hypothesis that ARNT plays a role in shuttling the ligand-activated Ah-receptor to the nucleus is not consistent with more recent data demonstrating that ARNT is exclusively a nuclear protein. Hence, it is now suggested that ARNT forms a heterodimeric complex with the Ah-receptor that allows it to bind to DNA.[579-581] Analysis of the primary amino acid sequences of the Ah-receptor and ARNT has suggested the presence of a basic helix-loop-helix (bHLH) domain in both proteins that may enable them to dimerize and form a DNA-binding complex similar to Myc/Max and MyoD/E2A.[582,583] Thus, binding of PAH to the Ah-receptor results in the dissociation of the activated complex from Hsp90 and its translocation to the nucleus. Translocation may involve interaction with ARNT, but recent evidence suggests that heterodimerization between ligand-Ah-receptor and ARNT occurs in the nucleus and enables the bHLH domains to interact with the XRE in the 5′-flanking sequence of PAH-regulated genes.

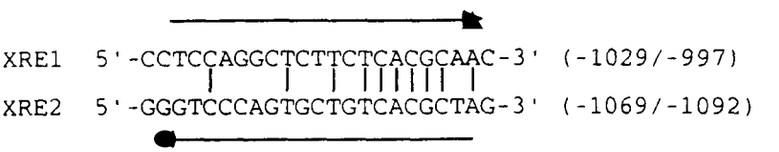
3. Structure of the XRE

The best example of regulation of genes by halogenated aromatic hydrocarbons and PAH is provided by the rat *CYP1A1* gene, which is induced 30-fold in response to treatment with such chemicals.[584] Within rat *CYP1A1*, two enhancer elements (XRE1 and XRE2) have been identified at approximately −1000 bp from the transcriptional start site that are responsible for inducibility by PAH.[585] Each of these elements was found to encompass about 18 bp (Figure 18). It was demonstrated that the two elements are tissue-specific inducible enhancers and, al-

A. Organisation of the rat P450 1A1 XRE1 and XRE2 within the gene enhancer.



B. Sequence comparison of XRE1 and XRE2 from rat cytochrome P450 1A1.



A. The relative orientation (indicated by arrows) and positions of XRE1 and XRE2 in the 5' flanking sequence of the rat cytochrome P450 1A1 structural gene enhancer.

B. Comparison of the DNA sequences of XRE1 and XRE2. Sequences from the sense and antisense strands of XRE1 and XRE2 respectively, are compared (arrows indicate orientation). Vertical lines represent matched bases between the two enhancer elements.

FIGURE 18. Structure of the XRE in the rat *CYP1A1* gene. (A) Organization of XRE1 and XRE2 within the rat *CYP1A1* gene. (B) Sequence comparison between XRE1 and XRE2. Sequences from the sense and antisense strands of XRE1 and XRE2 are compared (arrows indicate orientation). The vertical lines represent matched bases between the two enhancer elements.

though they are inversely orientated with respect to each other, they both contain the conserved sequence 5′-GCGTG-3′. Significant homology was seen over the entire length of both of the enhancers, suggesting they are derived from a common sequence. It was found that although the elements function independently of each other, in either orientation, highest enhancer activity is observed from constructs where the original

structure is retained. It was also demonstrated that, by increasing the number of copies of the XRE located in the 5′-flanking sequence, the activity from the promoter increased accordingly.[585] Mouse hepatoma cells also contain a CYP isoenzyme (Cyp1A1) that is induced in response to dioxin exposure.[586] Like the rat *CYP1A1* gene, the 5′-flanking region of the mouse *Cyp1A1* gene contains several regulatory sequences with homology to the XRE. Of the three XRE described (also known as DRE1, DRE2, and DRE3), all were shown to contain a conserved "core" sequence 5′-TA/TGCGTG-3′ that is essential for binding of the Ah-receptor to the enhancer. However, in addition to this observation, functional analysis indicated that nucleotides flanking the core sequence contribute to the enhancer function.[586,587] Table 13 shows the nucleotide sequence of the XRE from several different genes.

The XRE in the rat *GSTA2* gene contains the same core sequence as that found in rat *CYP1A1*. Furthermore, the XRE in rat *GSTA2* is found at approximately the same position in the upstream regulatory sequence with respect to the transcriptional start site as is the XRE in rat *CYP1A1*. However, unlike the *CYP* genes, the rat *GSTA2* gene does not contain multiple copies of the XRE.

C. The Role of Electrophiles, Antioxidants and Pro-Oxidants in the Regulation of Expression of GST

1. Monofunctional and Bifunctional Inducers

All the chemicals listed in Table 11 induce GST and other phase II drug-metabolizing enzymes, but large differences exist in their ability to induce the phase I CYP enzymes. Consequently, the xenobiotics that increase expression of both phase I and phase II enzymes have been classified as bifunctional inducers, whereas those that induce only phase II enzymes are designated monofunctional inducers.[32] The distinction between monofunctional and bifunctional inducers is possibly not as clear-cut as was at first thought. The definition of bifunctional and monofunctional inducers was originally made primarily on the basis of induction of Ah hydroxylase activity. Chemicals such as β-NF, tetrachlorodibenzo-*p*-dioxin, PAH, and azo dyes, which induce both phase II drug-metabolizing enzymes and Ah hydroxylase activity, were classed as bifunctional inducers, whereas diphenols, thiocarbamates, and isothiocyanates, which induce phase II enzymes but not Ah hydroxylase activity, were designated monofunctional inducers. According to this definition it is possible for monofunctional inducers to cause transcriptional activation of *CYP* genes other than those encoding enzymes with Ah hydroxylase activity.

Using NQO as the paradigm for the regulation of phase II enzymes, Prochaska and Talalay[32] showed that monofunctional inducers act independently of the Ah-receptor, whereas bifunctional inducers require competent Ah-receptors. Therefore, the essential difference between monofunctional- and bifunctional-inducing agents is that the former group of chemicals act via an XRE-independent process, whereas the latter group of chemicals act via an XRE-dependent mechanism. The fact that the induction of phase II enzymes by bifunctional inducers is dependent on the Ah-receptor suggests that CYP1A1 Ah hydroxylase activity is required to oxidize the xenobiotic before it is able to effect induction of phase II enzymes; thus, increased expression of phase

TABLE 13
XRE in Genes Encoding Phase I and Phase II Drug-Metabolizing Enzymes

Gene	Species	Strand	Position		Enhancer element	Ref.
CYP1A1	Rat	Sense	–1029/–997	XRE1	5′-CCTCCAGGCTCTTCT**CACGC**A**A**CTCCGGG**G**CA-3′	585
CYP1A1	Rat	Antisense	–1069/–1092	XRE2	5′-GGGTCCCAGTGCTGT**CACGC**T**A**GCTGGGG**G**AG-3′	585
Cyp1A1	Mouse	Sense	–906/–880	DRE1	5′-TGGAGCAGGCTTACG**CACGC**T**A**GCCTCAG**G**AA-3′	586, 587
Cyp1A1	Mouse	Antisense	–1048/–1074	DRE2	5′-GGGTCCCAGTGCTGT**CACGC**T**A**GCTGCTG**G**GG-3′	586, 587
Cyp1A1	Mouse	Sense	–997/–978	DRE3	5′-CCTCCAGGCTCTTCT**CACGC**A**A**CTCCGGG**G**CA-3′	586, 587
GSTA2	Rat	Antisense	–875/–925	XRE	5′-CTGGCCTCAGGGATG**CACGC**A**A**CATGCCT**G**CC-3′	573
NQO1	Rat	Antisense	–352/–393	XRE	5′-GGGAAATCGCCTTTG**CACGC**A**A**GGGGAAG**G**GT-3′	588
Consensus				XRE	5′-NN*K*NNNNNNNNNN*W*N*K***CACGC***WA.S*NNNNNNG**N**N-3′	

Note: The location of the enhancer element is indicated with respect to the transcriptional start site. Letters in bold typeface represent bases conserved between all sequences. Underlined bases represent Ah-receptor complex core-binding sequence. The consensus sequence shows those nucleotides that are conserved between all sequences (**bold type**), whereas letters in *italics* represent nucleotides that can be represented by one of two bases. Here *W* is either A or T; *S* is G or C; *K* is G or T; and *N* is any nucleotide. In the original paper describing the identification of XRE sequences in the rat P450c gene, Fujisawa-Sehara et al.[585] reported the consensus core sequence as 5′-CACGC-3′. For the purpose of this review, all sequences of XRE are presented with this in mind.

II enzymes by bifunctional inducers can be achieved indirectly by a metabolic cascade.

On the basis of these data, it was proposed that the induction of phase II enzymes by bifunctional inducers requires metabolism of the inducer by XRE-regulated CYP enzymes. According to this hypothesis, phase II enzyme induction by bifunctional inducers involves both the induction of CYP1A1 and the conversion of the inducing agent by these cytochromes into a Michael reaction acceptor (electrophilic olefin), which signals the induction of phase II enzymes (Figure 19). Thus, the compound that effects induction of phase I enzymes is distinct from that which causes induction of the phase II enzymes. Therefore, metabolism of the parental compound is required for induction of phase II enzymes by bifunctional inducers.

It should be emphasized that not all bifunctional inducers transcriptionally activate *GST* genes indirectly through the production of Michael reaction acceptors. In particular, the *GST* that contain a functional XRE are regulated directly by PAH via the Ah-receptor. In such instances, the situation can be complex as certain PAH and their metabolites can activate a specific *GST* gene through two separate *cis*-acting elements. Compounds that are metabolically inert, such as the halogenated aromatic hydrocarbon tetrachlorodibenzo-*p*-dioxin, can only transcriptionally activate *GST* through the XRE. Besides PAH and tetrachlorodibenzo-*p*-dioxin, barbiturates and glucocorticoids are bifunctional inducers that may activate *GST* genes through direct and indirect mechanisms.

By contrast with bifunctional inducers, the monofunctional inducers are structurally highly diverse. As proposed by Prochaska and Talalay,[32] monofunctional inducers all contain electron-deficient centers or are metabolized to such compounds; this definition includes Michael reaction acceptors as a major group of inducer. The xenobiotics benzyl isothiocyanate, catechol and cumene hydroperoxide, and the Michael reaction acceptors 1-nitro-1-cyclohexene, 5,6-dihydro-2*H*-pyran-2-one and 2-methylene-4-butyrolactone are all thought to be direct-acting monofunctional inducers, whereas BHA and ethoxyquin are indirect-acting monofunctional inducers, and require to be metabolized (but not necessarily by CYP1A1) to tBHQ and 6-hydroxy-2,2,4-trimethyl-1,2-dihydroquinoline,[530] respectively, to be effective. A number of synthetic antioxidants have been found to markedly induce CYP isoenzymes.[38,589,590] In particular, ethoxyquin (6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline) induces CYP1A2, 2B1, 2B2, and 3A4, but it is not known whether this effect is caused by the parental compound or by the major metabolite 6-hydroxy-2,2,4-trimethyl-1,2-dihydroquinoline, or even by the oxidation product 2,2,4-trimethyl-6-quinolone.

In addition to the generation of Michael reaction acceptors, the production of ROS can cause induction of phase II enzymes. Such species can arise during the metabolism of drugs by poorly coupled CYP reactions.[537,538] It is, however, unclear whether ROS act directly to induce phase II enzymes or react with other molecules within the cell to produce Michael acceptors (e.g., 4-hydroxynonenal or base propenals) that are the ultimate inducing molecules. It will be important to establish whether Michael acceptors and ROS modulate GST expression by separate pathways or a single common pathway.

It is becoming clear that the induction of detoxification enzymes by xenobiotics is highly complex and involves both the metabolism (activation and deactivation) of the inducing agent itself as well as the presence of multiple *cis*-acting elements in the flanking regions of the genes encoding detoxification proteins.

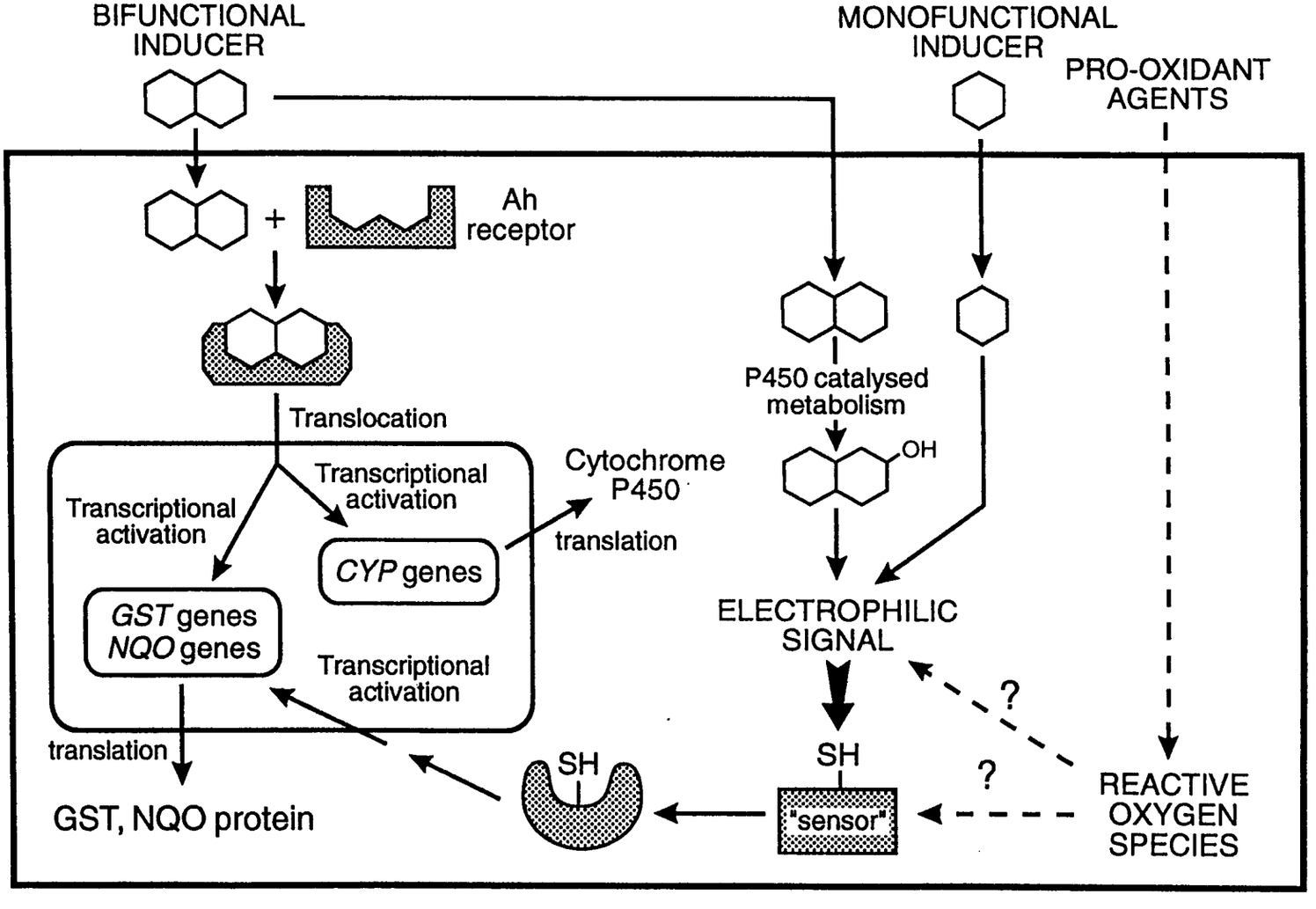


FIGURE 19. Mechanism of induction of GST by bifunctional and monofunctional inducers. This model is adapted from that proposed by Talalay and co-workers.[32,33] Bifunctional inducers can transcriptionally activate genes directly via the XRE, but require to be metabolized before they can effect induction of genes through the ARE. By contrast, monofunctional inducers and prooxidant agents transcriptionally activate genes solely through the ARE.

2. Identification of the Antioxidant-Responsive Element (ARE)

Computer-aided sequence examination and deletion analyses allowed Pickett and his co-workers[564,565] to identify a novel *cis*-acting regulatory element that mediates induction of the rGSTA2 subunit by monofunctional inducers. This element is now called the ARE, although originally it was isolated within a 41-bp sequence referred to as a "β-NF-responsive element," located between nucleotides –722 and –682, in the rat *GSTA2* gene.[564] The enhancer within this 41-bp sequence is now known to respond to monofunctional inducers, but it is ironic that it was initially located as a result of its responsiveness to the bifunctional inducers, β-NF and 3-MC, not through treatment with monofunctional inducers. The fact that these compounds could cause transcriptional activation through an enhancer other than the XRE was initially a surprise, as it suggested that PAH could operate through two separate *cis*-acting elements. It was demonstrated that the enhancer contained within the 41-bp sequence mediates responsiveness to bifunctional inducers only in cells that possess a functional Ah-receptor and active CYP1A1.[591] Although cells that lacked either the functional Ah-receptor or CYP1A1 did not increase transcription through the enhancer when exposed to PAH, increased transcriptional activity was observed in response to the monofunctional inducers tBHQ and 3, 5 di-*tert* butylcatechol.

Deletion analysis of the 41-bp sequence showed that an enhancer element within the –722 and –682 region provided both basal and xenobiotic inducibility to *GSTA2*. Stepwise 5′ deletion from nucleotide –722 to nucleotide –697 gradually abolished the basal level expression provided by the ARE, whereas 3′ deletions past nucleotide –688 completely abolished both basal and inducible activities.[565] The remaining sequence, 5′-TGACAAAGC-3′, was subsequently shown to be the minimal sequence required for inducibility by synthetic antioxidants. In addition to the deletion experiments, point mutation analysis defined 5′-TGACNNNGC -3′ as the core sequence of the ARE. Changes to any of the nucleotides 5′-TGAC-3′ within this core sequence abolished both basal and inducible activities of the ARE (Figure 20), whereas mutation of either of the 3′ G or C nucleotides (or both) of the core sequence abolished only the inducible activity.[592] Point mutations in the AAA nucleotides failed to alter either basal or inducible expression.[565] These results indicate that some of the nucleotides are important for both basal and inducible expression, and suggest that proteins involved in basal and inducible expression share overlapping DNA recognition motifs.

3. Characterization of the EpRE and its Relationship with the ARE

The 5′-flanking sequence of the mouse *GstA1* gene contains a 41-bp sequence that is closely similar to the 41-bp ARE-containing sequence in the regulatory region of the rat *GSTA2* gene (Figure 21). This murine 41-bp 5′-flanking sequence mediates responsiveness to electrophilic compounds, and the enhancer within this region flanking *GstA1* has therefore been called an EpRE.[569,593] Ligation of this enhancer to the minimal promoter of the *GstA1* gene revealed that the EpRE increased basal activity synergistically with copy number and conferred inducibility by a wide range of chemicals including tBHQ, β-NF, 3-MC, TPA, and PB. It has also been demonstrated that PAH need to be metabolized to induce *GstA1* expression via the EpRE.